Review Article

NANOTECHNOLOGY-BASED PRECISION TOOLS FOR THE TARGETED THERAPY OF TRIPLE NEGATIVE BREAST CANCER

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Abstract: Triple-negative breast cancer (TNBC) is a biologically aggressive and an immunopathological subtype of breast cancer that is characterized by absence expression of estrogen and progesterone receptors (ER/PR) and amplification of the human epidermal growth factor receptor 2 (HER-2) gene. Because of this, patients with TNBC do not benefit from hormonal or trastuzumab-based treatment strategies, which strongly demand the development of alternative and innovative treatment strategies. Other available approaches such as chemotherapy mainly lack the specificity which generates severe side effects regarding toxicity. Therefore, the development of targeted treatments, as well as early diagnosis is vital to ensure an adequate and timely therapeutic intervention in patients with TNBC. Recent years have witnessed an exceptional advancement in the development of nanotechnology-based approaches to tackle advanced tumors including TNBC. This article expounds latest nanotechnology-based precision tools that are employed to treat TNBC viz smart nanoparticles, liposomes, dendrimers, solid lipid nanoparticles and carbon-based nanomaterials, etc. and also provides a comprehensive outlook on emerging nano-based approaches that are under preclinical and clinical testing for the early diagnosis and theragnosis of TNBC along with their regulatory status.
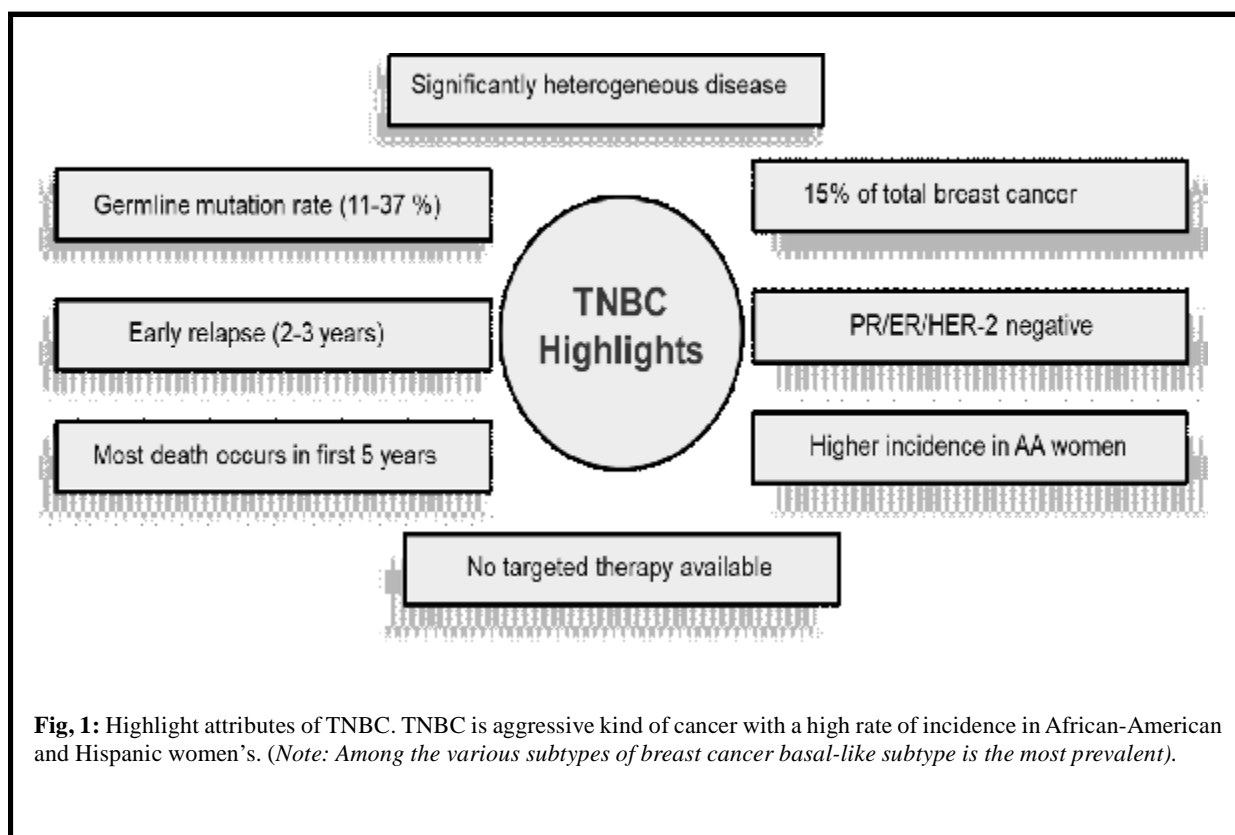
Key words: Triple-negative breast cancer, Nanotechnology-based approaches

INTRODUCTION

Triple-negative breast cancer (TNBC) is a kind of tumour in which cancerous cells verified as negative for estrogen receptors (ER-), progesterone receptors (PR-), and human epidermal growth factor receptor (HER2-) [1]. This cancer development and progression is independent of hormones, i.e., estrogen and progesterone, and characterized by the absence of too many HER2 receptors, hence named as triple-negative. Therefore, hormonal therapy like tamoxifen, selective estrogen receptor modulator, and aromatase inhibitors, e.g., Arimidex, aromatic, and Femara did not show any therapeutic benefits towards the treatment of TNBC [2]. The specific HER2 receptor targeted monoclonal antibodies, such as Herceptin (trastuzumab) also unable to provide therapeutic potential for treatment of cancer. Approximately, near about 10-20 % cases of breast cancer come from TNBC. There are other several medications also available to treat TNBC, but they are not efficiently used to treat the disease. Hence, researcher and doctors are finding new medicines or biological molecules that can be used as early stages of disease progression and development or which will interfere the processes of evolution of TNBC [3].

Another type of breast cancer called as basal-like

Fig, 1: Highlight attributes of TNBC. TNBC is aggressive kind of cancer with a high rate of incidence in African-American and Hispanic women's. (*Note: Among the various subtypes of breast cancer basal-like subtype is the most prevalent).*

breast cancer have many of clinical and pathological similarity with TNBC such as negative testing for ER and PR, but they are not identical. Basal-like cancer shows positive testing for HER2 which is negative tested for TNBC. Apart from this, basal-like breast cancer also indicates positive testing for cytokeratin 5/6, cytokeratin 8/18, and vimentin, and 75 % of this cancer is of TNBC [4]. Previous studies have evidence of 75 % TNBCs are categorized into BRCA1- like breast cancer. The statistical data shows TNBC has an increased likelihood of recurrence, distant metastasis, and mortality within five years of diagnosis, and the incidences were higher in first three years prove its severity and fatality [5]. Due to the close relation of TNBC and basal-like breast cancer, understanding and defining the TNBC from basal-like phenotype is vital to design treatment therapy, which could target TNBC with potential outcomes. A typical highlight about TNBC is shown in figure 1.

TNBC is identified through clinical and biological assays for ER, PR, and HER2; in contrast complementary DNA (cDNA) microarray technique used for initial refinement of basal-like cancer to study its molecular phenotype. The absence of proper understanding between the TNBC and the basal-like tumors, most of TNBC have categorized into basal-like cancer and *vice versa*. There are several intrinsic subtypes of breast cancer that clusters with TNBC. A summary of inherent subtypes of TNBC is represented in table 1.

To understand TNBC as well as its molecular and pathologic pattern, it is essential to understand the micro-anatomy of parenchymal cells present in the normal mammary gland, with their immune-phenotype [6]. The central luminal cells of mammary glands are responsible for expression of some lower atomic weight cytokeratins such as cytokeratin 7

Table 1. Intrinsic subtypes of breast cancer. Note: Among the various subtypes of breast cancer basal-like subtype is the most prevalent.

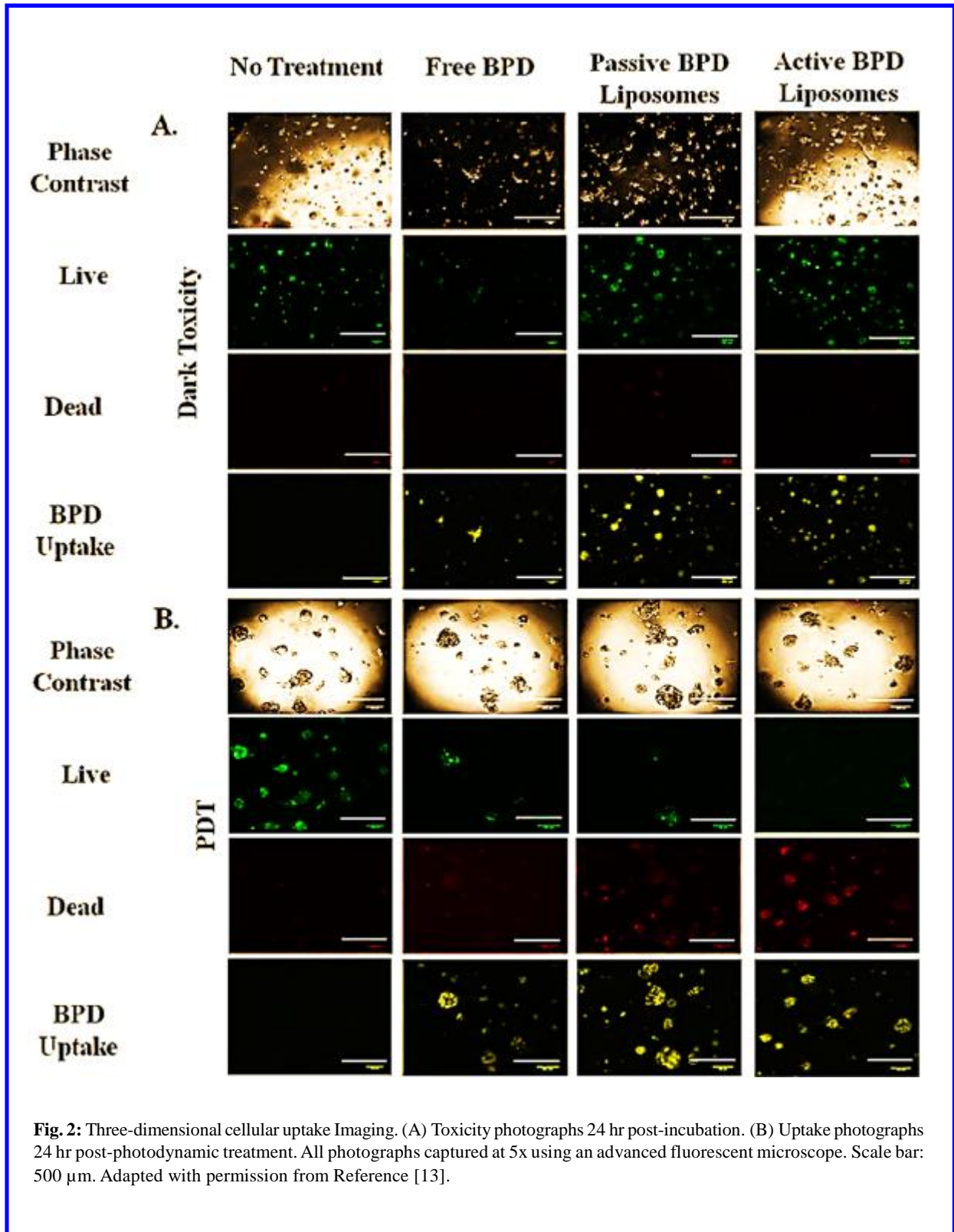| Subtype	TNBC (% incidences)
Basal-like	49
Claudin-low	30
HER-2 enriched	9
Luminal B	6
Luminal A	5
Normal breast like	1

Fig. 2: Three-dimensional cellular uptake Imaging. (A) Toxicity photographs 24 hr post-incubation. (B) Uptake photographs 24 hr post-photodynamic treatment. All photographs captured at 5x using an advanced fluorescent microscope. Scale bar: 500 µm. Adapted with permission from Reference [13].

(CK7), cytokeratin-8 (CK8), cytokeratin-18 (CK18), and cytokeratin-19 (CK19). They also express other proteins and receptors such as cell surface associated Mucin 1 (MUC1) alpha-6 integrin, BCL1, BCL2, ER, PR, and GATA3. Expression of different higher atomic weight cytokeratins like CK5, CK14, and CK17 is a function of myoepithelial cells. These cells are involved in the role of the construction of base cell layer of the basement membrane, and also expresses smooth muscle-specific markers, calponin, caldesmon, p63, beta-4 integrin, laminin, maspin, CD10, P-cadherin, caveolin-1, and nerve growth factor receptor (NGFR) and S100. However, basal-like breast cancers express a high level of CK5, CK14, caveolin-1, Carbonic anhydrase IX (CAIX), transformation-related protein 63 (p63), and epidermal growth factor receptor (EGFR/HER1) with a minimal level of ER, PR, and HER2. This dissimilarity of expression of proteins and receptors can be applied in diagnosis field to avoid cluster formation between TNBC and basal-like breast cancer [7].

Available treatment approaches and their limitations: Lack of specific targeted therapy creates limited options for treatment of TNBC patients and lefts as the only option of conventional systemic chemotherapy. The anti-hormonal treatment or any other targeted treatment such as anti-HER2 based medicine does not provide any therapeutic benefits for TNBC patients. Targeting to DNA repair mechanism and microtubules, p53 (taxanes), cell proliferation (anthracycline containing compounds) can be used as TNBC management tools with combination or without the combination of chemotherapy [8]. Some targeted agents with chemotherapy like poly-ADP ribose polymerase (PARP) inhibitors, EGFR inhibitors, anti-angiogenic agents and Chk1 inhibitors improved moderate gain patient survival, but the effect was limited to metastatic TNBC. A summary of the current status of therapeutic strategies towards the treatment of TNBC is represented in table 2.

Evolution of nanotechnology-based approaches to treat TNBC: With the growing research in TNBC, many new classes of targeted therapy are being evaluated. TNBC patients could potentially benefit from these therapies. Next-generation therapeutics is big hope and will provide a robust blockbuster platform to resolve the issues associated with TNBC treatment. There are many novel therapeutic targets and platforms are under the clinical investigation. Some of the novel Nanoplatforms are discussed here.

Liposomes: A liposome is a self-assembled vesicular system, showing its potential benefits in cancer therapy due to their non-toxic, bio-degradable, and non-immunogenic nature [9,10]. This nano-vesicular and other comparable system provide a platform for entrapment of both hydrophilic as well as hydrophobic drugs into formulation due to the amphiphilic property of phospholipids [11]. Liposomes can be targeted by use of passive targeting and active targeting mechanism. The passive targeting mechanism is based on the enhanced permeability and retention (EPR) effect while active targeting mechanism associated with receptor-mediated endocytosis [12]. The utilization of the leaky tumor vasculature for diffusion and accumulation of liposome at tumor tissues through EPR effect mechanism leads to drastic improvement in target selectivity and patient safety.

Sneider et al. [13] formulated polyethylene glycol (PEG) coated liposome with folate conjugation, to deliver the benzoporphyrin derivative (BPD), for treatment of TNBC through photodynamic therapy (PDT) approach. The conjugation of liposome with folate provides a more significant targeted therapy, because of overexpression of folate receptor on

Table 2. Novel strategies for treatment of TNBC. Therapeutic strategies for TNBC mostly rely on the chemotherapy in the current scenario. Data obtained from clinicaltrails.gov. Here: DOX, Doxorubicin; PTX, Paclitaxel.

Strategy	Clinical Trials Status
Anthracyclines (DOX)	Proven effective with combination therapy (Phase II)
Taxanes (PTX, DOX)	Proven effective with combination therapy (Phase III)
Platinum agents (carboplatin)	Active as well as active cytotoxic agent (Phase III)
Sunitinib + PTX	Proven effective in combinational therapy (Phase I)
Niraparib	Proven effective with combination therapy (Phase II)
pembrolizumab	Proven effective with combination therapy (Phase II)
Gemcitabine	Proven effective with combination therapy (Phase III)

TNBC cells. Hence, these liposomes specific binds to overexpressed folate receptor on cancer cells. PDT works on the principle of reactive oxygen species generation after irradiating laser light of specific wavelength with use of photosensitizer. In this study, investigators irradiated light at 690 nm as a trigger for BPD to generate reactive oxygen species that results in tumor cells death. The BPD also can be used as a theranostic agent because of its fluorescence signal properties after excitation by light. During this study, investigators used free BPD, non-targeted (without folate conjugated) and folate-targeted (folate conjugated) PEGylated and BPD-loaded liposomes to target a metastatic breast cancer cell line (MDA-MB-231). The prepared liposomes were synthesized in reproducible manner and characterization for size, polydispersity index (PDI), zeta potential, stability, and BPD release kinetics was carried out for determination of physicochemical properties. Folate competition test and fluorescence confocal imaging used to observe, while (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay is used for determination of active targeting concentration of BPD. The cytotoxicity of BPD before and after PDT treatment was observed in monolayer and 3D *in vitro* cultures (Fig. 2). This study suggests that nanoparticle-based approach by using PDT may provide a better platform to target TNBC. Some of the dead cells were observed in the deep toxicity dishes; it may be due to the hypoxic core in the tumor. The combined BPD and PDT approach leads to induction of cell death with a decrement in tumor size, but some of living cells were observed in the acini.

Feng et al. developed a phospholipid-mimic OXA prodrug (Oxalipid), having the ability of self-assemble within liposomal formulation [14]. This approach of the phospholipid-mimic oxalipid liposome (PMOL) showed higher encapsulation ability, increased systemic circulation time, and enhanced accumulation at the tumor site, which resulted in the enhanced anticancer effects of the drug against the metastatic TNBC tumor. *In vivo* study concludes that PMOL showed more efficacy against the development of TNBC and treatment towards its lung metastasis as compared to free OXA. This study suggests the use of liposomal formulation via prodrug-based approach can be used as a potential tool for the metastatic TNBC treatment. They investigated *in vivo* pharmacokinetic parameters (blood clearance half-

life ($t_{1/2}$), bioavailability (AUC0–t) of PMOL. The half-life and the bioavailability of PMOL were observed to 16.4 h and 80.1 mg·L^{-1}h^{-1} respectively. Then these results were compared with free OXA, and it was found that half-life and the bioavailability of PMOL were 6-times and 5-times greater than concerning free OXA respectively. This increased blood clearance half-life and increased bioavailability of OXA indicates the increased circulation time of OXA. The distribution of PMOL at tumor site was determined after 24 hr of injection, and Pt concentration was measured using ICP-MS (Fig. 3). The level of PMOL was observed 8.8-fold higher as compared to that OXA group.

Dendrimer: Dendritic molecules are featured by structural perfection [15]. Dendrimers and dendrons are monodisperse spherical molecules and usually possess high symmetry [16]. The dendritic particles can be broadly divided into low-molecular-weight and high-molecular-weight species. Recently dendrimers are shown to be involved in delivering the drugs for TNBC. Finlay et al. [17] recently reported an of siRNA-based (double-stranded RNA composed of 20-25 base pairs) TWIST1 silencing strategy for delivery by employing an altered poly (amidoamine) (PAMAM) dendrimer. After the treatment of formulation, authors observed that the SUM1315 TNBC cells efficiently takes PAMAM-siRNA complexes, and thereby results in significant knockdown of Twist-related protein 1 (TWIST1) and EMT-related target genes. The results they obtained that Knockdown TWIST1 and EMT-related target genes continue seven days post-transfection, which causes a substantial decrease in invasion of cancer cells, and they acquired these results through wound healing and transwell assays. After all, they employed this approach to xenograft orthotopic tumors, and from that they revealed, siRNA was at tumor site minimum for 4 hrs after treatment. However, using the dendrimer-based siRNA delivery to silence TWIST1 will promote the development of valuable TNBC treatment.

Solid lipid nanoparticles: A solid lipid nanoparticle is typically spherical nanoparticle system ranging size from 10 and 1000 nm [18]. Solid lipid nanoparticles comprised of a solid lipid core matrix that enhances the solubility of lipophilic/hydrophobic molecules. The different types of surfactants are used to stabilize the lipid core [19].
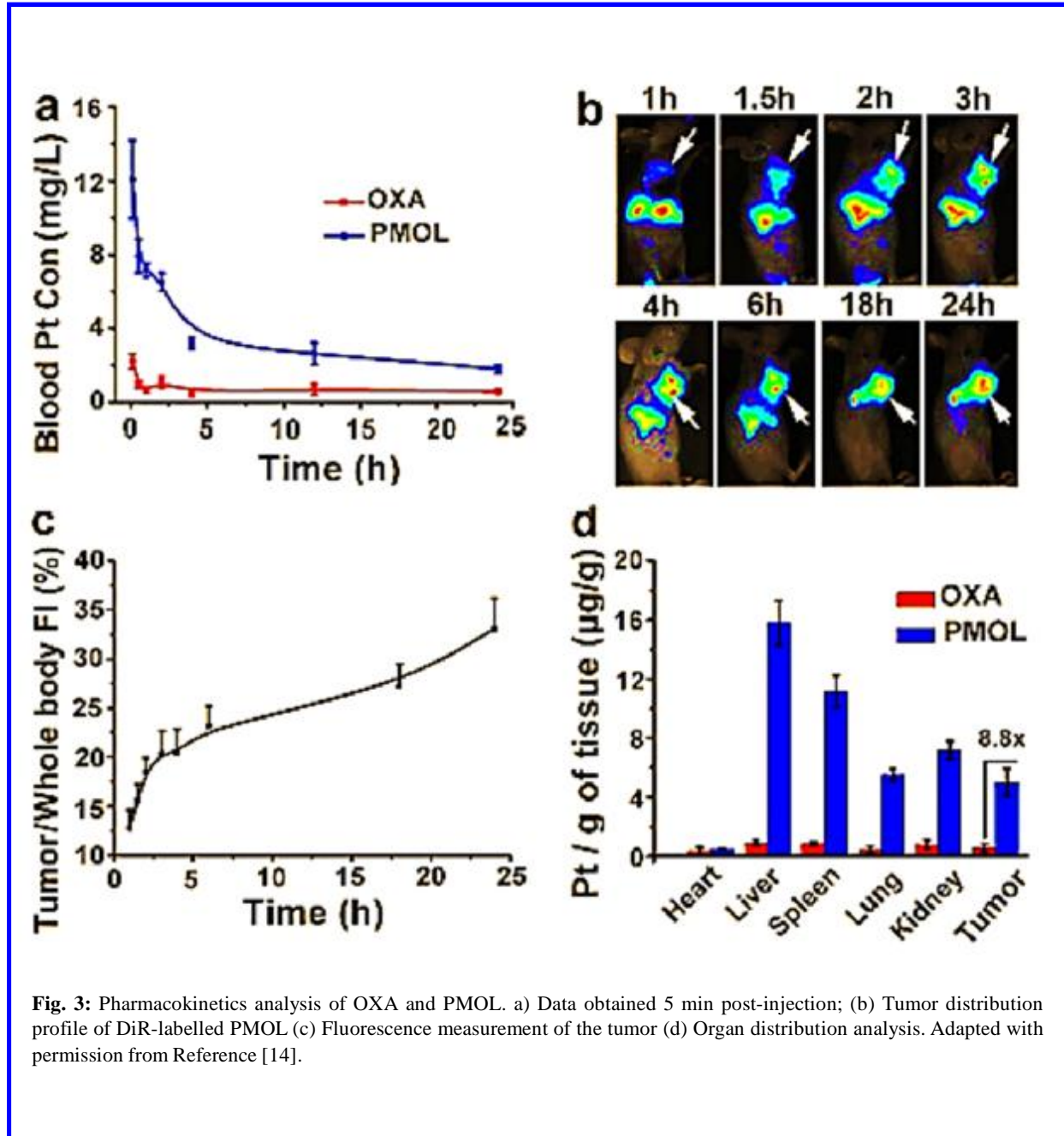
Fig. 3: Pharmacokinetics analysis of OXA and PMOL. a) Data obtained 5 min post-injection; (b) Tumor distribution profile of DiR-labelled PMOL (c) Fluorescence measurement of the tumor (d) Organ distribution analysis. Adapted with permission from Reference [14].

Siddhartha et al. formulated solid lipid nanoparticle formulation of cytotoxic agent di-allyl-disulfide (DADS) to overcome its bioavailability issues [20]. Then, the surface modified DADS-loaded solid lipid nanoparticles (DADS-SLN) with Receptor for advanced glycation endproducts (RAGE) antibody to achieve site-specific delivery of DADS to TNBC cells. They found a significant cellular internalization of RAGE surface modified DADS-SLN (DADS-RAGE-SLN) when compared to DADS-SLN. The cytotoxic effect of DADS was also significantly improved with DADS-RAGE-SLN by downregulating anti-apoptotic proteins and upregulating pro-apoptotic proteins as observed by western blot analysis. RAGE-targeted delivery of cytotoxic agents can be, therefore, a promising approach for improving anti-tumor activity and reducing off-target effects.

Other nanoparticles: Son et al. developed Trop2 antibody-conjugated bioreducible nanoparticles (ST-NPs) for active targeting TNBC, allowing for rapid drug release at the intracellular compartment via the reduction of disulfide bond [21]. As controls, the Glutathione (GSH) sensitive nanoparticles without Trop2 antibody (SS-NPs), GSH-insensitive nanoparticles without Trop2 antibody (CA-NPs), and GSH-insensitive nanoparticles with the Trop2 antibody (CT-NPs) were also prepared to investigate the potential of Trop2 antibody and bioreducible bond for TNBC-targeted therapy. Confocal microscopic images and flow cytometry analysis demonstrated that DOX-ST-NPs were selectively taken up by MDA-MB-231 as the representative Trop2-expressing TNBC cells. Consequently, DOX-ST-NPs exhibited higher toxicity to Trop2-positive MDA-MB-231 cancer cells, compared to DOX-loaded control nanoparticles without the disulfide bond or anti-Trop2 antibody. Overall, ST-NPs might be a promising carrier of DOX for targeted TNBC therapy.

Obayemi et al. demonstrated the required adhesion forces between components of model conjugated magnetite nanoparticle systems for selective and specific TNBC target improvement [22]. From the study, they observed adhesion forces between chemically synthesized magnetite nanoparticles (CMNPs), biosynthesized magnetite nanoparticles (BMNPs), and their conjugated systems with TNBC cells (MDA-MB-231 breast cancer cells) and normal breast cells (MCF 10A). The BMNPs showed higher adhesion forces to breast cancer cells as well as healthy breast cells in comparison to CMNPs. The LHRH-conjugated BMNPs or BSA-conjugated BMNPs was adhered to cancer cells by six times higher than normal breast cells. They revealed the reason behind excessive adhesion of conjugated BMNPs to breast cancer cells is due van der Waals interaction between peptides/antibodies from conjugated nanoparticles and overexpressed receptors on the surface cancer cells.

Cerqueira et al. [23] tried to enhance the chemotherapeutic efficacy of paclitaxel (PTX, a potent anticancer drug) to treat breast cancer. They prepared PLGA (lactic-*co*-glycolic acid) nanoparticles with conjugation of HA (hyaluronic acid) to form hyaluronic acid-poly (lactic-*co*-glycolic acid) (HA-PLGA) nanoparticles. They used o/w emulsion technique to synthesize PTX-HA-PLGA nanoparticles. They optimized the formulation parameters to get desired physicochemical properties of nanoparticles. They observed the effect of HA coating on drug release profile and cytotoxicity by incubating HA-coated and HA-non-coated nanoparticle formulation into MDA-MB-231 cells. The HA-coated nanoparticles showed sustained drug release profile with increased PTX cytotoxicity. Apart from this, HA-PLGA nanoparticles exhibited improved cellular uptake concerning non-coated PLGA nanoparticle, may be due to receptor-mediated endocytosis through interaction between HA and CD44 receptors. Furthermore, investigators studied for the non-hemolytic potential of the nanoparticles to check the suitability of formulation for intravenous administration.

Zhou et al.[24] synthesized polymeric nanoparticle delivery system to treat TNBC. In that, they encapsulated two chemotherapeutic agents having different physicochemical properties [erlotinib (Etb), and doxorubicin (DOX)]. The complexation of DOX, a hydrophilic molecule with 1,2-dioleoyl-*sn*-glycerol-3-phosphate (DOPA), an anionic lipid through ion pairing leads to the formation of a single entity with hydrophobic properties. The DOX is encapsulated with hydrophobic Etb, which was incorporated into poly (L-lactide)-*b*-polyethylene glycol (PLA-*b*-PEG) nanoparticles. Co-encapsulation of DOX and Etb was carried out by using nanoprecipitation method. Researchers were found that the complexation of DOX with DOPA increases the encapsulation of DOX, and sustains the release pattern. The overall nanoparticle-mediated burst release of Etb

and provides sustained release of DOX. The fluorescent imaging technique was used to observe accumulation of nanoparticles in the tumor, and results revealed that there was higher nanoparticle accumulation in tumor tissues.

Regulatory concerns: Nanoparticle-based products are comprised of complex structures and have sizeable potential diversity, which makes them as a challenging task to come into the market after all facing regulatory pathway. Nowadays, the regulatory agencies and regulatory bodies such as U S Food and Drug Administration (US-FDA), European Medicines Agency (EMA), are following strict norms for the individual new nanoparticle-based drug on a product-to-product basis. The unavailability of a standard protocol for examination of nanomedicines as a unique therapeutic agent category is leading to the development of new guidelines and definitions for proper manner regulation [25].

The increased complexity of nanoparticle-based products is due to multiple ingredients which are used to control the behavior of the active pharmaceutical ingredient in the formulation. These inactive ingredients (other than active pharmaceutical ingredient) may alter the pharmacological actions of active agent and may serve as less potential effective than standard drug. This complexity of nanoparticle-based products is enforcing the regulatory agencies and bodies to concrete complicated regulatory, organizational strategies [26].

A personalized approach to the treatment of nanoparticle-based products by identification of subgroups of patients that will likely respond to therapy may reduce the overall cost of clinical trials by reduction of the size of clinical trials [27]. The reduction in the effective size of clinical trials ultimately reduces the burden of regulatory filings followed by a rapid approval for the product for market launch. This approach has also been employed for nanoparticle-based medicines that is specifically targeted towards selectively expressed receptors/markers on the surface of cancer. For instance, the tumor is characterized by overexpression of various receptor or proteins such as folate receptors, HER2, transferrin receptor, lectin receptors, glucose [GLUT] transporters etc.

CONCLUSION

TNBC is a well-established subset of breast cancer which is also known as aggressive cancer type. Till date, the cytotoxic chemotherapy is the only solution for this type of cancer. The arrival of novel therapeutics such as liposomes, dendrimers, solid lipid nanoparticles and polymeric nanostructures has unlocked the opportunities of targeted therapy in a subset of breast cancer that is deficient in classic biomarkers (ER, PR, and HER2) in breast cancer. In this review, we saw the involvement of different types of nanotechnology-based approaches to treat and manage TNBC. The delivery of therapeutic agents using novel carriers has shown promising results at laboratory scale. However, their transformation from lab to the bedside of the patient is challenging due to complex regulatory needs.At present, there is not a transparent, proven effective single agent that targets a defining vulnerability in triple-negative breast cancer. To develop a suitable formulation for TNBC, a complete understanding of the clinical problem of triple-negative disease, potential prognostic factors, and efficacy of currently available therapeutic options, and advantages of new potential therapies required. Apart from that, the most significant hurdle is the amount of time necessary to carefully translate promising preclinical findings to the bedside, with the ultimate goal being FDA approval of new drugs that yield both superior survival and quality of life outcomes for patients with TNBC. The future challenge is to further identify specific targets within subsets of patients diagnosed with TNBC tumors, with the aim of improving the outcome of this aggressive disease.

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