Review

PEGylation in anti-cancer therapy: An overview

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ABSTRACT

Advanced drug delivery systems using poly(ethylene glycol) (PEG) is an important development in anti-cancer therapy. PEGylation has the ability to enhance the retention time of the therapeutics like proteins, enzymes small molecular drugs, liposomes and nanoparticles by protecting them against various degrading mechanisms active inside a tissue or cell, which consequently improves their therapeutic potential. PEGylation effectively alters the pharmacokinetics (PK) of a variety of drugs and dramatically improves the pharmaceutical values; recent development of which includes fabrication of stimuli-sensitive polymers/smart polymers and polymeric micelles to cope of with the pathophysiological environment of targeted site with less toxic effects and more effectiveness. This overview discusses PEGylation involving proteins, enzymes, low molecular weight drugs, liposomes and nanoparticles that has been developed, clinically tried for anti-cancer therapy during the last decade.

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1. Introduction

Cancer is one of the leading causes of death worldwide. Metastases are the primary cause of death from cancer. Cancer cells proliferate at much faster rate than the normal cells. The available traditional cancer chemotherapy is not essentially selective as it depends on the kinetics of the cell growth. Targeted cancer therapies are expected to be more effective and beneficiary in comparison to available conventional treatment procedures.

The last few decades of research in the particular area are focused on exploring the treatment of cancer at its molecular level. This will be helpful in developing better therapeutics. Polymer therapeutics is establishing as an innovative and reliable method for its ability to conjugate with protein, enzymes, nanoparticles, liposomes and low molecular weight drugs. In this regard, polyethylene glycol (PEG), a water-soluble and biocompatible polymer, is the most commonly used non-ionic polymer in the field of polymer-based drug delivery [1]. Passive targeting with PEGs in combination with active targeting (entry into the tumor cell via ligand receptor, antigen antibody interaction) delivery systems has been effectively employed to achieve better therapeutic index of anti-cancer drugs. Further, with the introduction of stimuli-responsive chemical moiety in PEGylated prodrugs, the sensitiveness of the drug molecule toward the pathophysiological environment of tumor cells in vivo evolved as a new era of site-specific targeted drug delivery system. Hence this overview deals with the development and recent advancement in various aspects of PEGylation.
in cancer treatment and its future prospective in a comprehensive way.

2. PEGylation and its significance

The technique of covalently attaching polyethylene glycol (PEG) to a given molecule is known as “PEGylation” and is now a well-established method in the field of targeted drug delivery systems. The general structure of monomethoxy PEG (mPEG) can be represented as CH\(_3\)O–(CH\(_2\)-CH\(_2\))\(_n\)–CH\(_2\)-CH\(_2\)–OH. At the beginning of PEG chemistry, in the late 1970s, Professor Frank Davis and his colleagues had shown that the immunological properties as well as the stability of bovine serum albumin and bovine liver catalase can be successfully altered by covalently linking them to methoxy PEG (mPEG) using cyanuric chloride as an activating agent. The process of PEGylation can be extended to liposomes, peptides, carbohydrates, enzymes, antibody fragments, nucleotides, small organic molecules and even to different nanoparticle formulations. mPEG is the most useful unit for polypeptide modification. Now several derivatives of PEG molecules are available that vary in molecular weight and structure, such as linear, branched, PEG dendrimers and more recently multi-arm PEGs. The first step of PEGylation is to activate PEG by conjugating a functional derivative of PEG at one or both the terminals of PEG chain.

PEGylation conjugation techniques can be classified into two categories: i) first-generation random PEGylation, and ii) second-generation site-specific PEGylation. Thanks to the second generation PEGylation processes that resulted in well-defined conjugated products with improved product profiles over those obtained through non-specific random conjugations.

Irreversibly conjugating PEGs had some adverse effects on the specific biological activity of many therapeutics. Thus, to minimize the loss of activity, a reversible (or releasable prodrug) PEGylation concept has been formulated. Reversible PEGylation concept deals with attachment of drugs to PEG derivatives through cleavable linkages (Fig. 1). The release of drug occurs by therapeutic agents through enzymatic, hydrolytic cleavage or reduction in vivo at a predetermined kinetic rate over a time period.

The objective of most PEG conjugation techniques aims at increasing the circulation half-life without affecting activity. It is to be noted that the distinct advancement in the PEG conjugation processes and diversity in the nature of the PEGs used for the conjugation has attributed to the increased demand for PEGylated pharmaceutical products.

PEGylation enhances the therapeutic efficacy of the drugs by bringing in several advantageous modifications over the non-PEGylated products. The systematic classification is illustrated in Fig. 2. Increase in the serum half-life of the conjugate is the major way of enhancing therapeutic potential of the PEGylated conjugate. PEGylation prolongs the circulation time of conjugated therapeutics by increasing its hydrophilicity and reducing the rate of glomerular filtration. Few factors such as protection from reticuloendothelial cells, proteolytic enzymes and decreased formation of neutralizing antibodies against the protein by masking antigenic sites by formation of a protective hydrophilic shield are the key components of PEG molecule that attributes to the improved pharmacokinetic profile (PK) of the conjugates. It has also been reported that PEGylation increases the absorption half-life of subcutaneously administered agents and is associated with a decreased volume of distribution. PEG is a non-biodegradable polymer.
that puts limits on its use. It has been shown that PEGs (up to molecular weight 20 kDa) is primarily excreted through the renal system, whereas higher molecular weight PEG chains get eliminated by fecal excretion [17]. PEGylation proved to be the most promising approach for increasing the serum half-life of the conjugated therapeutics, which is related to enhancement of efficacy of the conjugate. However, PEGylation imposes certain disadvantages on liposomes, especially for the delivery of genes and nucleic acids in anti-cancer therapy as its surface hydrophilic shield reduces the cellular uptake and improves the stability of the lipid envelope, and the process results in poor endosomal escape via membrane fusion and degradation of cargos in lysosomes [18,19]. Hence the use of PEG in gene and nucleic acid delivery to cancer cell is referred to as “PEG dilemma” [20]. The issue can be efficiently addressed by designing pH-sensitive and tumor-specific targeted PEGylated therapeutics [21–23].

2.1. Role of PEGylation in passive and active targeting of drugs

Passive targeting drug delivery technique, as illustrated in Fig. 3, mostly depends upon the concentration gradient between the intracellular and extracellular space, created due to high concentration of the drug in the tumor area [24]. PEG conjugates takes the advantage of enhanced permeation and retention (EPR) effect executed by the tumors and gets accumulated in the pathophysiological environment of tumor vessels through leaky vasculature and poor lymphatic drainage. However, this effect cannot be studied with low molecular weight drugs that freely extravagate causing systemic toxicity, and this is a size-dependent effect. PEGylation increases the solubility, size, molecular mass and serum stability of the drugs. For all these reasons, PEGylation is considered to be one of the best methods for passive targeting of anticancer therapeutics.

The concept of active targeting of drugs is based on the idea of conjugating drug molecules to targeting entities (antibodies, ligands, etc.) for specific interaction with the structures present on the cell surface for targeted delivery of the anticancer agent [25,26]. The fate of the pro-drug is dictated by the targeting molecule and the linker molecule present on the pro-drug. The targeting moiety essentially decides the type of cancer cell for the act of therapeutics. Further, depending on the linker molecule, the drug gets entry into the tumor cell by either of the two ways: (i) receptor-mediated internalization of the whole pro-drug by endocytosis and subsequent degradation by endosomal/lysosomal pathway (Fig. 4), or (ii) receptor-independent internalization of the drug into targeted cells after extracellular cleavage of the pro-drug (Fig. 5) [27]. PEGylated pro-drugs can be efficiently conjugated to targeting moieties by different conjugation chemistry in order to achieve the goal of active targeting. The targeted delivery of the PEGylated drugs at the desired site causes high bioavailability and low systemic toxicity.

3. PEGylated proteins in anti-cancer therapy

PEGylation of proteins is a well-established method in the pharmaceutical field, but the significance of PEGylated peptides and proteins for anti-cancer therapy has only been realized in the last several years as more and more PEG conjugates make it to late-phase clinical trials. Enzymes, monoclonal antibodies and cytokines are the three major class of proteins used in anticancer therapy or as adjuvant therapy (Table 1).

Fig. 3 – A schematic illustration of passive targeting with acid-sensitive PEG-prodrugs that cleave in the extracellular space.
In the field of anti-cancer therapy, monoclonal antibodies represent the major class of protein therapeutics. Antibodies act by binding to the specific antigens/cell surface receptors. This task is taken care by the fragment antigen-binding (Fab') region on an antibody. Depending upon the receptor and the binding site on the receptor against which the antibody is designed, it can either activate cellular signaling pathways leading to apoptosis, cell growth arrest, or block the pathways leading to cell growth that eventually causes tumor cell death (apoptosis). This event is illustrated pictorially in Fig. 6. The major drawback associated with Fab’s antibody fragment is its short serum half-life as it lacks the Fc region of the antibody that limits its potential as a therapeutic agent. Hence, suitable PEGylation methods and PEGs are used to ensure minimal loss of the antibody-antigen/cell surface receptor interaction keeping in view the enhancement of serum half-life. It has been reported that the hinge region cysteine residues on immunoglobulin G (IgG antibody isotype) Fab’ antibody fragments can tolerate attachment of one or two PEG moieties (up to a total of 40 kDa molecular weight) with little effect on antigen binding affinity. This process also enables significant increase in the half-life of the circulating plasma antibodies by reducing the
glomerular filtration and lower immunogenicity than the parent IgG [28].

The example of use of PEG-antibody fragment angiogenesis inhibitor (CDP791) is illustrated as follows: CDP791 PEGylated diFab antibody fragment antagonizes the effect of vascular endothelial growth factor receptor-2 (VEGFR-2), which is a prominent angiogenesis stimulatory molecule responsible for tumor progression. The short plasma half-life of the unmodified CDP791 antibody fragment, which lacks Fc region, has a low molecular weight and responsible for low therapeutic index. PEGylation of the cysteine amino acid present at the C-terminus of the native antibody could be able to resolve this issue by reducing its kidney clearance. This is demonstrated by the clinical studies for patients with colorectal, ovarian, renal cancer or other tumors [29].

### 3.2. PEGylated cytokines

Cytokines represent another class of protein therapeutics employed mainly as adjuvant therapy in classical anti-cancer chemotherapy protocols either to control or bring improvements in patient conditions. These small secreted proteins belong to the immunotherapy category and mobilizes the body’s immune system to fight cancer. The process is illustrated pictorially in Fig. 7.

#### 3.2.1. PEG-interferon-alpha conjugates

This process is illustrated as PEG-interferon-α2b (PEG-INTRON®/Sylatron™) and PEG-interferon-α2a (Pegasys®), which are discussed as follows:

##### 3.2.1.1. PEG-interferon-α2b (PEG-INTRON®/Sylatron™)

The PEGylated version of interferon-α2b was synthesized by conjugating interferon-α2b, with a single chain 12 kDa PEG-SC via a urethane bond [30]. It displayed a half-life of 27–37 h with 10-fold lower clearance and minor change in the volume of distribution in comparison to native form [31]. Based upon the outcome of clinical studies in the year 2011, the PEGylated drug peginterferon alfa-2b (PEG-IFN) got FDA approval for adjuvant treatment of melanoma patients with microscopic or gross

### Table 1 – PEG protein/enzyme conjugates in clinical development as anticancer therapy.

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Conjugate</th>
<th>Protein/Enzyme</th>
<th>FDA approved date/clinical trial status and use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sylatron™</td>
<td>PEG-interferon α-2b</td>
<td>PEG-interferon α-2b</td>
<td>March 9, 2011, approved as adjuvant therapy for resected stage III melanoma</td>
</tr>
<tr>
<td>Pegasys®</td>
<td>PEG-interferon α-2a</td>
<td>Interferon α-2a</td>
<td>Phase I for melanoma and phase II as for chronic myelogenous leukemia</td>
</tr>
<tr>
<td>Neulasta®</td>
<td>PEG-filgrastim</td>
<td>G-CSF(granulocyte-colony stimulating factor)</td>
<td>2002, to treat neutropenia during chemotherapy</td>
</tr>
<tr>
<td>Oncaspar®</td>
<td>PEG-asparaginase</td>
<td>Asparaginase</td>
<td>February 1994, acute lymphoblastic leukemia, and in July 24, 2006 first-line treatment for acute lymphoblastic leukemia</td>
</tr>
</tbody>
</table>
nodal involvement following definitive surgical resection including complete lymphadenectomy [32]. Sylatron™ is another brand name for peginterferon alfa-2b exclusively approved by FDA for adjuvant therapy in cancer treatment.

3.2.1.2. PEG-interferon-α2a (Pegasys®). Another PEGylated interferon, Pegasys®, is prepared by mono-PEGylation of interferon-α-2a with an N-hydroxysuccinimide (NHS) activated 40 kDa branched PEG molecule [33]. PEGylation prolonged the serum half-life from 3.8 to 65 h, slowing down the clearance by more than 100-fold. This has also reduced the volume of distribution to fivefold with respect to the native interferon-α2b [31]. PEGASYS® was efficient in improving the patient compliance by enabling once-weekly dosing while maintaining acceptable safety, tolerability, and activity profiles in clinical studies [34]. Currently, PEGASYS® is under evaluation as adjuvant therapy for patients with intermediate and high-risk melanomas [35].

3.2.2. PEG-granulocyte colony stimulating factor (PEG-filgrastim)
PEG-filgrastim was synthesized by conjugating a linear 20 kDa mPEG-aldehyde derivative to an N-terminal methionine residue of filgrastim through reductive alkylation under mild acidic conditions [36,37]. It is to be noted that a single dose of PEG-filgrastim per chemotherapy cycle could be able to reduce the risk of febrile neutropenia significantly with respect to the native protein (11% vs. 19%) [38–40]. Currently, PEGylated-G-CSF (Pegfilgastrim, Neulasta®) is used as an adjuvant therapy for patients with non-myeloid malignancies receiving myelo-suppressive chemotherapy (bone marrow suppression as a side effect of chemotherapy) associated with a 20% risk of febrile neutropenia [41,42].

3.3. PEGylated enzymes in anti-cancer therapy

Therapeutic enzymes represent a growing class of biopharmaceuticals, and PEGylation has played a major role in improving several of these products [43]. Many depleting enzymes are active against tumors. Enzymes’ intrinsic property of degrading amino acids is essential for cancer cells existence. The fate of the tumor cell is dictated by the different cellular pathways regulated by the substrate (amino acid) to be degraded, and the process of degradation is illustrated in Fig. 8. The normal cells are not affected because the normal cells can synthesize the amino acids for their growth. This situation is particularly the most advantageous aspect of using depleting enzymes in cancer therapy (Table 1). Therefore, during PEGylation procedure, a combination of these enzymes, low molecular weight (5–10 kDa) PEGs and random amine conjugation strategies are employed.

3.3.1. PEG-arginine depleting enzymes
Arginine is a nonessential amino acid in humans. It has been reported that arginine deficiency inhibits tumor growth, angiogenesis and nitric oxide synthesis [44]. Two types of arginine degrading enzymes, i.e. i) arginine deiminase (ADI) and ii) arginase (ARG), which can be utilized as antitumor agents, are discussed below:

3.3.1.1. PEG-arginine deiminase. The PEGylation of arginine deiminase proved to be a better therapeutic approach for anticancer treatment. Among the several PEGylated ADI formulations the ADI-PEG20000, formulated by conjugating 10–12 chains of 20 kDa PEG with ADI by using the succinimidyl succinate linker, is proved to be the acceptable one from in vivo study results [45]. Clinical studies have shown better efficacy of ADI PEG 200,000 in terms of antitumor activity and tolerability [46,47]. Currently, ADI PEG 200,000 versus placebo is under phase III clinical trial for advanced hepatocellular carcinoma. Further, Phase II for acute myeloid leukemia/non-Hodgkin’s lymphoma and Phase I (for metastatic melanoma in combination with cis-platin; for solid tumors in combination with docetaxel) are under trial [48].
3.3.1.2. PEG-arginase. The depleting enzyme arginase is an endogenous protein expressed in humans. The conjugate, PEG-rhArg, has 10 to 12 polymer chains of PEG 5000 per protein molecule that is covalently attached via a succinamide propionic acid (SPA) linker. This conjugate remains in fully active condition [49]. The PEGylated form executes sufficient catalytic activity at physiological pH with a prolonged plasma half-life of 3 days in comparison to the native form, which has a half-life of several minutes only. Currently, this conjugate is under phase I/II clinical trials.

3.3.2. PEG-asparagine depleting enzyme (PEG-L-asparaginase) Depletion of asparagine eventually results in leukemic cell death. Leukemic cells lack the enzyme asparagine synthetase, an enzyme required for asparagine synthesis, and depend on the exogenous supply of asparagine for their growth and survival. Therefore, asparaginase, the depleting enzyme for asparagine, plays a critical role as a therapeutic enzyme in treating acute lymphoblastic leukemia (ALL). Oncaspar is a modified form of the enzyme L-asparaginase approved by FDA in 1994. Oncaspar consists of tetrameric enzyme L-asparaginase derived from E. coli, and it is covalently conjugated with approximately 69–82 molecules of monomethoxy polyethylene glycol (mpeg), each having molecular weight of 5 kDa [50]. Oncaspar proved to be a better treatment option for patients who were allergic to the native form of the drug. The U.S. Food and Drug Administration granted approval to pegaspargase (Oncaspar, Enzon Pharmaceuticals, Inc) in July 2006 for the first-line treatment of patients with acute lymphoblastic leukemia (ALL) as a component of a multi-agent chemotherapy regimen [51].

4. PEGYLATED low molecular weight anticancer drugs

Various PEGylated low molecular weight anti-cancer drugs are currently under development. For example, topoisomerase I inhibitor camptothecin-based drugs (irinotecan, topotecan, SN38, exetecan, etc.) is reported to be useful in the treatment of many solid tumors. However, the hydrophobicity of such material limits their therapeutic efficacy. Few of the examples, listed in Table 2, are discussed below:

4.1. PEG-SN38 (EZN-2208) EZN-2208, the product Enzon Pharmaceuticals, Inc, is a PEGylated SN38 (10-hydroxy-7-ethyl-camptothecin (a derivative of camptothecin)). SN38 is the active moiety of CPT-11.

Table 2 – PEGylated low molecular weight drugs/liposomal derivatives/thermo-sensitive conjugates and nanoparticles in clinical development as anticancer therapy.

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Conjugate</th>
<th>Parent drug</th>
<th>FDA approved date/clinical trial status</th>
</tr>
</thead>
<tbody>
<tr>
<td>EZN-2208</td>
<td>PEG-SN38</td>
<td>SN38 (camptothecin derivative)</td>
<td>Phase II, various cancer</td>
</tr>
<tr>
<td>NKTR-102</td>
<td>PEG-irinotecan</td>
<td>Irinotecan</td>
<td>Phase III, metastatic or locally recurrent breast cancer and Phase II, solid tumor malignancies, including ovarian, colorectal, glioma, small cell and non-small cell lung cancers</td>
</tr>
<tr>
<td>Doxil (Caelyx)</td>
<td>PEG-liposomal doxorubicin</td>
<td>Doxorubicin</td>
<td>November 1995 for ovarian/breast cancer and Kaposi’s sarcoma</td>
</tr>
<tr>
<td>ThermoDox</td>
<td>PEG-thermosensitive liposomal doxorubicin</td>
<td>Doxorubicin</td>
<td>Phase III, hepatocellular carcinoma</td>
</tr>
<tr>
<td>CALLA 01</td>
<td>Pegylated cyclodextrin nanoparticle</td>
<td>SiRNA</td>
<td>In clinical phase I for solid tumors</td>
</tr>
</tbody>
</table>

Fig. 8 – Representing PEGylated depleting enzymes in anticancer therapy.
Nanoparticles (NPs) are synthetic materials with dimensions from 1 to 1000 nano-meters. NPs have large payloads, stability and the capacity for multiple, simultaneous applications due to their unique size and high surface area to volume ratio. Despite these advantages, the major drawback associated with NP drug delivery system for clinical studies are associated with short circulating half-life due to uptake by the reticuloendothelial system (RES) for larger NPs, whereas smaller NPs are subjects to tissue extravasations and renal clearance. Liposomes, solid lipids nanoparticles, dendrimers, polymers, silicon or carbon materials, and gold and magnetic nanoparticles are examples of nano-carriers that have been studied as drug delivery systems in cancer therapy. Therefore, surface modification of the nanoparticles with PEGs of various chain length, shape, density, molecular weight and incorporation of different targeting moieties (ligands, antibodies, etc.) is emerging as a more promising and technologically advanced drug delivery system in anti-cancer therapy. There are currently more than 35 US FDA-approved PEGylated NPs, with a larger number in preclinical studies for both imaging and therapy. Among several PEGylated nanoparticle formulations for anticancer therapy, liposomes have been most extensively studied.

5.1. PEGylated liposomes in anticancer therapy

Liposomes are spherical, self-closed structures formed by one or more concentric lipid bilayers with an encapsulated aqueous phase in the center and between the bilayers composed of natural or synthetic lipids. The development of long-circulating liposomes with inclusion of the synthetic polymer poly-(ethylene glycol) (PEG) in liposome composition could be able to solve the issue of low serum half-life associated with liposomes. PEG can be incorporated on the liposomal surface in a number of ways. However, anchoring the polymer in the liposomal membrane via a cross-linked lipid, PEG-distearylophosphatidylethanolamine [DSPE], is reported to be the most widely accepted method. Preclinical studies with PEGylated liposomes reported that the cytotoxic agents entrapped in PEGylated liposomes tend to accumulate in tumors. However, recent preclinical studies of anti-cancer drug enclosed in PEGylated liposomes in rodents and dogs have shown the rapid blood clearance of the pegylated drug carrier system due to the increased anti-PEG-\text{IgM} production. An example of PEGylated liposomal formulations, PEGylated liposomal doxorubicin (PLD), and most extensively studied, is discussed below:

5.1.1. Doxil (PEGylated liposomal doxorubicin)

Doxil is the trade name for PEGylated liposomal doxorubicin formulated to achieve better drug efficacy for cancer chemotherapy. This product contains doxorubicin (Adriamycin) enclosed in an 80-90 nm size uni-lamellar liposome coated with PEG. The modification increases the circulatory half-life of the drug leading to its enhanced bioavailability at the tumor site. PEGylated liposomal doxorubicin has fewer side effects on healthy cells than regular doxorubicin. PLD has improved pharmacokinetic features, such as long circulation time of about 60-90 h for doses in the range of 35-70 mg/m2 in patients with solid tumors. After PLD administration, nearly 100% of the drug in the plasma remains in the encapsulated form. Moreover, in comparison to free doxorubicin PLD, plasma clearance is dramatically slower and its volume of distribution remains very small, which is roughly equivalent to the intravascular volume. After obtaining approval from FDA, PEGylated liposomal doxorubicin (PLD) (DOXIL/Caelyx) is currently used to treat Kaposi’s sarcoma and recurrent ovarian cancer.

6. PEGylated smart polymers in anticancer therapy

Smart polymers are defined as polymers that undergo reversible large, physical or chemical changes in response to small external changes in the environmental conditions, such as temperature, pH, light, magnetic or electric field, ionic factors, biological molecules, etc. Smart polymers show promising applications in the biomedical field as delivery systems of therapeutic agents. Among various smart polymers currently in use in biomedical field of research, the temperature sensitive systems are the most studied systems. The greater therapeutic index of the targeted drug delivery systems can
be achieved by adjusting the transition temperature (Tt) of thermally responsive polymers, i.e., between body temperature (37 °C) and the temperature approved for mild clinical hyperthermia (42 °C) [79]. Within the temperature ranges, these polymers facilitate tissue accumulation by localizing the aggregation of systemically delivered carriers to the heated tumor volume [80,81]. For example, ThermoDox, a temperature-sensitive doxorubicin-loaded PEGylated liposome (DPPC), releases encapsulated doxorubicin at elevated tissue temperature. DPPC has a transition temperature of 41.5 °C, which makes it suitable for temperature-sensitive technology [82]. The temperature can be achieved by radiofrequency ablation technique. For ThermoDox, the concentration of the drug is up to 25 times more in the treatment area than IV doxorubicin, and several fold the concentration of other liposomally encapsulated doxorubins [82,83]. Currently, it is under phase III clinical trial for hepatocellular carcinoma (Table 2).

7. PEGylated polymeric micelles in anticancer therapy

Polymeric micelles are colloidal dispersions prepared by block-copolymers, consisting of hydrophilic and hydrophobic monomer units. Self-assembling amphiphilic polymeric micelles represents an efficient drug delivery system for poorly soluble or insoluble drugs [84]. Different varieties of amphiphilic polymeric micelles (i.e. diblock AB type, triblock ABA type or graft copolymers) can be designed by arranging the monomeric units in different ways and orders [85,86]. The hydrophobic block constitute the core and the hydrophilic block makes the corona of the micelles. The water-soluble PEG blocks with a molecular weight from 1 to 15 kDa are considered as the most suitable hydrophilic corona-forming blocks [87]. Various preclinical and clinical studies have shown the potential use of PEGylated polymeric micelles with different hydrophobic blocks such as PLGA poly(D,L-lactide-co-glycolide) [88–90], poly aspartate [91], γ-benzyl-L-glutamate [92], polyglutamate (Pglu) [93], and poly(D,L-lactic acid) [94] in anti-cancer therapy. It is to be noted that five different PEGylated polymeric micellar formulations, as enlisted in Table 3, are currently under clinical trials for possible anticancer treatment [95,96].

8. PEGylated nanoparticles for SiRNA delivery in anticancer therapy

RNA interference is a natural phenomenon employed to selectively turn off the genes expressed in some diseases. Molecular therapy using small interfering RNA (siRNA) has shown great therapeutic potential for tumors and other diseases caused by abnormal gene over-expression or mutation. It is a highly specific process for gene silencing. However, naked molecules of siRNA are vulnerable to premature renal clearance and nuclease degradation. The negative charge and hydrophilicity of siRNA also limit its permeability through cellular and endolysosomal membranes. Therefore, in order to overcome these issues, siRNA requires a carrier system for effective delivery [97]. Modification of drug delivery systems with PEGs of suitable chain length, molecular weight and percent composition was proven to be efficient in overcoming intracellular and systemic siRNA delivery barriers [98]. CALLA 01, a nanoparticle formulation (Calando Pharmaceuticals) formulated by using cyclodextrin nanoparticles conjugated to transferrin and coated with PEG, is the first one to enter under phase I clinical trials for solid tumors [44,99], in addition to few more which are currently under development (Table 2).

9. Conclusions

PEGylation offers a great advantage for bioactive molecules in pharmaceutical and biological applications by way of reducing protein immunogenicity and increased serum half-life of the drugs. This overview highlighted on the use of PEGylated proteins, low molecular weight drugs and PEG micelles. PEGylation improves the therapeutic efficacy of a drug by passive targeting in a novel way. The process can also be combined effectively with active targeting and stimuli-responsive targeted therapies for the development of new methodologies for the treatment of cancer. It is important to note that the efficacy of PEGylated drugs depends on overall exposure and its relationship to the pharmacodynamics of the drug. Molecular weight of PEG chain and its structural modifications carries strategic importance for conjugation with drug molecule for effective PEGylation process. The research in this direction shall be helpful in effective cancer treatment process in near future.

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Table 3 – PEGylated polymeric micelles in clinical development as anticancer therapy.

<table>
<thead>
<tr>
<th>Trade name for polymeric micelle</th>
<th>Block copolymer</th>
<th>Parent drug</th>
<th>Clinical trial status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK105</td>
<td>PEG-(aspartate) Paclitaxel</td>
<td>Paclitaxel</td>
<td>Phase II, advanced stomach cancer</td>
</tr>
<tr>
<td>NK012</td>
<td>PEG-Pglu(SN-38)</td>
<td>SN-38</td>
<td>Phase II, breast cancer</td>
</tr>
<tr>
<td>NC-6300</td>
<td>PEG-(aspartate) Epirubicin</td>
<td>Epirubicin</td>
<td>Phase I, breast cancer, stomach cancer, lymphoma</td>
</tr>
<tr>
<td>NC-6004</td>
<td>PEG-Pglu(cisplatin)</td>
<td>Cisplatin</td>
<td>Phase I/II, solid tumors</td>
</tr>
<tr>
<td>Genexol-PM</td>
<td>PEG-P(D,L-lactide) Paclitaxel</td>
<td>Paclitaxel</td>
<td>Phase IV, breast cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phase II, pancreatic cancer</td>
</tr>
</tbody>
</table>
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