Utilizing AOH1996 with chemotherapy delivered via drug nanocarriers; an advanced novel approach for cancer treatment especially Inflammatory Breast Cancer

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Abstract

Inflammatory Breast Cancer (IBC) is a rare disease but tends to be more aggressive than more common types of breast cancer. It accounts for 1–5% of new breast cancer cases. The treatment of IBC is comprised of chemotherapy in addition to targeted therapy as a neoadjuvant treatment, but it can be challenging to cure since IBC is a late-stage cancer. AOH1996 is a novel small molecule that has shown staggering results in inhibiting tumor growth in Phase I clinical trials in multiple cancer types, including IBC. This compound is an orally active drug that was tested in vivo, inducing apoptosis in cancer cells without causing any discernable toxicity even at 6 times its effective dosage. Mechanistically, it enhances the PCNA-RPB1 interaction while interfering with TRC resolution, which results in DNA double-stranded breaks in a transcription-dependent manner. Besides apoptosis induction, AOH 1996 sensitizes tumor cells to cisplatin and topotecan treatments. Nonetheless, a promising approach to augmenting the efficacy of chemotherapeutic agents and decreasing their peripheral toxicity is delivering them via nanocarriers. Polymeric nanoparticles showed remarkable properties in delivering paclitaxel in the case of Abraxane (ABI-007). This paper investigates exploiting the AOH1996 drug with different chemotherapeutics delivered via nanocarriers to be used for most cancer types, especially IBC.

I. Introduction

Cancer is the primary cause of death around the globe, according to the World Health Organization (WHO) [1]. In addition, cancer accounted for 9,958,133 deaths in 2020 from a total of 19,292,789 new cases in 2020 worldwide [2]. Remarkably, the Centers for Disease Control and Prevention (CDC) states that the cancer mortality rate has declined from 196.5 to 144.1 deaths per 100,000 population (27%) from 2001 to 2020 in the US [3]. This can be attributed to the improvement in early cancer screening for some cancers, the augmentation of cancer treatments, and the increased health-awareness in patients [4], [5]. One of the most common cancers in the world is breast cancer. In 2020, 2.3 million breast cancer incidences – the number of new cancers cases during a specific time period in a population – occurred with 685,000 death cases globally in women [6]. Though women and men are susceptible to breast cancer, the cancer incidence for men is much smaller than that for women. Breast cancer is a disease in which some breast cells of the ducts, lobules or surrounding connective tissue start

proliferating abnormally. Multiple division of breast cells can cause them to metastasize to the circulatory and lymphatic systems. Since these systems carry fluids to the whole body, tumors may form in other parts of the body, leading to metastasis. The most common sites of breast cancer are the lungs, bones, brain, and liver [7].

In order to carry on mitigating the cancer incidence and cancer mortality, more efforts should be focused on enhancing both cancer treatments and cancer screening. The most used cancer treatment modality is chemotherapy. This is due to its beneficial cumulative potential; many combinations of chemotherapy drugs can be used with different forms of cancer. Chemotherapy works by inhibiting the division of rapidly growing cells, which is a hallmark of cancerous cells. Chemotherapy is delivered intravenously (IV) or enterally (orally), via bloodstream, making it reachable to nearly every target organ in the body, except the brain and testes.

However, this aspect of chemotherapy not only affects cancerous cells, but also normal cells with fast multiplication rates, like bone marrow, hair follicles, and gastrointestinal (GI) tract cells. This generates the characteristic side effects of chemotherapy. Consequently, the unselective destruction of normal cells, the toxicity of chemotherapy drugs, and the development of resistance to multidrug treatment; necessitate finding efficacious treatments that only attacks tumor cells based on the molecular abnormalities in their cells. From the previous definition, "targeted therapy" is the treatment responsible for such mechanisms. In fact, targeted therapy can be used in combination with chemotherapy to attack and deliver the chemotherapeutic agents to the tumor cells only while leaving the normal cells unharmed. . Through this approach, it decreases the peripheral toxicity and achieves higher cytotoxic molecules concentrations in tumors [8 – 12]. The drugs used in targeted therapy target the biochemical factors which control how cancer cells divide, spread, and grow.

Antibody drug conjugates are biopharmaceuticals that combine the targeting properties of monoclonal antibodies (a drug class of targeted therapy) with the cytotoxicity of chemotherapy drugs using a stable linker, resulting in a selective accumulation of anticancer agents in tumor cells [12], [13 – 14]. Alternatively, considering the new advances in peptide research, cytotoxic peptide conjugates are being regarded as an efficient surrogate to antibody drug conjugates. They combine small peptides – up to 100 times smaller than antibodies – of low peripheral cytotoxicity with the capability to bind selectively to overexpressed receptors of some tumor cells, all with the cytotoxicity of anticancer drugs. In both antibodies drug conjugates and cytotoxic peptide conjugates, the linker of the conjugates should be sufficiently stable during blood transportation to maintain prodrugs, drugs that metabolize and become active inside the body, owing to avoid normal tissues. The linker must be cleaved intra- or extracellularly in the target cells via chemical or specific enzymatic degradations, which release the active cytotoxic agents in the cancer cells [12], [15 – 16]. Notably, a promising approach in delivering chemotherapeutic agents similar to targeted therapy is nanocarriers. Nanocarriers are colloidal nano-scale systems that are able to transport anticancer agents – like macromolecules or small molecular weight drugs – in a similar manner of targeted therapy.

Given that targeted therapy drugs block specific biologic transduction pathways, a potential potent drug should target a common pathway in all types of cancer. Proliferating cell nuclear antigen (PCNA) is a protein found in all eukaryotic cells widely used as tumor progression bio-marker [17 – 20], plays a fundamental role in cancer cell growth and survival [17], [21] and is essential in regulating DNA synthesis and repair [22]. DNA replication stress is a main characteristic of cancer cells [23 – 25]. It is used as an anti-

cancer strategy by exploiting the cancer-associated features in the DNA of tumor cells such as preventing DNA repair mechanism, which causes catastrophic destruction and damage of cancer cells [23]. Since PCNA is implicated in DNA repair, it is a potential target for this anti-cancer strategy. In addition, the identification of a unique isoform of PCNA – caPCNA – which is expressed in cancer cells has opened a novel avenue for developing chemotherapeutics. Early effects resulted from targeting PCNA detected many molecules of interest such as peptide-based molecules and small-molecules. This indicates that direct targeting of PCNA for oncotherapy may be a potential approach for interfering with the signals of cancer cells growth and impede their proliferation. [23], [26 – 32]. Thus, PCNA represents an alluring molecular target to develop broad-spectrum anticancer drugs [33].

i. Cancer and IBC epidemiology

Cancer arises from genetic mutations, that leads to cells proliferating abnormally and uncontrollably. These mutations can be acquired through exposure to carcinogens such as chemicals, radiation, or viruses; or inherited mutations. On the molecular level, cancer originates from mutation or damage of proto-oncogenes and tumor suppressor genes and tumor suppressor genes. Proto-oncogenes' overexpression result in proteins implicated in the cell proliferation and differentiation induction, while that of tumor suppressor genes code for proteins to induce cell growth inhibition and/or stimulate apoptosis. In many tumors, tumor suppressor genes are lost or inactivated/altered resulting in the removal of negative regulators of cell proliferation causing abnormal multiplication [34]. Alterations in both types – oncogenes (mutated proto-oncogenes) and tumor suppressor genes – are necessary for tumor growth in addition to being favored by tumor susceptibility genes mutations. The latter gene type, when expressed, encodes for a family of proteins involved in the control of DNA damage. The mutations that initiate a tumor are clonally selected to advocate absence of excessive cell growth inhibition, cell death blockage, and accumulation and transmission of genetic material errors [12], [24], [35 – 40].

One of the most aggressive, though rare, type of breast cancer is inflammatory breast cancer (IBC). It accounts for 1 – 5 % of breast cancer cases and, despite its rarity, it accounts for up to 7 – 10 % of breast cancer mortality [41 – 45]. IBC is characterized by the presence of several dermal tumor emboli in the reticular and papillary dermis of the skin overlying the breast [46]. It exhibits frequent local and distant metastases, rapid progression, and lower overall survival compared with other breast cancer types [46]. The diagnosis of IBC is often late or misdiagnosed as mastitis, dermatitis or infective process and when giving antibiotics, it worsens quickly. [46]. IBC typically starts at stage III and rapidly develops to stage IV [47]. Nevertheless, the pathogenesis of IBC is tumor microenvironment-dependent, characterized by several lymphocytes and macrophages/monocytes [46]. Furthermore, many amplified or mutated genes are characterized in IBC such as KRAS, ERBB2, EGFR, BRAF, PTEN, PIK3CA, AKT3, and AKTI genes [46], which suggest a therapeutic stratification with using different targeted therapies. However, there is no specific biomarker for early and accurate diagnosis of IBC. This makes its treatment more challenging than most other breast cancer types. In most cases of IBC, the treatment of is a combination therapy comprising neoadjuvant chemotherapy and sometimes targeted therapy. This is followed by surgery, lumpectomy or mastectomy, and radiotherapy as an adjuvant treatment [41].

ii. Chemotherapy drugs classifications

Alkylating agents

Alkylating agents are antiproliferative drugs that bind to DNA and cause DNA damage, preventing cellular reproduction. Their mechanism of action is binding covalently via alkyl groups to DNA, and subsequently pausing the cell cycle in the G1 or the S phase – a process called cell arrest [48 – 50]. Because the alkylating agents form cross-linkage of DNA strands, cell division or replication will be hindered. Thus, the cell will either undergo attempting cell repair or apoptosis. Examples of alkylating agents include Cyclophosphamide and Cisplatin.

Another example that is worth mentioning is the nitrosoureas group. Nitrosoureas are a group of alkylating agents that have a different action. Unlike other alkylating agents, nitrosoureas can enter the brain since they can cross through the blood-brain barrier, an area where not all chemicals can enter. This mechanism of action makes these drugs viable in treating specific types of brain cancer. Examples of nitrosoureas include Lomustine and Streptozocin.

Anti-metabolites

Anti-Metabolites – or Antimetabolites – are cytotoxic agents that interfere with the metabolism of nucleic acids, thus interfering with DNA and RNA. These drugs act through disrupting DNA and RNA metabolism/production by interrupting the S phase of the cell cycle, preventing the cell from replicating the genetic material. Particularly, they interfere with the biosynthesis of purines and pyrimidines, which therefore induces inhibition of DNA replication [49 – 51]. Nonetheless, some antimetabolites are capable of being inserted fraudulently into nucleic acids, causing structural abnormalities resulting from other mechanisms in cell death like DNA double-strand breaks. Examples of antimetabolites are 5-fluorouracil (5-FU) and Methotrexate.

Anti-tumor antibiotics

Anti-tumor antibiotics are derived from compounds that are produced from Streptomyces bacteria, which are independent of the antibiotics used for treatment of bacterial infections. The main group of these drugs is called anthracyclines [49 – 50]. They have several mechanisms of action. The mechanisms of action of these drugs start with (a) inhibiting Topoisomerase II. Topoisomerase II is an enzyme responsible for maintaining the topology of DNA as it relaxes the DNA supercoils during replication by breaking both strands of the DNA, unwinding them, then rejoining them again. Henceforth, by inhibiting Topoisomerase II, DNA replication is hindered which stops the cell cycle progression. (b) inhibiting helicase. During DNA replication, DNA helicase separates double-stranded DNA into single strands, which allows each strand to be copied. DNA helicase also unwinds DNA at the "origins" areas where the DNA biosynthesis will be initiated. By inhibiting DNA helicase, DNA replication is hindered, which will stop the cell from growing. (c) inducing reactive oxygen species (ROSs) formation, which will result in cellular destruction and eventually apoptosis. Examples of anthracyclines include Doxorubicin and Epirubicin.

Topoisomerase inhibitors

As the name indicates, topoisomerase inhibitors inhibit the topoisomerase enzymes. There are two types of topoisomerases: Topoisomerase I and Topoisomerase II. Topoisomerase I breaks one DNA strand whilst Topoisomerase II breaks both DNA strands [49], [52]. Consequently, Topoisomerase I inhibitors (also called camptothecins) inhibit Topoisomerase I which will hinder DNA replication. Examples of Topoisomerase I inhibitors include Irinotecan

and Topotecan. Examples of Topoisomerase II inhibitors include Etoposide and Topotecan. Some anti-tumor antibiotics are also considered Topoisomerase II inhibitors like Doxorubicin.

Antimicrotubular agents

Antimicrotubular agents (also known as mitotic inhibitors) disturb the M phase of the cell cycle inducing cell arrest and then inducing apoptosis. Their mechanism of action is through either hindering the polymerization or depolymerization of microtubules [49], [53]. Antimicrotubular agents are classified into two main categories based on the sites they bind on to microtubule: the colchicine domain, vinca domain, or taxane site. When these drugs target the vinca or colchicine domains, they are called vinca alkaloids. By contrast, when they target the taxane site, they are called taxanes. Vinca alkaloids inhibit the formation of microtubules – hence the name is called microtubules destabilizers – leading to disrupting the M phase which will result in cell arrest and then apoptosis. Taxanes, on the other side, stabilize and bind to the microtubules that are already formed in the M-phase – that's why they are called microtubules stabilizers. In other words, once the microtubules are formed, these drugs inhibit their breakdown. This results in M-phase arrest, then cell arrest, and finally apoptosis. Examples of vinca alkaloids include Vinblastine and Vincristine. Examples of taxanes include Cabazitaxel and Paclitaxel.

Even though cancer treatment has improved since the first time of only using nitrogen mustards, indiscriminate normal tissues destruction, and the side effects produced from chemotherapy drugs toxicity are downsides to their use. This unspecific strategy was revolutionized with the discovery of the cell signaling networks implicated in cell growth and differentiation. Consequently, this allowed designing drugs that specifically target those networks, which opened the door to the utilization of targeted therapy, in the late 1990 [12], [54].

iii. Targeted Therapy

As the name suggests, targeted molecular therapies are designed to interrupt cellular abnormalities responsible for cancer cell growth and spread. These drugs work by targeting and blocking specific protein related to the development of cancer cells by interfering with their biochemical pathway by turning off the signals sent to activate the cells division. Targeted therapy not only kills cancer cells but may also cease the proliferation of cancer cells. Moreover, most targeted therapy drugs are either classified as small-molecule drugs or monoclonal antibodies. Small-molecule drugs are named so since they are sufficiently small to easily enter the cell, so they are used to block specific targets from inside the cell. Monoclonal antibodies (mAbs) are proteins synthesized in-vitro designed to adhere to precise targets on cell surfaces. This class has different mechanisms of actions in treating cancer. Some mAbs work by delivering molecules toxins, like chemotherapy drugs, into or onto the cell in order to kill it. Others work similar to immunotherapy as those mAbs mark cancer cells so that the immune system can better identify cancer cells and respond to attack them more beneficially. This mechanism of action can be illustrated **in Figure 1** [55]. Still others directly alter cancer cells to hinder their multiplication and stimulate apoptosis.

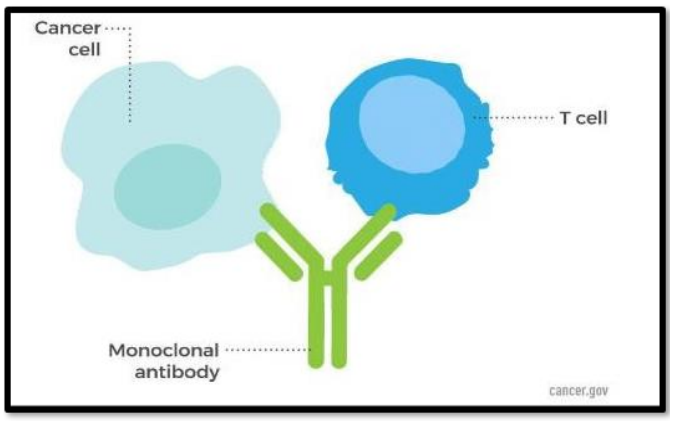

Figure 1: schematic representation of mAb marking cancer cells for the immune system, T-cell in this case.

iv. Small-molecule targeting PCNA

PCNA provides an anchorage for the several proteins implicated in DNA replication and repair pathways [23]. The cellular functions of PCNA can be regulated via post-translational modifications, the chemical changes undergone by covalently adding specific functional groups to proteins after translation, on the protein surface, altering partner interactions [56 – 57]. This takes place mainly through the outer hydrophobic PCNA surface, alongside its interdomain connector loop (IDCL) [58 – 59]. M121 to Y133 are the 8 amino acids present in the interdomain connector loop of PCNA [60]. It was reported that normal tissues express an isoform of PCNA – nmPCNA – with a basic isoelectric point/point of zero charge, the pH value at which the net charge of a particular molecule (protein in this case) becomes zero [61]. Conversely, cancer cells express not only the basic, but also to a much higher level, a distinct acidic PCNA isoform – caPCNA, which is not significantly expressed in nonmalignant cells [61 – 63]. Long Gu et al. mapped the specific antigenic site of caPCNA to the small eight amino acid peptide region, L126 – Y133, within the IDCL of PCNA [61]. The L126 – Y133 region is only accessible to immunohistochemistry (IHC), a process where monoclonal as well as polyclonal antibodies are used to indicate the tissue distribution of an antigen of interest in cells/tissues samples [64], staining by both monoclonal and polyclonal antibodies specific to this region in cancer cells [61]. This suggests that this region is altered structurally in tumor cells and causes expression of the caPCNA isoform in cancer cells instead of nmPCNA at the isoelectric point. Hence, interfering this region should block PCNA interactions, resulting in DNA replication stress.

Researchers exploited this approach and were able to selectively kill multiple cancers including breast cancer cells [17], [62], [65 – 66]. Long Gu et al. were the first to come up with the AOH drug family, which are oral targeted therapeutics with anticancer activity inhibiting PCNA [17]. The initial compound they identified was AOH39, a small-molecule compound that selectively kills many cancer types at a low micromolar concentration. AOH 1160 is the developed analogue of AOH39; it has a significantly enhanced potency and therapeutic window, the range of a drug dose that is considered effective with minimal side effects. Yet, this compound lacks metabolic stability and required further optimization to become a lead drug candidate. Afterwards, AOH1996 was identified as a novel analogue of AOH1160, which exhibits remarkable therapeutic properties. Unlike AOH 1160, AOH 1996 has superior metabolic stability, inhibits almost completely the xenograft tumors growth, and causes no discernible peripheral toxicity 6 or more times the effective dose in mice and dogs [17].

v. Drug Nanocarriers

Nanocarriers work by allowing anticancer agents to accumulate in tumors and preserve normal tissues as well as attaining a cytotoxic concentration several-fold higher in tumor tissues. This is also accompanied by reduced peripheral toxicity compared with free drugs. Therefore, nanocarriers indirectly mimic what antibodies and peptide-drug conjugates do without actually being a targeted therapy drug, besides augmenting cytotoxicity on tumor cells and decreasing toxicity for the rest of the body [11 – 12], [67 – 68]. Nanocarriers are used to improve the efficacy of anticancer drugs. They increase the payload of cytotoxic drugs, protect the drug from degradation, reduce the renal clearance and its half-life in the bloodstream. This allows the controlling of the release kinetics of the anticancer drugs and improves the solubility of insoluble/poorly-soluble drugs [11], [69 – 70]. As shown in **Figure 2** [12], nanocarriers can be delivered to tumors by passive tissue targeting or active cellular targeting. Passive tissue targeting exploits the increased tumor vasculature permeability and the poor lymphatic drainage of tumors (EPR effect), which the allow the release of chemotherapeutics in the tumor vicinity. Active cellular targeting is attained by the functionalization of the surface nanocarriers that contain chemotherapy drugs. Their targeting moieties increase their therapeutic efficacy, provide selective recognition of different antigens or receptors overexpressed in cancer cells, and overcome the multiple-drug resistance. Nanocarriers encompass mainly polymer therapeutics, where the drug is conjugated to a polymeric structure; and particulate drug nanocarriers, where the drug is physically encapsulated within molecular assemblies with different structures made from different materials. Polymer therapeutics contain polymer-drug and polymer-protein conjugates, while particulate drug nanocarriers comprise polymers, which further classifies to dendrimers, polymeric micelles, and polymeric nanoparticles; liposomes, or carbon nanotubes [12].

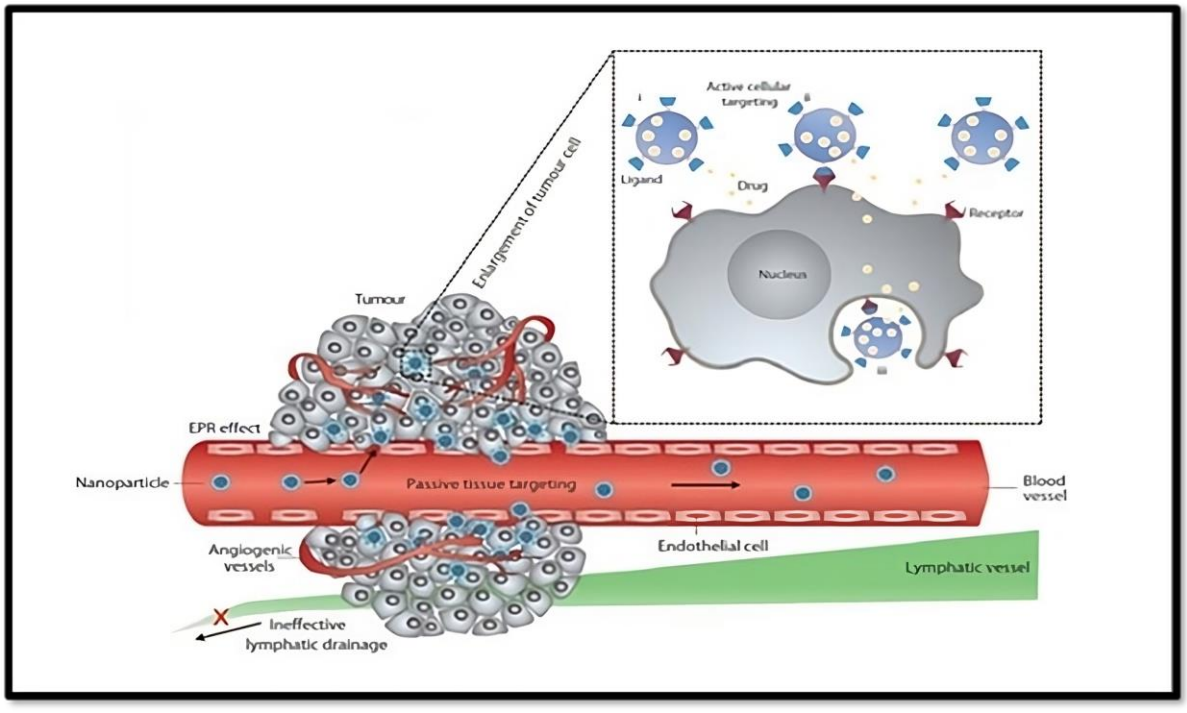

Figure 2: schematic representation of the interaction between nanoparticles and cancer cells via passive tissue targeting and active cellular targeting.

II. Results

i. AOH1996

The mechanism of action of AOH 1996 in eukaryotes is through stabilizing the interaction between the largest subunit of RNA polymerase II (RNAPII) – RPB1 – and PCNA, leading to intracellular RPB1 degradation [23]. AOH1996 dissociates PCNA from actively transcribed chromatin and induces double DNA strand breaks (DSBs) accumulation, without altering the presence of PCNA in the heterochromatin region, genome regions that are not transcribed and are highly condensed. This suggests that inhibition of caPCNA activity by AOH1996 results in transcription-associated collapse of DNA replication. Both point mutation, gene mutation that results from alteration of a single base pair, and transcription inhibition within the AlkB homolog 2 PCNA interacting motif (APIM) domain [71] of RPB1 debilitates the interaction between PCNA and RPB1; conferring AOH1996 resistance.

Correspondingly, transcription-replication conflicts (TRC), a process that occurs when two essential cellular machineries implicated in genome duplication and gene expression collide with each other on the same genomic location; are an intrinsic cause of genome instability and DSBs. Since DNA replication

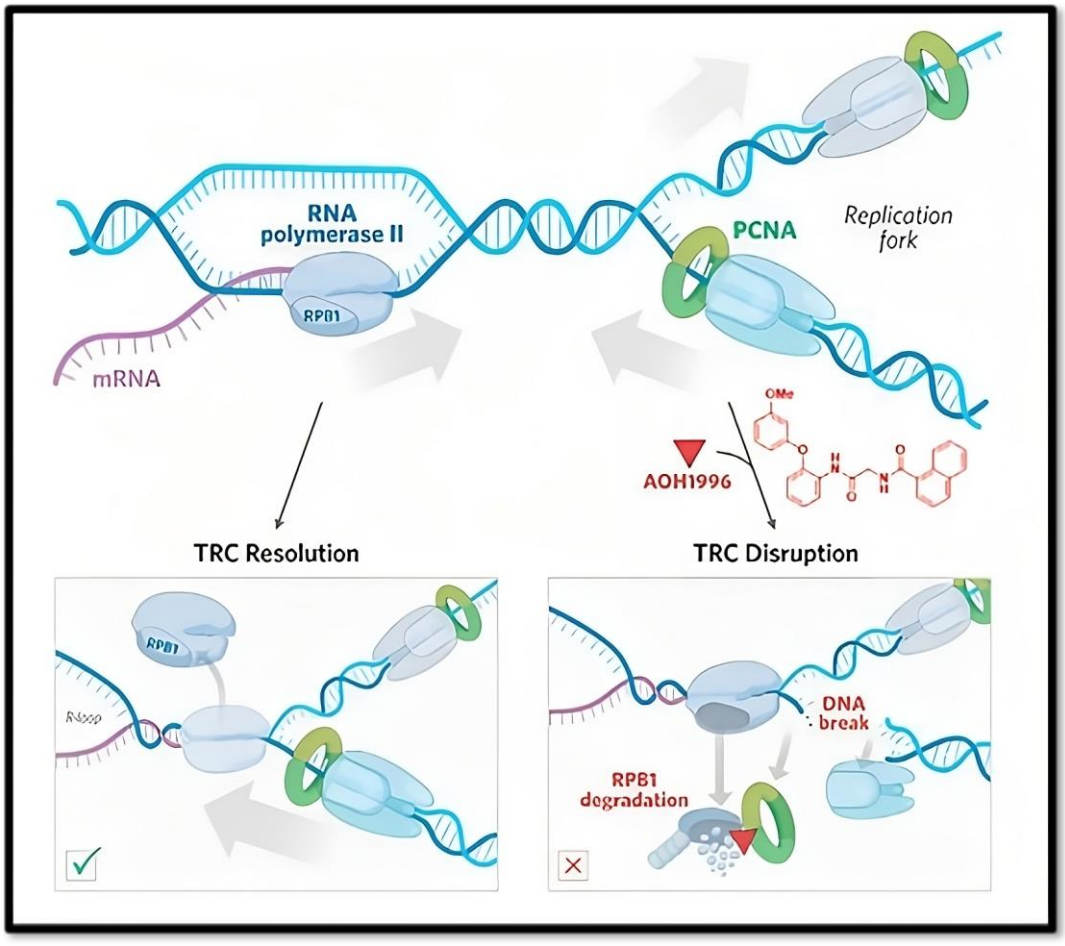

Figure 3: schematic representation of the mechanism of action of AOH1996: binding of AOH1996 to PCNA stabilizes PCNA interaction with RNA polymerase II and interferes with TRC resolution leading to dissociation of PCNA from chromatin in a transcription dependent manner.

and transcription are fundamental cellular processes, and that tumor cells likely increase interactions between the replication and transcription machineries, Long Gu. et al. suggest that this makes cancer cells more vulnerable to disruption of the process inducing TRC resolution. As shown in **Figure 3** [23], accumulating evidence points out that TRC resolution comprises removing RNAPII from the conflict sites, by degradation of RNAPII. This leaves the replication fork to go through the conflict site [72 – 73].

The evidence shows that AOH1996 induces a significant change in the cell cycle that indicates G2/M and/or S phase arrent in tumor cells, but not non-malignant cells, which suggests selective replication stress induction in cancer cells only. Given the TUNEL assay positivity, a method for detecting of DNA fragmentation undergoing apoptosis, results conducted by Long Gu et al. in **Figure 4** [23]; AOH1996 induces apoptosis in cancer cells, specifically neural crest stem cells (7SM0032) in that study [23]. Moreover, AOH1996 enhanced the sensitivity of cancer cells to genotoxic agents, encompassing cisplatin, which mainly causes Pt-GG adducts (62 – 75 %) [74] in open chromatin areas [75], genomic regions that are associated with basic physiological activities, as shown in **Figure 5**. A similar synergy profile was observable between AOH1996 and topotecan. As aforementioned in "Topoisomerase Inhibitors", topotecan inhibits topoisomerase I, which subsequently prevents the nicked DNA strand repair and causes DSBs during DNA replication [76].

Based on the AOH1996's target, caPCNA, broad expression in cancer cells, Long Gu et al. tested this compound in more than 70 cell lines and many normal control cells [23]. 300 nM was the median concentration to achieve 50% growth inhibition (GI50), the drug concentration required to reduce by 50% total cell growth, across more than 70 cancer cell lines tested. In comparison, AOH is not significantly toxic to nonmalignant cells including small airway epithelial cells (hSEAC) neural crest stem cells (7SM0032), and human peripheral blood mononuclear cells (PBNCs), even up to at least 10 μM concentration. This result demonstrates no less than a 30-fold sensitivity difference between cancer and normal cells. Consistent with these findings, the γH2A.X levels, a sensitive biomarker for DSBs, imply that the AOH1996 treatment resulted in DNA accumulation in lung cancer cells and neuroblastoma cells, but not in many nonmalignant cells. Readers may refer to the specific cell lines used in that study through published articles [23]. Essentially, AOH1996 is not a genotoxic mutation since it does not induce frameshift mutation, a type of mutation that results from insertion or deletion of nucleotide bases in a number that is not divisible by three, or base pair substitution in Ames test [77], a screen used in determining the mutagenic potential of new drugs and chemicals.

ii. Drug Nanocarriers and Chemotherapy

In this article, the results collected here demonstrate the drug nanocarriers combined with their chemotherapeutics agents that are FDA-approved for breast cancer treatment. Applying these guidelines, the groups and subgroups of these drugs are liposomes, polymeric micelles, and polymeric nanoparticles.

Initially, liposomes particulate drug nanocarriers are colloidal self-assembled vesicles with lipid bilayer-membranes, which are composed of amphiphilic phospholipids. This not only permits the encapsulation of multiple hydrophilic siRNAs and anticancer drug in its aqueous core, but also can host lipophilic cytotoxic agents in its hydrophobic membrane. Nevertheless, the drug loading capacity of poorly soluble drugs is limited because of the destabilization effect of the drug on the outer space and the presence of the small space available in the membrane, making it mostly as water-soluble drugs only, albeit with low loading limits, as well [12], [78

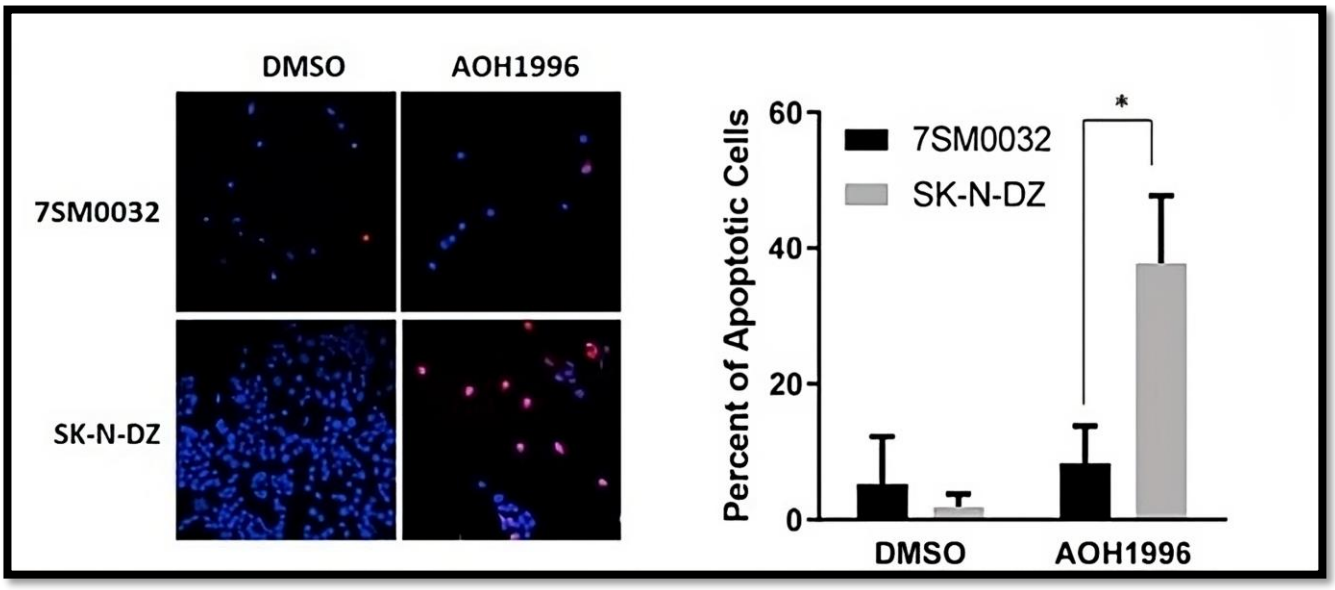

Figure 4: SK-N-DZ neuroblastoma cells and nonmalignant 7SM0032 stem cells were incubated with 500 nM AOH1996 for 24 h. Then, after being fixed on slides, cell apoptosis was analyzed by a TUNEL assay. Left image: TMR fluorophore (red) attached to the free ends of DNA indicates cells undergoing apoptosis. Blue indicates DAPI stained nuclei. Right: Average abundance ±S.D. of apoptotic 7SM0032 (black histogram) and SK-N-DZ (gray histogram) cells relative to the total number of cells are shown in 5 randomly selected fields.

*p < 0.01.

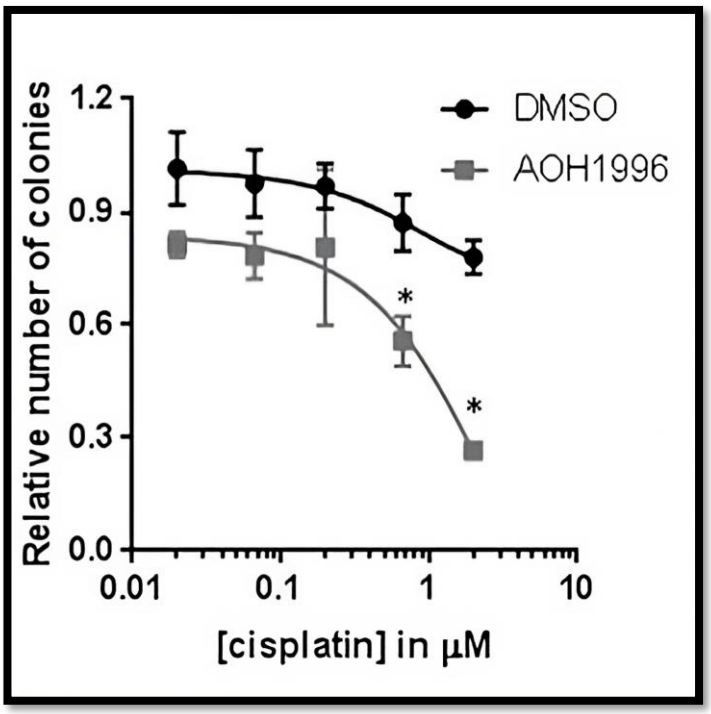

Figure 5: Human SK-N-DZ neuroblastoma cells were treated for 18 h with or without the indicated concentrations of cisplatin in the absence or presence of 500 nM AOH1996. Cells were washed twice with growth medium and cultured in fresh media for 18 days to allow colony formation. The colony counts in dishes treated with cisplatin but not AOH1996 (black) were normalized to the colony counts in dishes untreated by either agent. The colony counts in dishes treated by both cisplatin and AOH1996 (gray) were normalized to the colony counts in dishes treated with 500 nM AOH1996 alone. The relative number of colonies determined in triplicates for each treatment condition were averaged and graphed ± SDs (*p < 0.01).

– 79]. Moreover, liposomes exhibit advantageous properties like biocompatibility and nearly all biological inert patient profiles, which as a result don't cause toxic or antigenic reactions in a high percentage of cases. In contrast, it is worth noting that IV injection of liposomal drugs can cause complement activation-related pseudoallergy, drug-induced immediate immune toxicity evident in hypersensitivity reactions [80 – 82]. Liposomes exhibit long time circulation in blood, which can even be enhanced by polyethylene glycol conjugation to the surface of the liposome; and an easy tunable surface. However, the drawbacks of liposomes include the oxidation of phospholipids, problems with stability and industrial reproducibility, difficulties in sterilization, and the limited drug release control by the conventional formulations, which have profiles of release in rapid burst [12]. Additionally, liposomes can be used as active targeting carriers by binding antibody fragments or mAbs to the liposomes surface (immunoliposomes) [83 – 86], which will increase the anticancer activity and selectivity, reducing the free drug systemic toxicity [86 – 87]. Immunoliposomes are able to entail many antibodies and other targeting moieties, increasing the antibodies targeting avidity [88], the measure of the overall binding strength between an antibody and antigen. Conversely, the carriers' limited circulation in blood (that can be enhanced with PEG coating [89]) may impede their penetration in solid tumors [90]. Lipoplatin is a PEGylated liposomes with cisplatin that is used for the treatment of breast cancer [12], [91].

Polymeric micelles are promising carriers for delivering poorly soluble cytotoxic drugs which allow a controlled drug release [12]. They are composed of amphiphilic block copolymers, which form nanosized spheroidal micellar structures; with a hydrophobic core that can incorporate poorly-water soluble anticancer drugs; as well as a hydrophilic shell that allows the encompassment of hydrophilic drugs while providing stability to the micelle. This results in long time circulation of the drug in blood and makes this formulation an appropriate carrier for IV administration [92 – 94]. The reduced size of the micelle (20 – 80 nm) and their uniformity increase the drug circulation time even more in blood and provide better permeability to the anticancer drugs. This enhances their delivery from the blood vessels into the tumors and generates a uniform cytotoxicity distribution throughout the tumor tissue [95 – 96]. Nonetheless, polymeric micelles exhibit some limitations such as the premature drug leakage, which may cause a decrease in effectiveness and side effects, and their insufficient stability in systemic circulation [92], [97]. Genexol-PM is a polymeric micelle composed of paclitaxel encapsulated in monomethoxy-PEG- block-poly(D,L-lactide), which is used for the treatment of advanced, recurrent or metastatic breast cancer [12], [98].

Polymeric nanoparticles may be the most beneficial carriers for prolonged and controlled delivery of anticancer targeted drug delivery [12]. They are biodegradable colloidal systems that comprise spherical nanosized polymeric particles., where cytotoxic drugs can be encapsulated in a polymeric matrix or incorporated into a cavity surrounded a polymeric membrane. This makes it possible to conjugate between the anticancer drug to the core of the surface of the particles [99 – 100]. In the case of insoluble drugs, a hydrophobic interaction can be produced between the drug and the particle core, which increases its solubility [101]. When a drug is bound to the particle, the linkers properties enact an important role in the pharmacological properties of the complex. For instance, the linkers can make them stable in the bloodstream, but impose cleavage in the tumors by lysosomal enzymes [102]. Furthermore, polymeric nanoparticles possess advantageous properties like long circulation times, multiple synergic drugs delivery, enhanced cancer cells uptake, toxicity reduction with a limited interaction with healthy cells. They also show more

homogenous size distribution, better stability and controllable physicochemical properties, more controlled drug release via erosion and degradation of the particles or by diffusion through the polymer matrix, compared with other colloidal systems like liposomes and polymeric micelles [103]. Abraxane (ABI-007) is an albumin-bound paclitaxel nanoparticle polymeric nanoparticle that is used for the treatment of metastatic breast cancer [12], [104].

III. Discussion

The presence of the cancer-associated isoform of PCNA, specifically the L126 – Y133 region disrupts the TRC-PCNA interaction in cancer cells, which is an attractive target for therapeutic agents. This article demonstrates the AOH1996 enhancement on the interaction between RBP1 and PCNA, which leads to total degradation of RPB1 and collapse of DNA replication forks in actively TRC regions. Therefore, resolving TRC is of paramount importance to the survival and growth of cancer cells. Given that the level of replication stress TRC consequences can result in genomic instability and lethal DNA damage, that is where AOH1996 exerts its therapeutic potent, selective anticancer effect, while maintaining a staggering clinical safety profile. Yet, the mechanisms of resolving TRC still need to be fully elucidated, and TRC has not been selectively targeted as a viable therapeutic effect. Additionally, the results imply that AOH 1996 can be used for receptor-specific cancers, including inflammatory breast cancer. Like non-IBC, IBC can be classified into four subtypes: HR+/HER2+, HR+/HER2-, HR-/HER2-, and HR-/HE2+. In spite of this, AOH1996 is viable as a monotherapy or more efficiently in a combination with other treatments for IBC since it disrupts the cell-cycle profile rather than selectively targeting certain receptors.

Long Gu et al. indicated that AOH makes cancer cells more susceptible to cisplatin and topotecan treatments. In comparison, these chemotherapeutics can be more efficacious if they were delivered by nanocarriers. Regarding the cisplatin treatment, Lipoplatin is a good candidate for IBC, considering that it is a PEGylated liposome with cisplatin. However, since most anticancer drugs that were used for advanced and metastatic breast cancer involved paclitaxel, Abraxane (ABI-007) is nearly an ideal candidate for IBC since it is albumin-bound paclitaxel besides that it is a polymeric nanoparticle, which shows advantageous properties among the other nanocarriers drug types. Though, future research should evaluate the potency of AOH1996 in Phases II, III, and IV clinical trials. In addition, future research should check for chemotherapeutic agents delivered with drug nanocarriers and check for any adverse effects while using them in combination with AOH1996 in IBC.

The next section of the paper demonstrates potential novel approaches that contribute to the field of cancer treatment which are developing a new system for enhancing drug optimization and introducing a beneficial and accurate cancer screening method via simple blood test.

i. Improving clinical drug development

Drug discovery and development is a long and very expensive process that can take over 10–15 years with an average price of 1–2 billion US Dollars for each novel drug to be approved for clinical use. **Figure 6** shows that drug development follows a specific process [105]. Studies have shown that 9 out of 10 drug candidates fail during phase I, II, III clinical trials after they have passed the preclinical studies [105 – 107]. In particular, analyses of clinical trial data from 2010 to 2017 demonstrate four possible reasons assigned to the high failure incidence of clinical drug

development: lack of clinical efficacy (40% – 50%), unmanageable toxicity (30%), poor drug-like properties (10%–15%), and lack of commercial needs as well as poor strategic planning (10%) [105 – 106], [108].

This raises the question whether some aspects in drug optimization and target validation are left unconsidered. Duxin Sun et al. claims that current drug optimization overemphasizes drug's specificity/potency by using structure-activity-relationship (SAR), a technology designed to understand and find relationships between the drugs' chemical structure-related properties and their biological activity [105]. Though, drug optimization overlooks tissue exposure/selectivity in either disease or normal tissues by using structure-tissue exposure/selectivity-relationship (STR). This could impact the balance of clinical drugs' dose/efficacy/toxicity and might mislead the drug candidate selection. Particularly, throughout the drug optimization process, the nearly-ideal compounds are significantly optimized through SAR in order to achieve high specificity and affinity to their molecular targets besides limiting the off-target effect as much as possible [105], [109 – 113]. Nevertheless, the validation of the pharmacological effect (efficacy and toxicity) of a drug molecule may not only be a result of the drug's inhibition of its desired molecular target but may also be due to some unknown targets inhibition [114]. This makes using drug optimization based on SAR only challenging.

One possible approach in enhancing the drug optimization process is developing AI-aided computation models to evaluate both SAR and STR. In fact, AI-aided computational tools have widely been used in the field of drug development, specifically in the design of drug molecules for molecular target inhibition, in predicting the 3D protein structural shape of molecular targets, and in studying drug molecule interaction with its targets in SAR [105], [115 – 118]. For instance, AI- based computation mode can be exploited in analyzing the relationship between tissue exposure and molecular descriptors, the numerical representation of a molecular structure; plasma/tissue partition coefficient, the measure of the lipophilicity of a drug and an indication of its capability to cross the cell

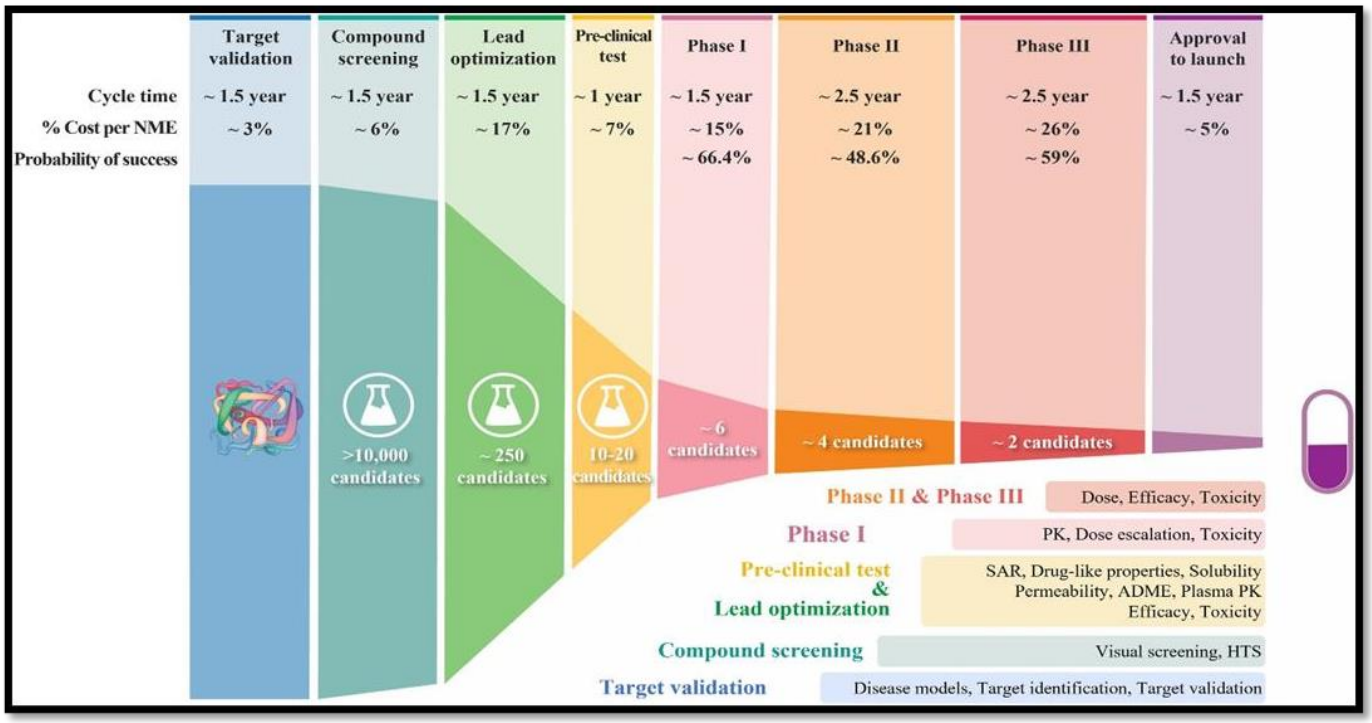

Figure 6: Drug discovery and development process, and the failure rate at each step.

membrane; and tissue selectivity for a selected set of compounds with *in vitro* and *in vivo* STR data [105], [119]. Providing that these information of sufficient number of compounds have been established by AI-aided computation modeling, the prediction of STR and SAR for any newly designed compounds will be performed by utilizing AI-based computation analysis before synthesis. This would reduce effort, time, and cost during drug optimization.

The balance of clinical dose/efficacy/toxicity of drug candidates in human trials is also determined by its exposure/selectivity in disease-targeted organs versus normal organs, not only by its specificity/potency to inhibit its molecular targets [105]; meaning that drug optimization process is determined by both SAR and STR studies. Consequently, another system should be used in evaluating the drug optimization process. Duxin Sun et al. proposed a structure-tissue exposure/selectivity -activity relationship (STAR) system to enhance the drug optimization process. As shown in **Figure 7** [105], this system classifies drug candidates into 4 categories, I– IV, based on 3 aspects which are: dose requirement for balancing clinical efficacy/toxicity, drug selectivity/potency to inhibit the molecular target by using SAR studies, drug tissue exposure/selectivity by using STR studies [105]. The four classes of drug candidates require different strategies to select the nearly-ideal drug candidates balance clinical efficacy/toxicity, and optimize clinical doses. If this system is applied successfully, it will boost the efficacy of the drug optimization process and clinical studies for 4 different classes of drug owing to improve the success rate of the clinical drug development and discovery. Thus, applying this system will aid in evaluating the potency/selectivity/dosage of anticancer drugs, which will contribute to determining personalized treatment for cancer patients.

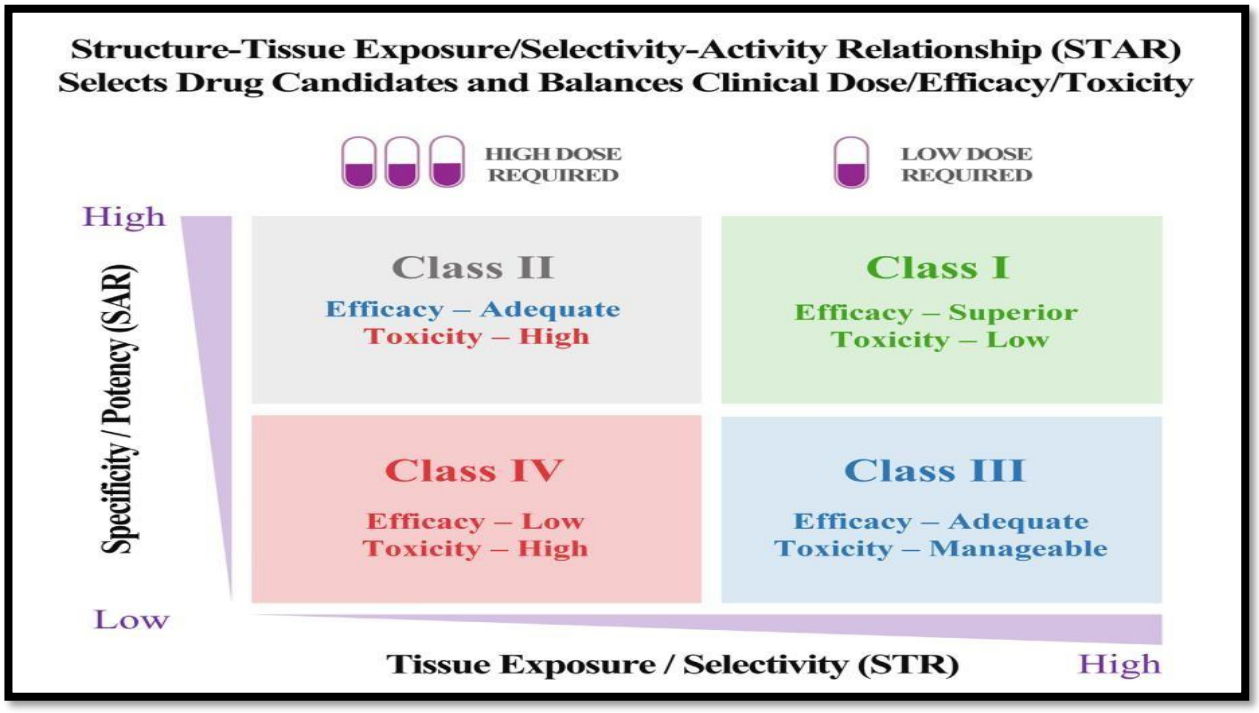

Figure 7: Structure–tissue selectivity/exposure-activity relationship (STAR) selects lead drug candidates and balances clinical dose/efficacy/toxicity to improve drug optimization for successful the clinical drug development process.

ii. Potential Novel Early Cancer Detecting/Monitoring Candidate Strategy

Multiple questions remain a mystery in cancer biology on how cancer initiates, develops, metastasizes, or recurs. Answering these questions may provide insights into developing the underlying pathomechanisms for treating cancer effectively. Early cancer detection contributes to identifying the presence of cancer and which specifically which form to aid in determining the most effective treatment for each patient. Nonetheless, most cancer detection methods aren't always suggestive for accurate diagnosis. Not all negative reports imply the absence of cancer, considering *"absence of evidence is not always evidence of absence."* [120] For instance, current multiple solid cancers detection is done through liquid biopsy, based on the presence of circulating tumor cells (CTCs) or clusters, or circulating tumor DNA (ctDNA). Though, the quantity of starting material is considered adequate when the tumor has grown beyond a certain size, and in some cases, would result in late-stage cancers. Circulating ensembles of tumor-associated cells (C- ETACs) detection method, on the other side, are heterotypic clusters encompassing tumor cells, immune cell, fibroblasts, and their presence, whether singly or in clusters, suggests the eloquence of malignancy. This urges the need of finding new detection methods since a screening test should be able to detect nearly all cancer types at early stages with equal sensitivity in detecting for a test to be effective.

Debate is still proceeding as to whether the somatic mutations theory (SMT), or tissue organized field theory (TOFT) is responsible for carcinogenesis [120]. SMT hypothesizes that successive genetic mutations in a single cell cause a clone of malignant cells and that quiescence is the default state of all cells in multicellular organisms [121 – 122], while TOFT proposes that cancer is tissue- based disease that proliferation is the default state of all the cells [122]. Interestingly, there are several existing imponderables as if cancer is initiated by somatic mutations or cancer stem cells (CSCs), if they exist. If yes, do they originate from current adult tissue-resident stem cells, or originate through reprogramming and de-differentiation of differentiated progenitor cells to a pluripotent, stem-like state [120]. Studies have claimed that cancer stem cells may not necessarily be rare or/and quiescent and may originate through somatic cells de-differentiation and reprogramming, which may indicate CSCs as a potential and more effective tracing material for detection [120], [123 – 125].

CSCs share many common characteristics with very small embryonic stem cells (VSELs), small stem cells with self-renewal properties which exist in all adult tissues. VSELs are developmentally correlated to primordial germ cells and serve as a backup reservoir that induce tissue-committed stem cells (TCSCs; also called progenitors), types of cells that are committed to forming specific cells from their original tissue, and cannot form other types of cells. Therefore, VSELs play an essential role in maintaining long-term homeostasis. Moreover, many malignant tumor express pluripotent markers linked to embryonic stem cells. Since VSELs express embryonic genes that are scattered in epithelial cells, this suggests that the growth of VSELs induce cancer (similar to CSCs) [120]. In fact, further studies suggest that several insults give rise to epigenetic changes and lead to the transformation of VSEL into CSCs, which grow and proliferate rapidly. Readers may refer to reviews providing evidence for the previous hypothesis, one of which that both CSCs and VSELs express similar cell surface markets entailing CD45-ve, CD133+ve, and LIN-ve in humans and SCA-1+ in mice [120], [126 – 127]. Further studies identified the role of the octamer-binding pluripotent marker in multiple types of cancer including breast

cancer. It was shown that OC-4 was responsible for tumor growth and chemotherapeutic resistance and other treatments in breast cancer CSCs [128 – 130], which should influence future researchers to provide stem cell-based targeted therapies in breast cancer since controlling OC-4 is a promising approach in for beneficial elimination and suppression of breast cancer stem cells. Henceforth, rather than CTCs or ctDNA, CSCs (epigenetically converted VSELs) better elucidate and answer the ambiguous questions in the field of cancer like tumor initiation, metastasize, and recurrence, as shown in **Figure 8** [120]. Future studies should focus on blocking CSCs because this is potentially the key to cure cancer.

Deepa Bhartiya et al. developed a method to enrich CSCs, VSELs, and impacted organs' progenitors from peripheral blood [120]. This strategy is based on stem/progenitor cells in a liquid biopsy: HrC (named after Himanshu Roy Cancer test), and AOB (All Organ Biopsy). HrC evaluates peripheral blood for markers specific for CSCs/VSELs, while AOB evaluates tissue committed progenitors during NGS studies (Next Generation Sequencing) for delineating changes in transcriptome and exome where the tissue provides information regarding impacted organs, type/subtype of cancer, driver/germline somatic mutations, and gene expression and dysregulated pathways. A published study used 100 clinical samples, where 500 were normal control and the other 500 were cancer samples [131]. Deepa Bhartiya et al. exploited HrC based on the aforementioned study, and the test enabled them to detect 25 types of cancers types comprising sarcomas, solid, and hematological cancers, with >99% accuracy. According to the results of the test and the study of common set of cancer-related biomarkers entailing epigenetic, pluripotent, proliferation, and tumor suppressor genes, a scale has been developed in order to classify whether a cancer presents, and its likelihood if the cancer is positive [120], as shown in **Table 1**.

This is considered an advantageous asset for fighting cancer. Accurate diagnosis will be given to patients for most types of cancers, which will offer describing the best personalized treatments , and; subsequently, appropriate and well-matched prognosis will be provided. Numerous hours and huge money will be saved, which will help both the health care institutions and patients considering that this will increase the available seats and number of treatments offered to patients as well as people will be informed of their diagnoses from a single test. IBC is not an exception since in about 1 of 3 cases IBC has already metastasized at the time of diagnosis [47]. This will aid in treating this aggressive form of breast cancer more successfully.

Possibility	Score	Implication
Absent	<2	Indicative of cancer absence
Low Likelihood of Cancer	2 – 6	Suggests a low likelihood of cancer
Moderate Likelihood of Cancer	6 – 10	Suggests a moderate risk of developing cancer
High Likelihood of Cancer	>10	Indicative of cancer presence

Table 1: Possibility-of-forming-cancer groups based on HrC done by Deepa Behartiya et al.

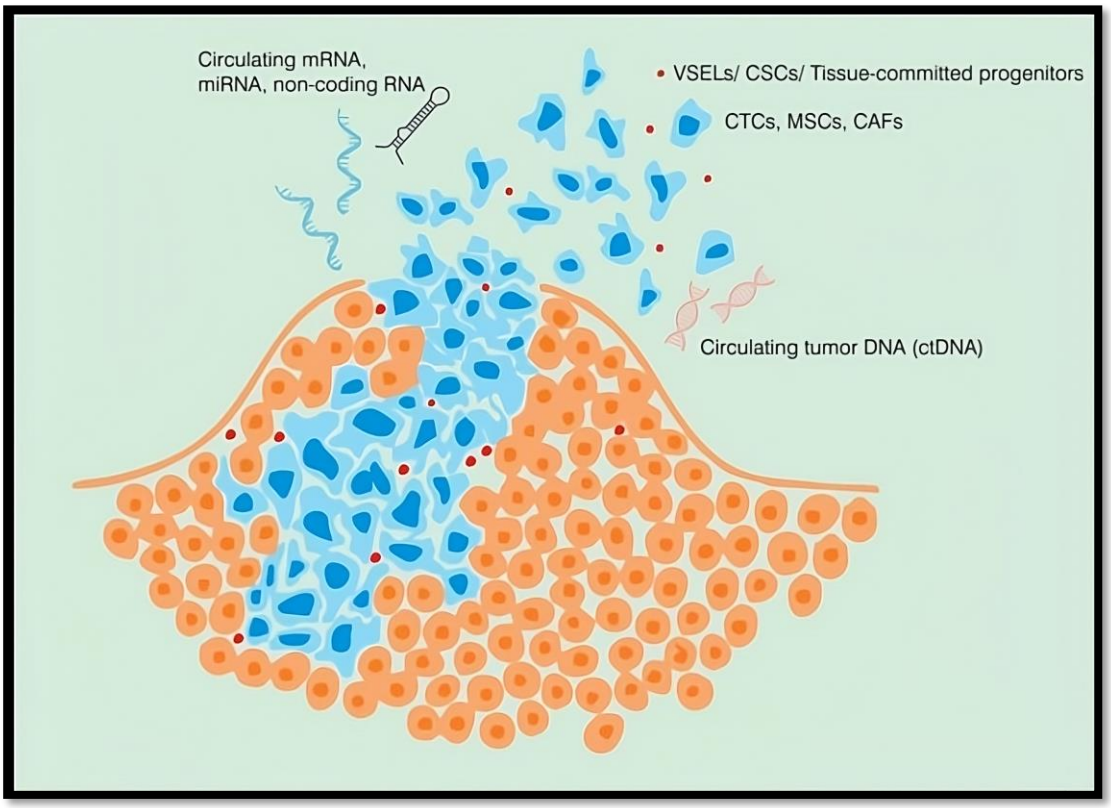

Figure 8: CSCs/VSELs are ideal candidates in blood for cancer detection.

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