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RESEARCH ARTICLE

POTENT NANO CARRIERS FOR TARGETING BREAST CANCER

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Abstract

Breast cancer (BC) is the most common cancer in female population. Triple-negative breast cancer (TNBC) is the subtype of breast cancer in which the three major receptors are absent. TNBC has very aggressive phenotype and high spreading rate/metastasis which often results in resistance to chemotherapy. Nano-medicines offer various promising approaches to BC and TNBC therapy because of its unique characteristics *viz.*nanometric size, high drug payload, targeting capabilities and capability to accommodate multiple therapeutic moieties.This review focuses on the conventional therapies, novel approaches and recent treatment strategies available for the therapeutic benefits to the patients. The role of cancer stem cell in the recurrence of BC and TNBC has also been highlighted. Moreover, the application of nanomedicines for the treatment of BC/TNBC and different molecular targets available for further exploration have also been discussed.Nanomedicines offers a right platform to develop multi-faceted treatment strategies to control the spread and recurrence of BC/TNBC. Researchers explored multiple pathways that can play a major role in controlling the progression of TNBC. Several chemotherapeutic agents were delivered using these nanocarriers with outstanding responses. Numerous targets were also reported that could stop/alter the specific pathway or receptor interaction.

Introduction:-

In India, Breast Cancer (BC) is the most common cancer in women and it is the most common cancer among all the cancer subtypes, globally. 70-80 % BC are histo-pathologically classified into 20 types and based on the molecular subtypes it is classified as luminal A, luminal B, human epidermal growth factor receptor 2 (HER2) type and estrogen or progesterone receptor positive.Nearly, 15-17% of the breast cancers lack HER-2, estrogen receptor (ER) and progesterone receptor (PR) expression which is known as Triple-negative breast cancer (TNBC). TNBC is a subtype of BC with similar characteristics of basal-like with an aggressive phenotype and high spreading rate.The high metastatic rate can be attributed to gene alterations and genomic instability in multiple tumour suppressor genes.(1)

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Approximately, 2 million cases were reported globally in 2018 and 627,000 women died due to BC which is 15 % among all cancer death in women in same year.(2) In India 162,468 new cases of BC and 87,090 death were reported in 2018. According to a report, theproportion of TNBC cases was in the range of about 7% to 28 % in different countries,while the highest TNBC incidence was reported in India.(3)

Irrespective of resemblances in standard clinic-pathological characteristics, TNBC remains a very heterogeneous disease which has a wide variety of biological, morphological and clinical behaviour.(4)Once metastasis occurs, chemotherapy always fails to offer a selective and broader response which can lead to the death of the patient.(5)About 90% of the death caused by breast cancer is due to metastasis.(6)Recent advances in surgery, radiation, endocrine therapy and chemotherapy have declined the death rate of patients with BC, though 20-30% patients even diagnosed at early stages will eventually develop metastatic disease.(7)

The deteriorating environment and lifestyle flaws are raising the frequency of this cancer in which most of the risk factors are linked to oestrogen. The women with the age of 40-55 are at a greater risk of BC. Risk increases if there is a family history of BC.There are certain reports that suggest an increase in body mass index increases the risk of cancer in post-menopausal women.Consuming excess fat can also cause hormonally dependent BC by modifying levels of circulating oestrogens.Women having BC family background seems to be at higher risk of tumorigenesis when exposed to ionizing radiation compared to others.(8)The effect of ionizing radiation in the range of 1.1 to 2.7 at 1 Gy (gray) increases the risk of BC in the women exposed to radiation before the age of 40 years. Other factors which can induce BC are genetic, hormonal, behavioural, environmental and dietary factors. Post-menopausal hormone therapy and the use of oral contraceptive can also induce the disease.

The emergence of nano-medicines offers a paradigm shift in treatment approaches available for BC and TNBC therapy because of its unique characteristics like nanometric size, longer circulation half-lives, higher drug entrapment, ability towards surface modification and targeting capabilities. This review focuses on recent strategies and advancements available in conventional therapies to offer more reliable therapeutic outcomes to patients suffering from BC and TNBC.(9)

Treatments strategies for BC and TNBC:
Several strategies are available to treat the BC and TNBC such as conventional approaches like adjuvant and neo-adjuvant chemotherapy, surgery, endrocrine therapy and radiation therapy. Novel strategies include active and passive targeting by exploiting nanoparticulate carriers, and some biological molecules, which prevent the spread of tumour and eliminates the chances of recurrence of cancer in breast.

Conventional therapy:
During the initial phases of BC, surgery is performed to remove the tumor mass located in and around the breast or regional lymph nodes. Even after surgical procedures, there can be some deposits which are undetected and may cause recurrence. There can be a frequent problem in the arm after surgery which can cause psychological distress too.(10)

Chemotherapy is the short term conventional therapy because it kills cancerous as well as normal cells and majority of the available drugs are able to produce desired antitumor effect only at higher doses. Adjuvant chemotherapy for the treatment of the BC is carried out on the basis of two parameters, one being prophesied sensitivity for a specific method and the advantage for its use and another being personal threat of reversion. The adoption of the therapy depends upon the patient's condition. Neo-adjuvant therapy is recommended before surgery to access the effect of a drug in patients and to reduce the extent of surgery if the patients are responding well to the drugs.(11) However, the chemotherapy induces cytotoxicity to tumour cells, which prevents the progression of cancer. The higher doses of chemotherapeutic agents induce anaemia. Other side effects include discomfort, sickness, vomiting, loss of hair, fluctuations in weight, fatigue and nervousness.(12)

Currently, there are no specific guidelines for conventional treatment of TNBC because heterogeneity of this cancer depicts diverse pathological behaviour.(13) However, there are many chemotherapeutic regimens such as anthracycline-taxane combination used in high risk cases of TNBC. Similarly, epirubicin, 5-fluorouracil, cyclophosphamide regimens followed by docetaxel (DTX) or paclitaxel (PTX) are also used in moderate to high risk cases as adjuvant chemotherapeutic regimens. Platinum-based regimens such as cisplatin, carboplatin and anthracycline compounds are used also as neo-adjuvant therapy.(14) Among neo-adjuvant and adjuvant therapies,

the former has higher rate of pathologic complete response (PCR) which prevents the recurrence of cancer in TNBC patients. The neo-adjuvant therapy is more effective in the TNBC compared to adjuvant therapy.

Radiation therapy given during the treatment iscost-effective as well as clinically effectiveand offers a reduced risk of recurrence hence improves the survival rate of the patients. Radiation therapy induces dermatitis on exposed area where the skin turns red in colour, itchy and painful.(15)

Novel Approaches:

Novel approaches include Nano-particulates, receptor targeting molecules and antibodies used in the treatment of the hormone positive and hormone negative BC. The targeting carrier may act as passively or actively to reach at the cancer site. Nano-medicine is an emerging field expecting to create nano-drugs with improved drug delivery. It is an integration of material science, pharmaceutics, medicine, engineering,molecular biology and information technology. The use of nano-science with medicine creates a way to study the biological system in a better manner and helps to understand various mechanisms involved. Nano-medicines can easily cross cell membrane and other barriers helping in drug conveyance. For clinical application, the most important factor to be considered is stability, circulation time and biocompatibility which can be effectively achieved by employing nanomedicines. Nanomedicines such as drug loaded nanoparticles, micelles, and liposome possess specific optical, magnetic, chemical and structural properties that impart a capability to cross tissue barriers, un-coat and deliver drug cargo inside the cells (Figure 1). The most common use of nanoparticle as a carrier with a core containing the specific drug is to bypass the side-effects associated with it. Nanoparticles offer an advantage of protecting the drug or encapsulated molecules from exposure to external environment that offer protection to drug itself and prevents undue exposure to surrounding cells. Due to this advantage, NPs can be used for both vaccination to elicit an immune response and for gene delivery.(16) The factors that determine cellular uptake, bio-distribution patterns and clearance mechanisms are particle size, shape and surface chemistry.(17)

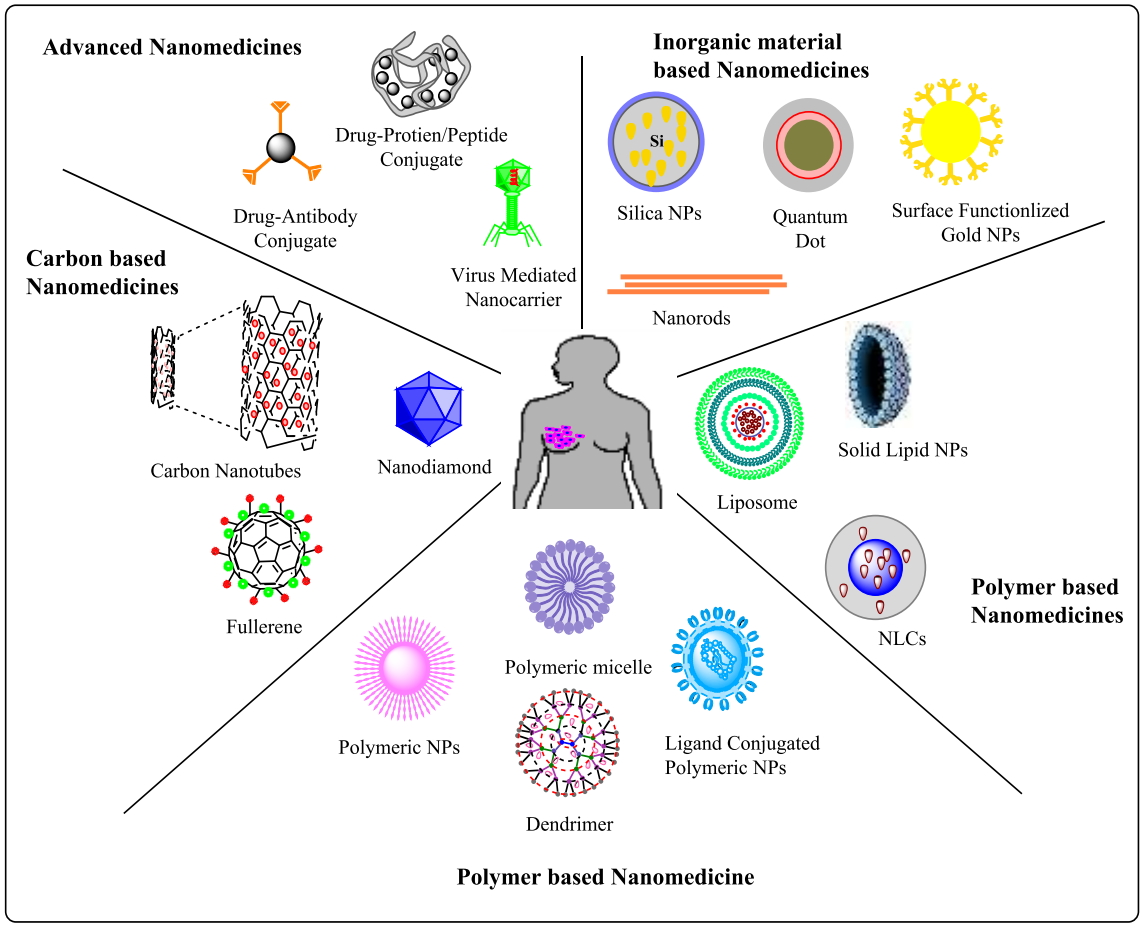

Fig. 1:- Types of nanomedicine used for the diagnosis and treatment for BC and TNBC.

Passive targeting:

Currently, passive targeting is being used as tumor targeting approach because the nano-particulates delivered passively possess tendency to differentiate between tumour tissues and normal tissues. (18) The tumor mass have less developed vasculature. The molecules with particle size less than 500 nm can easily penetrate the vasculature and reach the tumour, this phenomena is known as Enhanced Permeability and Retention (EPR) effect and the passive targeting is based on this phenomenon. (19) Nano-formulations such as polymeric NPs, micelles, fullerenes, nanotubes and lipid based formulation such as liposomes, solid lipid nanoparticles (SLNs), and nano-emulsion etc. can penetrate leaky vasculature easily. The complexity of tumor microenvironment in the hormone negative breast tumour exhibits heterogeneity of tumor endothelial cells, dense collagen rich tumor, extratumor matrix and intratumor pressure resist the passive diffusion of the nano-particulates.(20)However, few researchers have succeeded in the delivering the drug by passive targeting through EPR effect. For example, Palma and co-workers developed the DTX loaded Polyethylene glycol -poly (epsilon-caprolactone/ PCL) nanoparticles, which exhibited prolonged drug release up to 30 days. These developed NPs revealed to be effective against MDA-MB-231 cells *in vitro* and depicted similar efficacy and improved survival in TNBC animal model.(21) Similarly, PTX conjugated with modified Polyethylene glycol (PEG) micelles was targeted to TNBC. In this study, the researcher modified the PEG to PEG containing eight hydroxyl groups and conjugated with PTX using linker 4-nitrophenyl chloroformate. The micelles were conjugated with iNGR peptide to target NRP-1 receptor in TNBC. These PTX micelles showed higher uptake in TNBC than the non-targeted micelles and Taxol®(22). Another study on PTX bounded albumin liposome (Abraxane®), revealed to be an effective nano-carrier to deliver the drug in TNBC. This lipidicnanomedicine with particle size around 400 nm has shown effective accumulation at the tumor site. (23)

Active targeting:

Active targeting involves site-specific tumor targeting. Generally, active targeting is achieved by functionalizing the nano-particulates with specific ligands which recognise and bind at the particular receptor or molecule at the targeted site.Functionalization of nanomedicines will enhance their deposition in certain regions such as an ischemic tissue, tumour region or inflamed area. The release of the drug is based on activation by pH, redox potential, the presence of certain enzymes and temperature. Similarly, surface functionalized NPs like DTX loaded di-block copolymer of Polyethylene glycol-poly(lactic-co-glycolic acid) (PEG-PLGA) NPs; single walled carbon nanotube surface coated polyidoaminedendrimer and doxorubicin (DOX) loaded PEG liposome have also been investigated. (24)

The active targeting can be achieved by addressing tumor microenvironment (vessel, extracellular matrix) by the use of monoclonal antibodies, pH sensitive drug carriers, non-platinum metal complexes and magnetic nanoparticles (MNPs) to cure the metastatic condition as well as imaging of TNBC (**Figure 2**) (25). The active targeting helps to evade the uncertain off-target toxicity of drugs and improves the drug uptake and accumulation in tumour tissue.

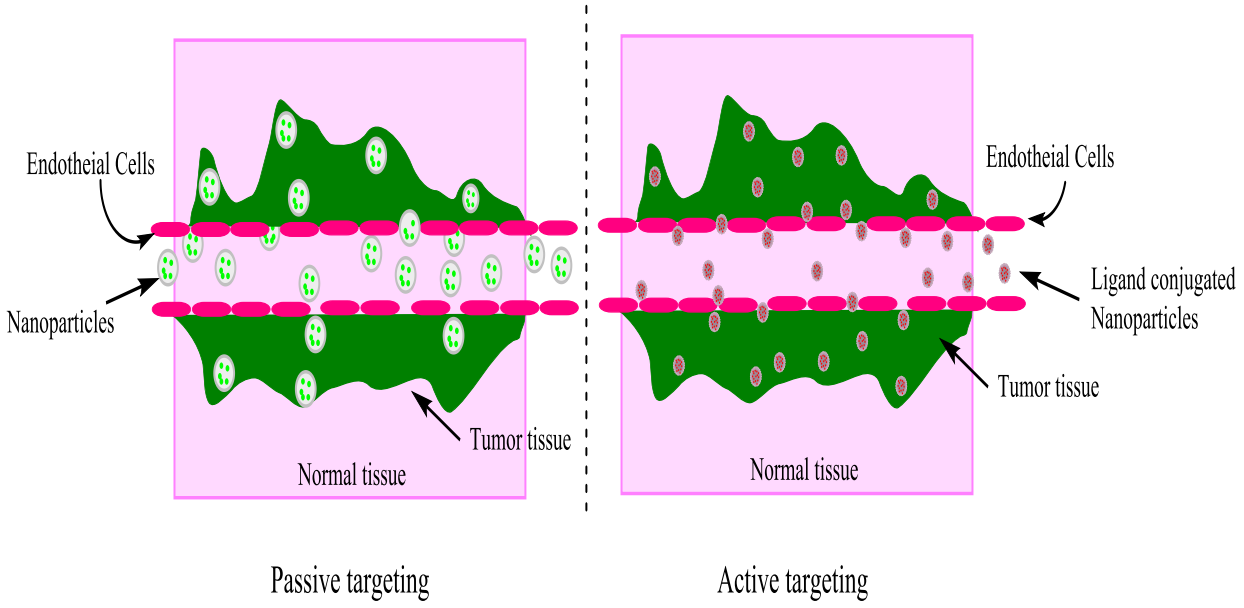

Fig. 2:- Targeting approaches of nanomedicine in Cancer therapy.

Potent Nano carriers for targeting breast cancer:
Nanotechnology is a developing area of science with potential for imaging, monitoring, diagnosing and delivering chemotherapeutic drugs to tumour site. NPs help to deliver drugs with enhanced efficacy and reduced toxicity and are able to overcome biological barriers, thus improving anticancer activity. Nano-medicine is an emerging approach to deliver the drug by improving its therapeutic efficacy. It plays significant role in the development of nano-medicine in the management of breast cancer.(26)

Carbon nano-materials associated carrier:
The carbon nanomaterials such as CNTs, fullerenes and graphenes have been the subject of interest for researchers due to their biocompatibility, versatile chemical functionalization, effective drug delivery strategy and stable physio-chemical properties. The recent reports have shown that these materials can be used for controlled drug delivery of the therapeutic agents and also as contrast agents for diagnosing, imaging and locating tumours. Fullerenes, CNTsand graphenes can be functionalized on its surface with imaging agents and chemotherapeutics to diagnose and treat cancer.Nurunnabiet al.(27) formulated carboxyl functionalized graphene which revealed as a potential imaging agent of tumours in deep tissue via a non-invasive technique that could also kill tumor cells through combined photodynamic and photo-thermal effects.Amine-functionalized fullerenolnanocarrier present excellent fluorescence properties which also possess the ability to easily penetrate into the BC cells (MCF-7) in-vitro.(28)

In another study, PEG nano-sheets modified with graphene oxide (PEG-GO) was coupled with lipophilic aromatic molecule. The PEG-GO nano-sheets containing SN38, an analogue of camptothecin is soluble in aqueous solvents. This nano-carrier exhibits anticancer effectwith improved biocompatibility and enhanced stability in aqueous solvents.(29).*In vitro* studies on nano-sheets exhibited anti-proliferative effects on cancer cells such as MDA-MB-436, SK-BR-3, MDA-MB-231 and MCF-10A cells. Researchers suggested that PEG-GO nano-sheets altered the oxidative phosphorylation in mitochondria of BC cells without affecting normal cells. A labelling strategy used to understand the mechanism of graphene oxide nano-sheets modified by PEG on BC cells as well as on normal cells is termed as SILAC (Stable Isotope Labelling by Amino acids in Cell culture) which quantify the expression of the protein. PEG-GO selectively down-regulated PGC-1a protein expression in tumor, which leads to the modification of proteins involved in the generation of energy by inhibiting the oxidative phosphorylation in mitochondria causing the alteration in the F-actin cytoskeleton assembly and decreases ATP synthesis.The suppression of ATP synthesis leads to inhibition of cancer cell invasion and metastasis.(30).

Gloriosasuperba mediated Nanoparticles:
The new era nanomedicines containing phytoconstituents or plant derived products are the emerging approach based on green chemistry to target medicinal plant extracts which exhibit potent anticancer effects. Rokade et al. utilized this approach against breast cancer in which, they synthesized the phytogenic platinum nanoparticles (PtNPs) and palladium nanoparticles (PdNPs) using Gloriosasuperba tuber extract (GSTE). It was reported that the synthesis of these NPs was rapid, efficient and environmentally safe. The NPs revealed the potent anticancer activity against MCF-7 cells *in vitro*. PtNPs and PdNPs exhibited cytotoxicity as 49.65 ± 1.99% and 36.26 ± 0.91%, respectively; and it induced the apoptosis by externalization of phosphatidyl serine and membrane blebbing mechanism.(31).

In another study, Ghosh et al. reported the preparation of gold (AuNPs) and silver nanoparticles (AgNPs) using GSTE. The size of AuNPs was found in the range of 3-20 nm and the AgNPs in the range of 20-120 nm. Both AgNPs and AuNPs depicted anticancer activity against MCF-7 cells. Majorly, the AgNPs exhibited potent anti-cancer activity, while the combination of AgNPs with AuNPs revealed the synergistic effect on breast cancer cells *in vitro*.(32)

Similarly, Muthukrishnan et al. synthesized the Gloriosasuperba leaf extract containing AgNPs, which was assessed against DLA tumor cells. The developed NPs have shown effective anticancer activity by 77 µg as ED_{50} whereas the Gloriosasuperba leaf extract showed the ED_{50} value at 80 µg. The studies thus demonstrated NPs to be environment friendly to deliver the potential active drugs against triple negative breast cancer (33).

Bismuth lipophilic Nanoparticles:
Bismuth is known as a "green" element and the heaviest member of the pnictogen group. It was found that despite of no direct application of bismuth on breast cancer cells, bismuth loaded compounds like thiosemicarbazone, hydrazone, and dithiocarbamate depicted potent anticancer activity. The study on Bi (III) hydrazine complex was evaluated against MCF-7 cell lines and the results of *in vitro* evaluation suggested them to be effective against these

cancer cell lines.[86]In another study, the Bismuth lipophilic Nanoparticles (BisBAL NPs) were assessed against the human breast carcinoma MCF-7 cell line. These NPs induced the dose-dependent inhibition of tumor growth and growth inhibition was reported to be 51% at 1µM in MCF-7 cells. It also causes the cell apoptosis by the mechanism that includes loss of plasma membrane integrity and genotoxic effect on the genomic DNA. It is an innovative therapy in cancer nanomedicine.(34)

Multifunctional core-shell nanomedicine:

The multifunctional core-shell nanomedicines have the capability to inhibit the metastasis of tumor with enhanced cytotoxicity by photodynamic action. Malarvizhiet al. reported that the development of core-shell nanomedicine consisting of PLGA nanocore encapsulating m-tetra (hydroxyphenyl)chlorin (mTHPC) as a photosensitizer and albumin nanoshell encapsulating Dasatanib (a tyrosine kinase inhibitor) with 20 nm of particle diameter. The *in vitro* studieson MDA-MB-231 showed the disruption of Src kinase protien which is involved in the migration of metastatic cancer cells. The combinatorial delivery of Dasatinib with mTHPC in PLGA nano core produced the synergestic cytotoxicity in both MDA-MB-231 metastatic cell and MCF-7 (non-metastaic tumor cell) by the photoactivated oxidative stress mechanism. With the use of photosensitizer, it leads to the inhibition of Src that in turn causes apoptosis in metastatic cancer cells. The photodynamic killing using nanomedicine is an emerging strategy against TNBC. (35)

Natural bio-actives for cancer treatment:

Natural products are the main sources in the development of anti-cancer drugs. Phytochemicals derived from plants have the ability to inhibit carcinogenesis. It has been suggested that many bioactive natural compounds prevent metastasis by inhibiting the various signalling pathways,thus playing an important role in drug discovery and development. It is been reported that most of the anti-cancer drugs approved by the USFDA are natural products. (36)Nano-particles interact with various enzymes/proteins and thus possesses biological activity at relevant target of the vast chemical space.Phenolic compounds such as flavonoid, lignins, tannins, and coumarins exhibit anticancer activity by the mechanism of cell cycle arrest, inhibition of angiogenesis, induction of p53 and inducing apoptosis (**Table 1**). (37)

Table 1:- Various bio-actives and their possible target molecules for cancer treatment.

Flavonoids	Cancer Type	Cell lines	Comments
Lycopene	Breast Cancer	MCF-7 & MDA-MB468 (Human)	Epigenetically promotes expression of GSTP1 tumor suppressor gene.[134]
Resveratrol	Breast Cancer	MCF-7 & MDA-MB231 (Human)	Suppression of fatty acid synthase (FAS) gene expression alongwith induction of genes like BNIP3, DAPK2 and pro-apoptotic genes.[135]
	Breast Cancer	MCF-7 (Human)	Suppression of the NF-κβ activation and restricting the cell proliferation at S-G 2-M phase.[52]
Resveratrol (combined with Salinomycin)	Breast Cancer	MDA-MB231 (Human)	Suppressed Chronic inflammation markers (COX-2, NF-kβ, and p53) InhibitAutophagy markers (Beclin and LC3) Down regulate apoptotic markers (Bax, Bcl-2).[53]
Apigenin	Breast Cancer	MDA-MB231 (Human)	Integrin beta 4 function inhibition along with inhibition of matrix–cell adhesion, cell- endothelial cells adhesion and PI3K/Akt pathway by phosphorylating Akt.[54]
Cyanidin-3-o-glucoside	(TNBC) co-expressing ERα36/EGFR	MDA-MB436 BT-20 MDA-MB231 (Human)	Inhibits EGFR/AKT signalling and promotes EGFR degradation, thereby promoting the apoptosis of TNBC cells both in vivo and in vitro.[55]

	Breast Cancer	HS-578T (Human)	Cause cell cycle arrest at G2/M phase leading to inhibition of cell growth. Down regulates the expressions of cyclin B, CDK-1, cyclin D1 and CDK-2 induce caspase-3 dependent cell death.[89]
Fisetin	TNBC	MDA-MB-231 & BT549	reversing of EMT by inhibition of PTEN-Akt-GSK-3β signalling pathway.[136]
	Breast Cancer	MCF‑7 & MDA‑MB‑231	regulation of the phosphatidylinositol‑3‑kinase/protein kinase B/mammalian target of rapamycin pathway.[137]

Studies revealed that nanotechnology can produce effective nano-medicines and these nano-formulated bio-actives possess potent ability against various cancer cells with better efficacy and bioavailability under in vivo conditions. The physicochemical properties of the nutraceutical or phytoconstituent directly affects the pharmacokinetics in the body. When the drug is encapsulated in nano-biomaterial, it forms a colloidal system that comes in contact with the biological system directly.The beneficial aspect of nano-biomaterial is that it reduces the toxic effects associated with the drug.

Nano-carriers made up of polymers are known as polymeric micelles and polymeric NPs. These are employed in delivering bio-actives and drugs through oral route of administration. These polymeric nanocarriers possess several advantages like the stability of the drug in the GI tract which is due to varied structural characteristics. Polymeric nanocarriers are used to deliver a widerange of bio-actives and drug molecules due to the hydrophilicity and hydrophobicity of polymeric system. Polymeric NPs tend to show sustained release of drug molecules, which is the major advantage of polymeric NPs.(38)Nano-particles made of resveratrol and Dox depicted significant cytotoxic effects on MCF-7/ADR and MDA-MB-231/ADR cells.Nano-liposomes loaded with quercetin depicted enhanced solubility, bioavailability and cellular uptake in MCF-7 human breast cancer cells.Tamoxifen and quercetin combination when encapsulated in PLGA polymeric NPs and administered orally, reduced the growth of breast tumour by inducing apoptosis.Quercetin loaded PLA NPs were used to treat BC and it has shown a significant reduction. (39)

Few other studies have shown that a soy-rich diet can prevent cancer since it possess a phenolic compound genistein (Gen). It mainly act by altering apoptosis, angiogenesis, inhibiting metastasis and the cell cycle. It has been assessed as an anti-cancer agent in different BC cell lines such as MCF-7 and MDA-MB-231. It was also assessed to possess synergistic activity in combination with other drugs. (40) Even though the in-vitro and in-vivo results were promising, poor water solubility, rapid metabolism, higher excretion and low oral bioavailability have been the main drawbacks.Hence,nanotechnology can be used for the delivery of this hydrophobic drug.(41) Several nano-formulations of Gen for the treatment of TNBC and other types of cancers are in the stages of investigation.

Recently, a flavonoid, fisetin (Fis) (3,3',4',7-tetrahydroxyflavone) is found to possess anti-cancer activity on several types of cancers such as prostate cancer, lung cancer, colon cancer, melanoma and breast cancer. Increasing evidence has validated its apoptotic effect which probably stems from the inhibition of ERK phosphorylation, topoisomerase activation, PI3K/Akt/mTOR signalling and NF-κB expression.Feng et al. formulated Fis loaded polylactic acid (PLA) NPs for anticancer therapy which demonstrated enhanced solubility and therapeutic efficacy. Several nano-formulations of Fis for the treatment of TNBC and other types of cancers are in the stages of investigation. (42)

Breast cancer therapy with mesoporous silica nanoparticles (MSNs):

A combination of drugs is mainly intended for cancer patients when the drugs are resisted by the tumour cells. Themesoporous silica nanoparticles (MSNs) are used as an emerging nanocarrier to overcome this limitation associated with other delivery carriers. A report by Menget al. suggested that the co-administration of DOX and siRNA by MSNs were more effective in inhibitionof tumour growth *in vivo* and also produced synergistic effects compared to naïve drug and siRNA treatment alone. (43).

The MSNs show proficient intracellular protection because of the narrow pore size distribution. The adsorption and release of siRNA can be altered by modifying the surface of siRNA. siRNA is adsorbed onto the surface of NPs and modified catatonically since it is difficult to load into the core. Xia et al. coated MSNs with a polyethylene imide (PEI) layer, which is a gene transfection agent.It was observed that the particles or nucleic acids in the ratio of 10-100 showed site-specific delivery and optimal adsorption. Modifying the surface of siRNA with organophosphate is not as effective as PEI. But these methods are beneficial only for *in vitro* observation. The large part of siRNA absorbed is destroyed rapidly when administered through in vivo route due to the presence of nucleases in the plasma. (44). In order to prevent this, the optimization of loading condition is done along with usage of MSNs consisting of bigger pores. There are several attempts carried out by researchers to make MSNs such as larger pores of size ranging from 10 to 24 nm or by utilizinglarge number of amino groups to immobilize and maximise interaction of siRNA's in them. It also improves the circulation time of siRNA or cytotoxic drugs loaded mesoporous. MSNs externally coated with a layer of lipid or polyethylene glycol makes them excellent carriers in effective cancer treatment.(45).

Drug Targeting to Molecular level in TNBC:

Inhibition of the metastasis of TNBC requires the therapeutic targeting up to the molecular level. The molecular therapeutics diverse the function of programmed overexpressed receptors, proteins, hormones or alter the DNA repair mechanisms. There are some molecular therapies available to treat the TNBC such as PARP inhibitors, EGFR Inhibitors, VEGF inhibitors with a greater selectivity and efficacy.

Poly(ADP-ribose) Polymerase (PARP) inhibitors have shown improved and synergetic benefit in chemotherapy. PARP helps to repair single strand breaks in DNA. The germline BRCA1/BRCA2 mutant genes acts as tumor suppressor proteins that repair double strand breaks in DNA. PARP inhibitors inhibit the DNA repair mechanism in tumorous cells thus causing DNA damage and arrest the DNA replication fork to stop the replication of DNA. PARP inhibitors such as Olaparib, Talazoparib have shown better clinical effect in TNBC patients and are under investigation in the clinical phase III (46). Similarly, Veliparib in the combination with carboplatin and PTX is being investigating in phase III. PARP inhibitors administered with the immunotherapy demonstrated better clinical efficacy to inhibit the germline BRCA1/BRCA2 mutant genes and DNA repairs in the TNBC patients. It also depicted prolonged survival and improved quality of the life of BC patients.(47)

Anti-angiogenic agents such as Bevacizumab in combination with first-line chemotherapy like PTX and DTX suggested the higher clinical benefits in BC. Bevacizumab is a monoclonal anti-VEGF-A antibody that supress the angiogenesis. Unfortunately, there haven't been any clinical evidence for predicting benefit on TNBC. However, bevacizumab is being tested to treat TNBC.(48).

In TNBC, it is observed that the EGFR is overexpressed in 70% of population. This overexpression is associated with poor prognosis of tumor and also resist the conventional chemotherapy. Administration of chemotherapeutics in combination with EGFR inhibitors produces synergistic effect. For example, Cetuximab a monoclonal anti-EGFR antibody in combination of carboplatin demonstrated overall response rate of 17% with clinical benefits of 10% over the population, which is higher than the single treatment of Cetuximab or carboplatin that showed 6% clinical benefits.(49)

Unfortunately, the study on the molecular targeting using these molecular therapeutics on combination with conventional chemotherapeutics via nanomedicine delivery have been assessed limited. However targeted NPs for the delivery of peptide, aptamers, miRNA and siRNA have been explored earlier.(50). Therefore, the nanocarrier based delivery of therapeutic agents for the treatments of TNBC is the subject of interest to the researchers.

CSCs targeted therapy:

Compared to single agent, combinational chemotherapies by cytotoxic agents depicted greater efficacy. Nanomedicines incorporating chemotherapeutic agents can effectively inhibit CSCs using target specific biomarkers such as CD44, CD133, CD90 and ALDH. It can also target specific signalling pathways like Notch, Hedgehog and transforming growth factor-b that have been implicated in the maintenance of the CSCs in cancer.(51). Generally, biomarkers combined with conventional anticancer therapies exhibit better effect against CSCs.CD44 receptors are over expressed on CSCs, which is a family of transmembrane glycoproteins which can be utilized a surface marking for providing a binding platform for targeted therapeutics. The CSCs consisting of the CD44 surface marker, is been involved in the number of processes like cell orientation, intercellular adhesion, cell-matrix signalling and cell

migration. To overcome this, hyaluronic acid (HA), hyaluronan and *heparanase* were used to target the CD44 receptors. HA functionalized NPs encapsulating salinomycin (SLM) were used for targeting cancer stem cells. P-glycoproteins are inhibited by this therapy of SLM and also can be used in PTX resistant cancers. Other delivery techniques were also developed for the delivery of salinomycin using recombinant polypeptide NPs and biodegradable polymeric NPsthat possess significant anticancer activity.(52) Similarly, CD133 which is known as prominin-1 is also a member of transmembrane glycoprotein, which is overexpressed in different kind of cancers. To target CD133 marker, Ni et al. developed SLM encapsulated PEGgylated-PLGA NPs conjugated with CD133 aptamers. Aptamer conjugated NPs revealed potential anticancer activity and were found effective in targeting and eliminating $CD133^+$ CSCs both *in vivo* and *in vitro*. CD90 is a glycosyl phosphatidylinositol-anchored membrane glycoprotein which was found responsible for tumorigenic activity. It was reported that the Notch pathways activated the $CD90^+$ CSCs and the inhibition of the same decreased the cell invasion and migration.[124]Another aspect of drug resistance by CSCs is the high level of ALDH activity, which enhances the tumorigenicity and chemoresistance. Studies have been reported that the chemoresistance was decreased by the inhibition of ALDH activity through NPs based drug delivery.(53)

As mentioned earlier, there are various signalling pathways associated with the proliferation, invasion and migration of cancer. Inhibition of these pathways affects the tumor invasion and metastasis. For example, a study on Notch pathway inhibition suggested that the decreased capacity of CSCs to undergo self-renewal resulted in reduced tumorogenicity. Inhibition of Notch pathways using γ-secretase inhibitors loaded MSNPs decreased the CSC-subpopulation and improved the susceptibility of CSCs to radiation induced apoptosis.(54) Similarly, Hedgehog (Hh) signaling pathway was down regulated by the anthothecol- loaded PLGA-NPs which significantly reduced the cell proliferation. In another study, it was reported that the transforming growth factor-b was inhibited by the siRNA and LY364947 (TGF-b signaling pathway inhibitor) encapsulated NPs, which depicted the tumor regression and decrease in CSCs proportion.(55)

Discussion:-

Breast cancer and its advanced form (triple negative breast cancer) accounts for major deaths in cancer patients. The emergence of resistance, absence of major receptors and the toxicity associated with available treatment options makes the disease as well as treatment more complicated. Chemotherapy followed by surgery and radiation are the suitable option to suppress the tumour but relapse occurs in most of the cases. Nanotechnology based platforms offers promising strategy for effective drug delivery of cytotoxic agents owing to its unique features like small particle size, high drug loading capacity, targeted delivery and stability to the encapsulated actives. In this review we discussed multiple strategies as well as recent advancements in the field of nanotherapeutics for the management of breast cancer/ triple negative BC.

Nanomedicines not only talks about the nanometric devices for delivering '*tough to deliver drugs'* but it also incorporates multiple strategies involving identification of novel molecules like nucleic acid based therapeutics, monoclonal antibodies, phytoconstituents, combinatorial drug regimens and many more. The identification of molecular targets as well as pathways responsible for resistance development and tumor progression/invasion will also open new paradigm for emerging with new treatment options. Addressing the role of stem cell in tumor progression and invasion provides multiple opportunities to control the relapse of tumor. For example, nucleic acid based therapeutics (siRNA, miRNA and ncRNAs) are used to down regulate cell proliferation, migration, invasion and propagation of CSCs, which eventually leads to recurrence-free survival of cancer patients.

The increased incidences of drug resistance with conventional chemotherapeutic agents were also resolved with the emergence of combinatorial therapeutic regimens and identification of new molecular targets. Numerous chemotherapeutic drugs are being investigated clinically along with monoclonal antibodies, pathway inhibitors and RNAs.(56). There are many molecular targets available which inhibits a particular cell signalling pathway or receptors. However, there is a lack of literature on the utility of molecular targeted therapeutics to be used as nanomedicines. A number of studies are available in the literature that suggests that the nanomedicines are emerging as an innovative approach in cancer treatment. Many more researchers are continuously exploring new strategies based on nanotechnology to provide more selective and promising options for treatment as well as diagnosis of breast cancer.(57) The use of the nanomedicines to deliver the drug/therapeutic agent efficiently to the targeted site is made possible by conjugation of target selective moieties with nanoparticles. Therefore, nanomedicines offer the most promising strategies for the management of breast cancer in current era as well as, in future.

Conclusion:-

Nano-medicine helps in bringing major advances in the diagnosis and treatment of hormone negative and hormone positive BC. A wide range of nanoparticle based formulations have been developed and are currently playing an important role in the treatment of the disease. Nano-particles that combine therapeutic agents, molecular targeting and imaging capabilities have been the next generation BC and TNBC therapies. Nanoparticles are conjugated with mAbs or cell signalling pathway inhibitor to improve the targeting efficiency. Green chemistry methods were also explored these days for designing nanomedicines. PtNPsand PdNPs depicted potent anticancer activity against MCF-7 cells and it is used for drug delivery and photo thermal therapy. Carbon nano-materials can be used as diagnostic as well as novel therapeutic agents. In case of BisBALNPsbased drug delivery to BC cells are more affected than noncancerous cells. Mesoporous silica nanoparticles are also used for targeted therapy and have revolutionized the field of drug delivery. There are numerous other types of therapeutic agents reported as potent inhibitors of the TNBC; however literature lacks finding of those agents being explored as nanomedicines. A better understanding of the biological processes and refinements of nanotechnology will certainly help to develop more compatible nanomedicines for the treatment of BC and TNBC in future.

Abbreviations:

Breast cancer (BC), human epidermal growth factor receptor 2 (HER2), Triple-negative breast cancers (TNBC), estrogen receptor (ER), progesterone receptor (PR), Nanoparticles (NPs), Epidermal growth factor receptor (EGFR) solid lipid nanoparticles (SLNs), cancer stem cells (CSCs), breast cancer stem cells (BCSCs), vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), carbon nanotubes (CNTs), paclitaxel (PTX), docetaxel (DTX), pathologic complete response (PCR), Enhanced Permeability and Retention (EPR), doxorubicin (DOX), genistein (Gen), fisetin (Fis), mesoporous silica nanoparticles (MSNs)Bismuth lipophilic Nanoparticles (BisBAL NPs), poly(lactic-co-glycolic acid) (PLGA), Polyethylene glycol (PEG).

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