Molecular Biotechnology (2021) 63:167–183
https://doi.org/10.1007/s12033-020-00296-2

REVIEW

Oncological Ligand-Target Binding Systems and Developmental Approaches for Cancer Theranostics

Jaison Jeevanandam[1] · Godfred Sabbih[2] · Kei X. Tan[3] · Michael K. Danquah[2]

Accepted: 21 December 2020 / Published online: 9 January 2021

Abstract

Targeted treatment of cancer hinges on the identification of specific intracellular molecular receptors on cancer cells to stimulate apoptosis for eventually inhibiting growth; the development of novel ligands to target biomarkers expressed by the cancer cells; and the creation of novel multifunctional carrier systems for targeted delivery of anticancer drugs to specific malignant sites. There are numerous receptors, antigens, and biomarkers that have been discovered as oncological targets (oncotargets) for cancer diagnosis and treatment applications. Oncotargets are critically important to navigate active anticancer drug ingredients to specific disease sites with no/minimal effect on surrounding normal cells. In silico techniques relating to genomics, proteomics, and bioinformatics have catalyzed the discovery of oncotargets for various cancer types. Effective oncotargeting requires high-affinity probes engineered for specific binding of receptors associated with the malignancy. Computational methods such as structural modeling and molecular dynamic (MD) simulations offer opportunities to structurally design novel ligands and optimize binding affinity for specific oncotargets. This article proposes a streamlined approach for the development of ligand-oncotarget bioaffinity systems via integrated structural modeling and MD simulations, making use of proteomics, genomic, and X-ray crystallographic resources, to support targeted diagnosis and treatment of cancers and tumors.

Keywords Oncotargets · Bioaffinity ligand · MD simulation · Cancer · Proteomics · Genomics

Introduction

Cancer is one of the most common non-communicable diseases among humans. It is characterized by an abnormal growth of cells, with ability to spread to other parts of the body [1]. The International Agency for Research on Cancer (IARC) reported about 18 million new cases of cancer comprising of 36 cancer types in 185 countries in 2018. Further, IARC reported about 9.6 million deaths due to cancer in 2018 globally [2]. Cancers can be categorized as carcinomas, lymphomas or leukemias, and sarcomas. Carcinomas are commonly associated with any type of epithelial cell of the skin or lining of organs, and about 90% of human malignancies are categorized as carcinomas [3]. Lymphomas and leukemias are malignancies of the immune cells and blood-forming cells, respectively. They constitute about 8% of human cancers. Sarcomas refer to any malignant tumor of connective tissues or non-epithelial cells, including bone, cartilage, fibrous, and muscle tissues. They account for less than 2% of reported cancer cases [4]. Cancers can also be further classified based on the affected cell type (e.g., erythroid leukemia, referring to red blood cell malignancy) or the affected tissues or organ (e.g., prostate and breast cancer). Although cancer continues to be a major global health issue that accounts for one in every seven deaths [5], there are only a few cancer types commonly diagnosed. Table 1 presents the ten most common cancers and their estimated cases of mortality in the USA in 2019. GLOBOCAN in 2018 reported that lung cancer is the most common cancer type, representing about 11.6% of global cancer cases. The mortality rate of lung cancer was reported to be the highest (18.4%), followed by colorectal

✉ Michael K. Danquah
michael-danquah@utc.edu

1 CQM—Centro de Química da Madeira, MMRG, Universidade da Madeira, Campus da Penteada, 9020-105 Funchal, Portugal

2 Chemical Engineering Department, University of Tennessee, Chattanooga, TN 37403, USA

3 School of Materials Science and Engineering, Nanyang Technological University, Singapore 639798, Singapore

Table 1 The ten most common cancers and their estimated cases of mortality in the USA in 2019

Type of cancer	Characteristics	Estimated mortality in 2019	References
Lung and bronchus	1. Lung and bronchogenic carcinoma are developed from malignant neoplasm which proliferate uncontrollably and irregularly from the atypical epithelial cells of bronchus or bronchiole. The neoplasm can spread into the bronchial wall or lumen and to other organs by invading the lymph nodes 2. Bronchogenic carcinomas include adenocarcinoma, squamous cell carcinoma, and bronchioloalveolar carcinoma	142,670	[26, 27]
Colon or rectum	1. Tumor formation starts from enhanced proliferation of colon epithelial cells and large-size benign adenomas. Consequently, tumor invasion occurs to the basal lamina, underlying connective tissues, other organs such as small intestine or bladder, and finally metastasizing to other body parts by invading blood and lymphatic vessels	51,020	[4]
Pancreas	1. Pancreatic cancers are divided into three types of non-invasive pancreatic neoplasia: mucinous cystic neoplasm, pancreatic intraepithelial neoplasia (the most common type in humans), and intraductal papillary mucinous neoplasm 2. Pancreatic cancers are mostly exocrine carcinomas such as heptoid, colloid, ductal, and acinar cell carcinoma. Ductal adenocarcinoma is the most prevalent type with moderately differentiated glandular structures and contributes to more than 80% of all pancreatic cancers 3. Pancreatic tumor can metastasize and spread rapidly to lymph nodes, liver, and peritoneal cavity	45,750	[28]
Breast	1. Breast cancers are either invasive or non-invasive. Non-invasive breast cancers do not invade away from the ducts or lobules where they are located. However, these 'in situ' breast cancers can proliferate and grow into invasive breast cancers. Non-invasive breast cancers include lobular carcinoma in site (LCIS) which grows into breast lobules; and ductal carcinoma in situ, which grows into breast duct and forms ductal co-medo-carcinoma 2. Invasive breast cancers include infiltrating lobular carcinoma, medullary carcinoma, tubular carcinoma, and infiltrating ductal carcinoma. Invasive breast cancers are developed when tumor cells proliferate from the ducts or lobules into close proximity of breast tissues and further invade to other body parts via systemic circulation or immune system	42,260	[29]
Urinary system (e.g., kidney, urinary bladder, ureter carcinomas)	1. Renal cell carcinoma of renal parenchyma is responsible for more than 90% of kidney cancers. There are diverse kinds of kidney cancers. These include collecting duct, papillary, oncocytoma, and medullary 2. Bladder cancer usually occurs via urothelial cell proliferation in the bladder lining. The tumor cells can also develop into the ureters and renal pelvis as upper tract bladder cancers 3. Chromosome 3p deletion and the inhibition of VHL tumor suppressor gene can lead to kidney cancer. The inhibition of tumor suppressor genes such as p53, PTEN, and Rb can result in bladder cancer	33,420	[30]
Liver and intrahepatic bile duct	1. The primary liver cancers are cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC). Malignant CCA is transformed from cholangiocytes, the epithelial lining of both intra- and extra- hepatic biliary epithelium. HCC is usually developed from fibrosis to cirrhosis and finally transformed into tumor cells. It is usually caused by hepatitis B and C viral infections as well as alcohol-related liver injury	31,780	[31]

Table 1 (continued)

Type of cancer	Characteristics	Estimated mortality in 2019	References
Prostate	1. Prostate cancer forms when prostate cells multiply uncontrollably in growth due to genetic mutation 2. Without proper diagnosis and treatment, prostate cancer can metastasize to other body regions such as the surrounding seminal vesicles and lymph nodes	31,620	[32]
Leukemia	1. Leukemia is a blood-related malignancy which comes from immature leukocytes such as myeloid and lymphoid. These abnormal blood cells grow rapidly and outnumber healthy blood cells due to genetic alterations and chromosomal abnormalities in lymphoid precursor cells 2. There are four types of leukemia depending on the malignant site (lymphoid or myeloid cells) and disease period. These are acute, chronic, lymphocytic and myelogenous	22,840	[33]
Lymphoma	1. Lymphomas include blood cancers originated from lymphocytes or cancer of the lymphatic system. There are two main types lymphoma; Hodgkin's and non-Hodgkin's lymphoma. Hodgkin's lymphoma is caused by malignant mature B cells whilst non-Hodgkin's lymphoma is caused by either B or T cells	20,970	[34]
Brain and nervous system (e.g., brain carcinoma)	1. Brain cancers originate from the central nervous system and remain as the primary malignant brain tumor 2. Malignant brain tumors can be categorized as either primary or secondary. Primary brain tumors originate from the brain and rarely invade to other body parts. Secondary brain tumors originate from other tumor cells, metastasizing from another body parts to the brain and surrounding nerve tissues	17,760	[35]

cancer (9.2%) and stomach/liver cancer (8.2%) [6]. Generally, the treatment of cancer is carried out by surgery to remove specific cancerous tissues [7], radiation therapy [8], and chemotherapy [9]. Surgeries are usually recommended if the spread of the cancer is rapid and difficult to control using conventional treatment methods [10]. Cancer relapse is associated with the presence of circulating tumor cells in cancer patients and this can result in unsuccessful surgery and patient mortality [11]. Chemotherapy is the most common cancer treatment method where chemical-based drugs at high concentrations are administered to inhibit rapidly growing cancer cells [12]. Even though chemotherapy has proven to be successful in retarding cancer cell growth, the lack of selectivity and specificity induce severe side effects through the inhibition of normal healthy cell growth [13]. General side effects of chemotherapy include hair loss, loss of appetite, diarrhea or constipation, nausea, fatigue, mouth sores, and skin problems [14]. Radiation therapy is introduced to overcome the limitations of chemotherapy. The treatment utilizes high intensity radiation, targeted at the cancer-affected site or organ, to inhibit the growth of cancer cells. However, radiation therapy can lead to alterations in the DNA of neighboring healthy cells and this may lead to side effects [15]. Hyperthermia, cryosurgery and magnetic therapy are some of the novel methods developed to address the challenges of conventional cancer treatment approaches [16–18] though issues relating to toxicity, inhibition of healthy cells and cancer relapse persists [19, 20]. Targeted treatment of cancer hinges on the identification of specific molecular receptors on cancer cells to inhibit growth; the development of novel ligands to target biomarkers expressed by the cancer cells; and the creation of novel multifunctional carrier systems for targeted delivery of anticancer drugs to specific malignant sites. There are numerous oncotargets that have been discovered as receptors or biomarkers for targeted delivery of anticancer drugs to control the growth of cancer cells [21]. For example, the secretion of oncotargets such as vascular endothelial growth factor (VEGF) and glutathione peroxidases has been exploited for targeted delivery of anticancer drugs to inhibit cancer cell growth and modulate metastasis [22, 23]. Genomics, proteomics, and bioinformatics are effective tools to identify specific cancer inhibiting molecular targets [24, 25]. Moreover, MD simulations offer opportunities to structurally design novel ligands and optimize binding affinity for specific oncotargets binding. This article discusses the development of ligand-oncotarget bioaffinity systems via integrated structural modeling and MD simulations, making use of proteomics, genomic, and X-ray crystallographic resources, to catalyze targeted cancer therapy.

Oncotargets for Different Cancer Types

The efficacy and specificity of cancer diagnosis and treatment since the disease occurs at the cellular or tissue level [36, 37]. For example, chronic blood cancer is a common tumor disease among the elderly and it is attributed to the switching or reciprocal translocation of genetic materials between chromosomes 9 and 22. There is a biomarker known as ph1, expressed by 95% of chronic blood cancer patients and synthesized from the combination of the gene Bcr and oncogene Abl as an activated protein kinase [38], which is effective for diagnosis. By targeting and inhibiting the ph1 biomarker, the balance of cell proliferation and apoptosis is interrupted, and this facilitates the inhibition of tumor growth.

Several studies [39–41] have reported the potential of hypermethylated promoters as novel biomarkers for all human cancer diagnosis, prognosis, and therapy owing to their presence in specific CpG regions that affect cancer induction. CpG promoter is an ideal biomarker as it influences all cellular pathways with a cancer-type specific profile and it is associated with genes such as tumor suppressor, microRNAs, and DNA repair genes with growth inhibitory effects [41]. The CpG islands of the CpG promoter of these genes hypermethylate if the cell is undergoing transformation, suggesting its suitability as a cancer biomarker.

A recent study by Whitwell et al. [42] has revealed the use of longitudinal multi-biomarker models to enhance the early diagnosis of ovarian cancer. The findings demonstrated the high sensitivity, 85.7%, and high specificity, 95.4%, of this model, which comprises of various overexpressed biomarkers, including CA125, HE4, CHI3L1, PEBP4, and/or AGR2 to target ovarian carcinoma. This provided a better detection of ovarian carcinoma at early-stage disease screening as compared to the conventional test that uses serum cancer antigen 125 (CA125) alone. The novel longitudinal model is capable of detecting cases of ovarian cancer, which were undiagnosable using CA125 test alone, showing its enhanced detection lead times and performance up to 1 year prior to clinical diagnosis. It was also demonstrated in another study [43] that combined biomarkers (both cancer CA125 and immune interleukin 7, IL-7 biomarkers) can perform better in the early detection of ovarian cancer with improved cancer detection times as shown in Fig. 1.

Bladder cancer is one of the most common cancers globally with high recurrence rates and, therefore, requires an effective biomarker for targeted treatment of malignant bladder tumor. It has been demonstrated that the competitive glucose metabolism can serve as a

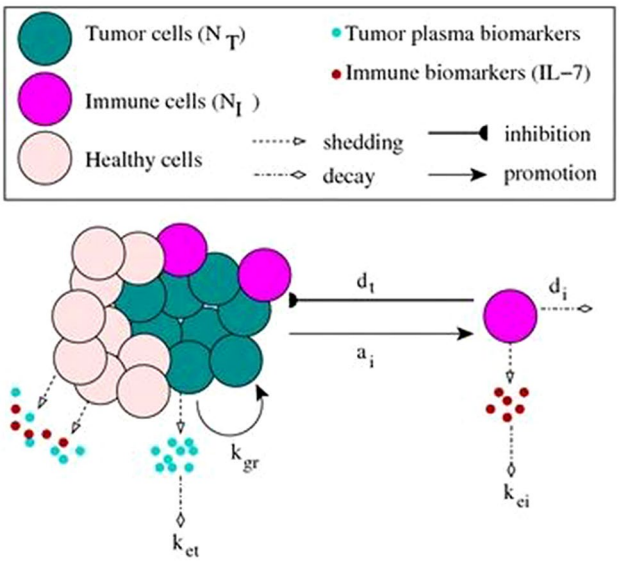

Fig. 1 Schematics of interactions between immune effector and tumor cells. Reproduced with permission from [43], under the terms of the Creative Commons Attribution 4.0 International License

specific target to kill tumor cells in addition to improving the immune system, especially the therapeutic efficacy of the immune checkpoint inhibitors (ICIs) in the case of metastatic bladder cancer [44]. This is attributed to the presence of overlapping metabolic phenotypes between the activated immune T cells and cancer cells, which leads to the metabolic competition that impacts immune performance. By targeting the glucose metabolism in tumor cells, especially bladder tumor cells, glycolysis-linked receptors can be effectively targeted and destroyed by various inhibitors of glucose metabolism [44]. This is probably due to the implication of both glycolytic signaling pathways and metabolites in the bladder tumor, offering abundant putative therapeutic targets for the anticancer strategy.

Different cancer cells overexpress their own unique biomarkers which are distinct from other cancer types due to varying genetic compositions. For example, the epidermal growth factor receptor (EGFR)/ERBB2 kinase is associated with breast carcinoma [45]; vascular epidermal growth factor receptor (VEGFR) kinase for renal carcinoma [46]; EGFR kinase and protein kinase ALK surface receptors are for non-small cell lung carcinoma (NSCLC) [47]; and BRAF kinase from melanoma [48]. Some other reported specific biomarkers or extracellular surface receptors of different tumor cells are provided in Table 2. The presence of these surface biomarkers helps increase the targeting specificity for diagnosis as well as boost drug therapeutic indices by distinguishing between healthy and malignant cells for enhanced targeted treatment strategies.

Table 2 Examples of some overexpressed biomarkers of various cancer cells

Targeted biomarker	Type of cancer cells	References
Protein tyrosine kinase 7 (PTK 7)	Colon, lung, esophageal, and gastric carcinoma	[49, 50]
Prostate specific membrane antigen (PSMA)	Kidney and prostate carcinoma	[51, 52]
Nucleolin	Breast, lung and gastric carcinoma; leukemia; melanomas	[53, 54]
Epithelial cell adhesion molecule (EpCAM)	Breast, ovarian, pancreas, hepatocellular, and bladder carcinoma	[55, 56]
T cell immunoreceptor with Ig and ITIM domains (TIGIT)	Melanoma, acute myeloid leukemia, glioblastoma	[57]
NK cell frequency	Colorectal and prostate cancer, and melanoma	[58]

Advanced Methods of Oncotarget Discovery

With advancements in both molecular and bioinformatics techniques, more gene data can be rapidly generated to probe genetic disorders, particularly the role of mutated genes in specific cancer cell formation and growth. This is crucial to identify novel tumor biomarkers for early cancer diagnosis and to deliver appropriate therapies. There are other computational approaches such as ligand–protein docking used to generate novel ligands for specific target binding as well as analysis of the binding characteristics to develop promising drug candidates for targeted delivery. Conventional methods often require pre-existing information about the protein sequences and structures and this creates significant challenges due to the similarities among protein structures [59]. Therefore, advanced technologies in detecting gene transcript diversity and the impact of individual protein isoforms of drug effects are important in the design and development of targeted therapies. Thomas et al. [60] described the efficacy of high-throughput genotyping in querying 238 known oncogenes via a powerful mutation distribution spanning 17 types of cancer across a thousand of human tumor samples. The findings indicated a new advancement in tumor genetics whereby multiple mutations associated with various tumor genes can be interrogated simultaneously on a large-scale mutation profiling in a 'real-time' scenario to detect previously unknown oncogene mutations as well as formulating cancer classification and cancer therapeutic interventions.

Intratumor heterogeneity plays a vital role in the discovery of cancer biomarkers and clinical treatments, especially for targeted therapies. Intra-tumor heterogeneity is described as solid tumors consisting of cells with distinct genomic alterations within the same tumor. With the advent of sequencing technologies such as next-generation sequencing (NGS) technique, certain features of intratumor heterogeneity have been used in routine pathologic evaluation to optimize therapeutic outcomes and patient care. For instance, Ding et al. [61] have sequenced the primary tumor and relapse genomes of several patients with acute myeloid leukemia and illustrated that the presence of a founding clone in the primary tumor has evolved in the relapsed tumor. By identifying the distinct genomic architectures and tumor evolution within multiple patients at different cancer stages (from primary to metastatic tumor and during therapy), it is possible to tackle the mechanism of tumor recurrence for a better targeted therapy [36]. Also, a more tractable method and robust biomarkers such as the 'actionable mutations' which are ubiquitous and dominant driver events in all cancer sites can be developed for therapeutic targeting applications, based on the trunk-branch model [62]. This is useful, particularly for tumors with lesser branched events.

'Cell competition' has been reported to have strength in triggering cancer cell killing and it can possibly be modified to develop novel anticancer treatments with tumor-suppressive behaviors [63]. Cell competition is a type of cell-to-cell communication whereby competent cells are capable of eliminating neighboring cells within the microenvironment in order to survive, expand, colonize, and grow into targeted tissues. Many reported studies have demonstrated that gene mutation in cell signaling, cell growth, and endocytosis can induce cell competition whereby wild-type cells behave as winners against mutant cells to prevent tumor growth [64–66]. Cell competition occurs via several mechanisms, including mechanical interactions and molecular exchange. Mechanical interaction is utilized to remove cells with a higher crowding sensitivity due to enhanced p53 activation and subsequent apoptosis [67, 68]. On the other hand, molecular exchange is based on the capability of cells in capturing and sequestering growth factors such as Wnt ligands [69] or TGFβ [70], in order to activate the death-triggering pathways in cells receiving lower signals. Studies by Rhiner, Lopez-Gay, Soldini, Casas-Tinto, Martin, Lombardia, and Moreno [71] and Madan, Pelham, Nagane, Parker, Canas-Marques, Fazio, Shaik, Yuan, Henriques, Galzerano, Yamashita, Pinto, Palma, Camacho, Vieira, Soldini, Nakshatri, Post, Rhiner, Yamashita, Accardi, Hansen, Carvalho, Beltran, Kuppusamy, Gogna, and Moreno [72] showed a correlation between the Flower isoforms expression in *Drosophila* and the human cancer progression by investigating the human Flower protein expression and mouse cancer models. This further supports the promising

potential of cell competition as a target to develop novel anticancer approaches.

In addition, a network-based method is emerging due to its effectiveness in discovering cancer drug target at the isoform level as shown in Fig. 2. Ma et al. [73] reported credible detection of target genes at the isoform level by integrating the isoform co-expression (IIC) networks with perturbed genes. This cancer-type-specific IIC network was built according to the isoform expression data integrated from both gCSI and CCLE datasets. Perturbed isoforms of target genes were selected based on the response of the corresponding genes to the applied drug. Further examinations via docking tests and comparison of proteomics data and expression status were carried out to determine the major protein isoform drug targets. This method is able to discover targets for cancer drugs as well as improve the drug targeting capability. The study also illustrated that major isoforms of targets can be identified to study the mechanism of action of the drug. Besides that, tumor classifications based on the evolution of different tumors using evolutionary index (Evo-index) and ecological index (Eco-index) provide promising implications in cancer target discovery [74].

Furthermore, mathematical modeling is often employed together with computational methods to investigate the specific targets of different cancer cells. These methods can be used to study the biological mechanisms and interactions between tumor cells and complex immune systems as well as the effects on tumor cell biomarker expression [76–78]. As a result, numerous stochastic biomarker-network models have been developed and reported for the diagnosis of diverse cancer cells [79–81]. These networks are built up of a library of cancer-associated proteins and other protein types and are exploited to determine the most specific targets or biomarkers for cancer detection.

Multi-region exome sequencing (Mseq) is another promising approach used to examine therapeutic targets by studying the cancer evolution based on multiple mutational profiles of spatially different regions of a tumor. Loga et al. [82] reported the application of MSeq in identifying the extreme intra-tumor heterogeneity and evolution of mismatch repair deficiency (dMMR) gastro-oesophageal cancer. The evolution analysis and MSeq data revealed the promising potential of MSeq in the discovery of biomarkers for highly heterogenous cancers. MSeq is capable of estimating the truncal mutation loads more accurately and robustly to prevent sampling biases and illusion of clonality of driver mutations in order to develop effective immunotherapy biomarkers such as 'mutation burden' [82]. In short, advanced technologies in understanding the cellular and genetic characteristics of biological systems are a promising development in detecting powerful tumor biomarkers or therapeutic targets to boost the cancer target discovery, diagnosis, and therapy.

Role of Genomics, Proteomics, and Bioinformatics

The central theme of cancer medicine is prevention, detection, and treatment; and for these goals to be achieved, a clear understanding of the genomic and proteomic influences at the molecular level is critical. Such a knowledge is required to develop solutions in the treatment of cancer, especially in the areas of early detection and personal medicine [83, 84]. Oncotargets are an essential part of this understanding and genomics play a crucial role in their discovery, to revolutionize biomedical research with the promise of early diagnosis of cancer, identification of cancer risk, selection of required treatment therapy, and the detection of relapse as shown in Fig. 3 [85]. Two major categories of genomic oncotargets are prevalent: DNA and RNA biomarkers. DNA biomarkers include the measurement of serum DNA [sDNA] concentrations as an indication of the presence of cancer, and this can be used in the process of staging during detection and treatment of cancer. Researchers have demonstrated the feasibility of using serum DNA concentrations as biomarkers for cancer detection. A study conducted

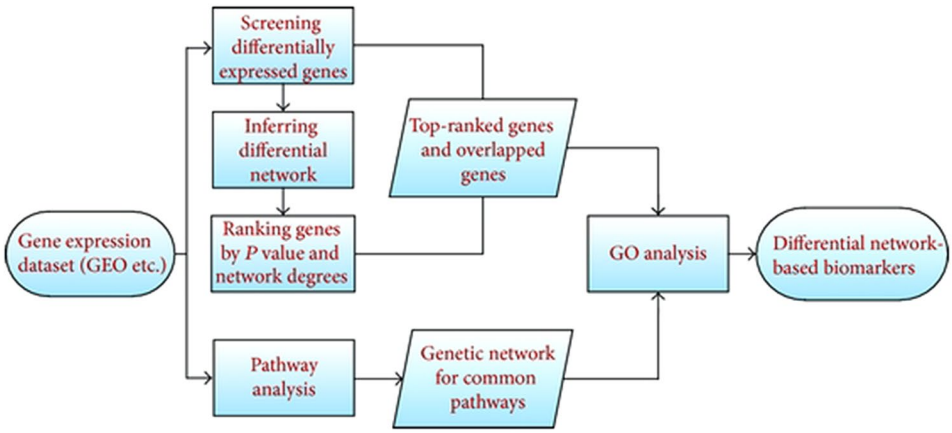

Fig. 2 Network-based method for the identification of cancer biomarkers. Reproduced with permission from ©Yang et al. [75], under the Creative Commons Attribution License.

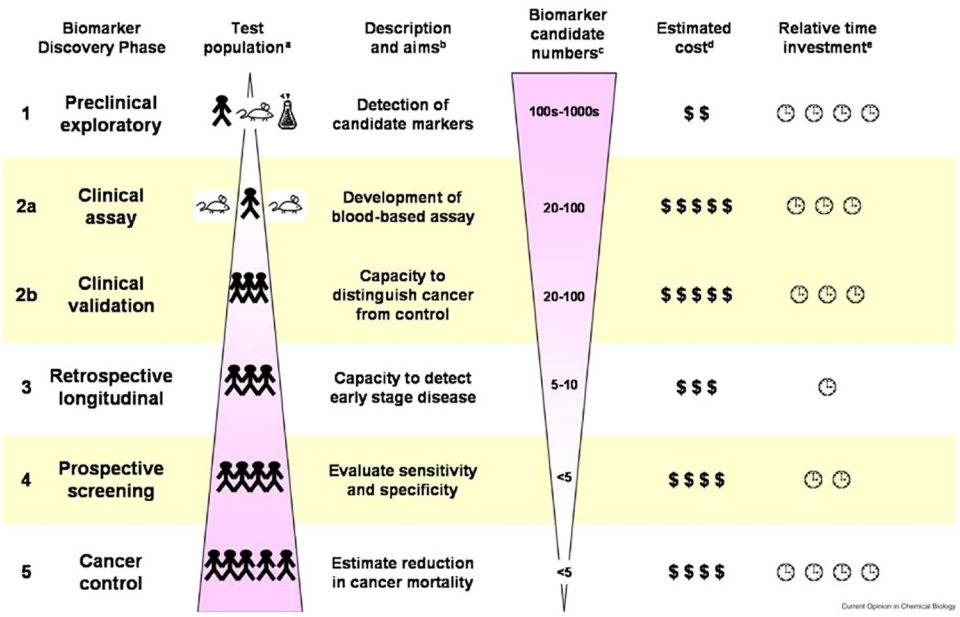

Fig. 3 Pipeline for the validation and discovery of biomarker candidates for disease diagnosis. Reproduced with permission from [85], ©Elsevier, 2008

by Gal et al. [86] showed a four-fold difference in the median levels of circulating sDNA between breast cancer patients and healthy controls (medians of 221 and 63 $ngml^{-1}$, respectively) using a real-time polymerase chain reaction (PCR) quantitative method [86]. The researchers found that 72% of patients with breast cancer (69 out of 96) had DNA concentrations of more than 100 $ngml^{-1}$, while only 12.5% of the control group had the same amount of sDNA concentration (3 out of 24). Again, using a receptor operating characteristic (ROC) curve analysis, the researchers demonstrated that there was a strong relationship between the DNA concentration and malignancy by observing an area of 0.92 under the ROC curve. Another group of researchers, Sozzi and coworkers, performed a similar experiment in lung cancer patients and discovered a similar correlation of circulating plasma DNA and cancer by using a ROC curve [87]. They obtained their results by comparing plasma DNA extracted from 84 patients with radically resected primary non-small cell lung cancer (NSCLC) (stages I–III) and that of 43 healthy blood donors as control.

Apart from circulating DNA in plasma/serum being used as biomarkers, mutations in oncogenes, tumor suppressor genes, and mismatch repair genes can also serve as DNA biomarkers [85]. This is made possible by the discovery of the HRAS gene, a gene that causes a glycine-to-valine mutation at codon 12, and subsequent identification of similar mutations in the family members of the KRAS and NRAS genes. Other genomic biomarkers have also been discovered. The RAS family encodes small proteins with enzymatic GTPase activities that couple external growth signals governing cell proliferation, and thus, a mutation in the RAS gene encodes a protein with normal guanosine triphosphate (GTP] binding, but no GTPase activity, causing the resulting RAS protein to be activated all the time as seen, leading to unregulated cell division [84, 88]. It is worth noting that the gain-of-function mutations of the RAS oncogene occur in approximately 30% of all human cancers and its isoform. The KRAS gene is the most studied and most frequently mutated oncogene and constitutes 86% of RAS mutations [89]. Recent studies have shown strong indications that the KRAS gene (as a cancer biomarker) and its expressed KRAS protein are potential targets for drug discovery and development for KRAS-targeted therapies [90]. Mutations can also occur in the tumor suppressor genes of cell to result in cancer. The detection of this defect can serve as an oncotarget. The pRb, p53, and p21 genes that make up the family of tumor suppressor genes encode proteins that restrain cell division. A defect in the RB1 gene (the gene that encodes pRb), for example, causes retinoblastoma in children and results in blindness if not removed.

RNA biomarkers have also gained traction in recent times, especially in the discovery of the functional and expression roles of piwiRNA (piRNA), messenger RNA (mRNA), small nucleolar RNA (snoRNA), long non-coding RNAs (lncRNAs), circular RNA (circRNA), and microRNAs (miRNAs), that can regulate gene expression by various mechanisms thus presenting a great potential for the development of diagnosis and therapeutic measures for several types of cancers [91–93]. For instance, miRNA in particular has been demonstrated as a biomarker for cancer, either causing malignancy in certain types of cancer or acting as a tumor suppressor in others. A study conducted by Akao et al. [94] found a significantly reduced expression of miRNA-143 and miRNA-145 in colon cancer cells and that by transfecting each precursor

miRNA into the cells, a notable growth inhibition of human colon cancer DLD-1 and SW480 cells was observed, indicating that the miRNA-145 acts as a tumor suppressor [94]. Furthermore, another supporting study published by Link et al. [95] found that miR-21 expression was higher in stool samples from patients with colorectal neoplasia than those with normal colonoscopies, demonstrating the use of miR-21 as an oncotarget [95]. These studies demonstrate the great role that genomics play or will play in the future of cancer classification, early detection, prognosis prediction, and therapeutic decision-making.

Cancer detection, diagnosis, treatment, monitoring, and prognosis have been bolstered by protein biomarkers under the broad area of proteomics. The underlying theme of oncological proteomics relates to understanding distinct changes that occur during neoplasm and the available technologies for probing these changes. The prospects of this can be seen with the many approvals obtained from the US Food and Drugs Administration for the use of single proteins as cancer biomarkers. For instance, the human epidermal growth factor receptor-2 HER2/NEU, EGFR, and KIT are used clinically to determine whether or not breast cancers, colon cancers, or gastrointestinal stromal tumors would respond to trastuzumab, cetuximab, or imatinib, respectively, as membrane protein-targeting drugs. Similarly, breast cancer cells that are ER positive or PR positive inform clinicians which hormonal therapy will be more effective for treatment [83, 96, 97]. Identification of protein biomarkers is done by techniques and assays including differential in-gel electrophoresis (DIGE), two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), multi-dimensional protein identification technology (MudPIT), reverse phase micro arrays, mass spectrometry, and field effect transistors (FET) that are coupled with molecular probes. FET is a promising technique as it increases protein marker sensitivity to several orders of magnitude [83]. FET-based protein detection, for example, has been demonstrated by Ohno et al. [98] where they immobilized immunoglobulin E (IgE] aptamers onto a graphene surface FET and showed selective electrical detection of IgE protein [98]. Such a technique will play a huge role toward rapid detection of protein oncotargets.

Bioinformatic tools are essential to process and extract meaningful information from the large proteomic and genomic data. It is particularly useful for the discovery of sensitive and specific biomarkers in cancer research [96]. The importance of bioinformatics in understanding biological macromolecules and their relation to the activity of diseases, especially cancer, cannot be underrated. Public genomic datasets like GEO (https://www.ncbi.nlm.nih.gov/geo/) provide large micro-array data of complementary DNA that has been used by researcher to discover new biomarker for diseases. For instance, micro-array datasets GSE19665, GSE33006, and GSE41804 from GEO databases were used by Li et al. [99] together with the open source bioinformatics tools STRING and Cytoscape to identify a total of 273 deferentially expressed genes (DEGs) and 16 hub genes involved in the carcinogenesis of hepatocellular carcinoma (HCC) and have been used as potential biomarkers for diagnosis and treatment [99]. Finally, apart from proteomics, which is well researched in the development of cancer drugs targeting expressed proteins, genomics research and applications in cancer therapy has a number of key challenges, both scientific and non-scientific, that have to be addressed in order to fully exploit the knowledge of cancer genomics to develop targeted therapeutics and informative biomarkers. The challenges mostly relate to logistical and regulatory issues associated with data acquisition and privacy as well as scientific challenges, such as the incorporation of early preneoplastic or intra-tumoral heterogeneity. The genomic data sharing policy of the National Institute of Health (NIH), for example, requires that publishers only make genomic data available after a period of time post publication or after an embargo period has been expended [84]. These policies, particularly, become bottlenecks in fields, including pediatric cancer research, that deal with rare tumor types and require a quick flow of available data to increase understanding of the disease characteristics and create new and improved theranostic strategies. Furthermore, it has been shown in recent times that genomic data can raise serious privacy concerns among individuals [100–102].

Role of Molecular Dynamics Simulation and Experimental Validation

Molecular dynamics (MD) simulations are well-established techniques for investigating the time-resolved motions of biological macromolecules at the atomic level. It provides information about the characteristics, stability, and binding features through fluctuations in the relative positions of atoms in protein and DNA macromolecules as a function of time [103, 104]. An understanding of these molecular motions is important in the area of drug discovery and provides the foundation for experimental validations, helping to refine experimentally determined structures of macromolecules (for instance, biomarkers) and performing conformational analysis and protein homology modeling as well as docking of biomolecular complexes.

MD simulations initially start with an understanding of the biological system followed by the acquisition of structural information from X-ray crystallographic or nuclear magnetic resonance (NMR) spectroscopy. This structural information contains all the possible atoms and their initial spatial coordinates. Publicly available databases such as the protein databank (RCSB PDB—with about 159,230 submitted structures as of January 2020), which collect

structures from X-ray crystallography and NMR spectroscopy, are the usual sources for such initial structures [105, 106]. The availability of these structures plays a major role in the modeling of numerous oncotargets, existing ligands, or druggable molecules. Force fields are used to calculate the accelerations of atoms, new coordinates, and velocities at each time-step of the simulation using special software, such as NAMD [107], CHARMM [108], AMBER [109], and GROMACS [110]. Rampogu et al. [111] applied molecular docking and molecular dynamics techniques to obtain a potential ligand that can repress cancer angiogenesis and growth [111]. The study demonstrated the role of MD simulations in predicting the behavior of macromolecules under various conditions in silico. We have recently proposed that aptamers can be a potential oncotargeting molecular probe for targeted inhibition of specific cancer cells [112]. The article also recommended that MD simulations can play a significant role in designing aptamers as probes to specifically target cancer cells for improved treatment and reduced side-effect to the normal healthy cells. Danquah et al. [113] proposed an integrated structural MD simulation approach to design RNA and DNA aptamers for targeting the outer membrane domain of CD19 protein as a biomarker for acute lymphoblastic leukemia [113]. Several other applications of MD simulations have been demonstrated by researchers. For instance, Kumar et al. [114] used MD simulations to detect the phenotypic characteristics of Single Nucleotide Polymorphism. The authors used a long time scale simulation of 200 ns to study the stability of a mutated Aurora-kinase protein and demonstrated the atomic alterations relating to the mutant protein in comparison with the native form using several assessment techniques including RMSD and RMSF that show unique structural conformations and adaptations associated with the expression of cancer. MD simulations have been used to refine top hit molecularly docked protein–protein or protein–ligand complexes in cancer research. Weako et al. [115] successfully examined the stability and efficacy of the unbound form of MetAP2 (an isoform of Methionine Aminopeptidases that is upregulated in various cancers), its complexes with fumagillin, spiroepoxytriazole, and the two best promising compounds (*N*-(4-[2-(2,5-Dimethylbenzyl)-1-pyrrolidinyl]sulfonyl phenyl and 1-(4-Benzyl-1-piperazinyl)-2-[3-(2-methylphenyl)-1,2,4-oxadiazol-5-yl] ethanone) obtained from molecular docking with compounds from the non-academic version of the OTAVA Chemical Library (https://www.otavachemicals.com). Further, Reddy et al. [116] also used MD simulation to explore the stability of gefitnib (a highly effective kinase inhibitor) and its derivative with Epidermal Growth Factor Receptor (EGFR) after docking in AutoVina [117] and found the best protein–ligand hits based on the highest binding affinity. MD simulations are a highly beneficial tool in drug discovery processes for identifying novel drugs for the treatment of non-small cell lung cancer.

However, it should be noted that computational simulations do not displace experimental methods as both are relevant to realize the full potential of MD simulations in the discovery of cancer oncotargets. Techniques such as surface plasmon resonance (SPR] analysis and circular dichroism spectroscopy are well suited to probe target binding relationships and thus serve as indispensable tools to verify computational results [118]. For instance, Suenaga et al. [119] used SPR analysis to verify the binding free energies of the interaction between the SH2 domain of the growth factor receptor-binding protein 2 (Grb2) and ErbB receptor-derived phosphotyrosyl peptides after performing MD simulations in Amber [120]. The comparative analysis indicated that the computationally estimated binding free energies were larger than that of the experimental data; however, both were highly consistent. Further analysis indicated that the dipartites in the data were as a result of the short simulation timescale of 1 ns and hence can be resolved by performing MD simulations at a higher timescale. Furthermore, Amiri et al. [121] also used various spectroscopic and electrochemical methods in addition to MD simulations to investigate the interaction between three oxovanadium (IV) Schiff base complexes (anticancer drugs) with bovine serum albumin (BSA). Experimentally, the authors used UV–visible absorption spectroscopy, fluorescence quenching, and circular dichroism to study the molecular interaction of the complexes and compared it to the computational data. They observed a good agreement between the experimental and computational results.

Latest Trends in Targeted Cancer Treatments

Currently, there are a significant number of cancer treatment technologies that are under extensive preclinical research or in transition states to clinical studies. Nanomedicines and genetic modification methods are widely employed in lab-based research that targets cancer cells and inhibits their growth. Rao et al. [122] reported that nanosized hyaluronic acid can be a potential therapeutic agent carrier for targeted tumor treatment. The efficient receptor-binding features and the biodegradable and biocompatible properties of hyaluronic acid conjugates make them effective carriers for controlling delivery of drug to target cancer cells. For example, biodegradable hyaluronic acid conjugates have been demonstrated to possess the ability to deliver small interfering ribonucleic acid to targeted cancer cells for treatment [122]. Plant indole-3-acetic acid (IAA) hormone activated horseradish peroxidase (HRP) enzymes are used for in vitro anticancer

agents. However, utilizing these enzymes in direct prodrug therapy has led to adverse effects in animal models. The plant enzymes are noticed to contain isoenzyme mixtures with a heterogeneous pattern of glycosylation that are not compatible with human systems. Bonifert et al. [123]. demonstrated that the recombinant variants of HRP can be a potential anticancer drug for targeted treatment of cancer complications. Two HRP isoenzymes C1A and A2A were recombinantly produced in *Pichia pastoris* strain after α-1, 6-mannosyltransferase (OCH1) was knocked out to limit hypermannosylation, producing a glycol-engineered strain. The results revealed that the recombinant isoenzyme HRP C1A is a promising targeted cancer treatment agent [123]. Dong et al. [124] showed that hydrophobic metal–organic framework can be a significant nanoplatform for targeted delivery of anticancer drugs. The authors introduced a novel strategy to fabricate camptothecin@zeolitic imidazolate framework-8 that is modified with Arginine-glycine-aspartic acid (RGD) as a hydrophobic nanoplatform-based drug delivery system. The results showed that the metal–organic nanoplatform framework possesses an excellent pH-mediated controlled and targeted delivery properties and improves the intracellular generation of reactive oxygen species (ROS) to inhibit cancer growth [124]. Gavande et al. (2016) discussed the potential of DNA repair targeted therapy as a next-generation cancer treatment method. It is worthy to note that cancer cells have the ability to repair DNA damages that are induced by therapies, affecting the efficacy of treatment modalities. Thus, the development of anticancer agents to target pathways of DNA and protein repair is useful for targeted and efficient cancer treatment compared to conventional chemotherapy [125]. Wang and Mooney [126] recently described biomaterial-assisted immune cell modulation as a potential targeted cancer treatment method. They reported that dendritic cells, tumor-associated macrophages, B cells, T cells, myeloid-derived suppressor cells and natural killer cells can be targeted and modulated using immuno-materials as a cell-targeted immunomodulated treatment strategy. These biomaterial-based targeted cancer treatment method can address several existing treatment limitations, including side effects and reduced patient response and are a promising replacement for chimeric antigen receptor-T cell therapy and immune checkpoint blockade therapy [126]. Even though, nanomedicines and genetic modification methods are proposed to be promising, there are some limitations. The lack of risk assessment procedures, and in vivo studies showing alterations in the anticancer ability and potential toxicity-based side effects are some of the challenges hindering the transition of these treatment approaches [127, 128]. The combinations of the latest, a novel and advanced aptamers and CRISPR technologies along with pre-existing methods may improve the cancer targeting ability and address some of the existing limitations.

There are various novel oncotargets and methods that are under preclinical and clinical trial to demonstrate efficacy for targeted cancer treatment applications. Vassilev et al. [129] validated the toxicity and biodistribution of chimeric oncolytic adenovirus named ONCOS-102 to obtain a regulatory approval for Phase I clinical studies. The toxicology and biodistribution profile were evaluated using 300 hamsters and revealed no adverse effects on body weight, hematology, food consumption, histopathology, clinical chemistry parameters and bio-accumulation even after repeated administration of ONCOS. The study confirmed that ONCOS-102 is highly safe with enhanced double targeting and granulocyte–macrophage colony-stimulating factor (GM-CSF) expressing property for advanced NCT01598129 cancer-type therapy [129]. Massacesi et al. [130] showed that phosphoinositide 3-kinase (PI3K) inhibitor, which inhibits the PI3K enzyme in PI3K/protein kinase B (Akt)/mTOR pathway, responsible for cell cycle regulation, can be a novel targeted anticancer agent. These inhibitors have been formulated into pan-PI3K buparlisib inhibitor (BKM120) and PI3Kα-selective alpelisib inhibitor (BYL719) by Novartis Oncology as a superior anticancer agent [130]. The article reported some glitches in the clinical development of PI3K inhibitors and the challenges in overcoming target enrichment and stratification based on the activation state of PI3K pathway [130]. Mirzaei et al. [131] discussed the potential of boron neutron capture therapy as an important targeted cancer treatment modality. This therapy is carried out by irradiating a stable boron-10 isotope with low thermal energy neutrons to obtain stripped nuclei of lithium-7 and helium-4, which eventually causes toxic reactions in cancer cells. The clinical and preclinical studies of this therapy showed efficacy in curing head, neck, thyroid cancer, melanoma and brain tumors [131]. Furthermore, polyethylene glycol (PEG) formulated anticancer drugs named as PEGylated advanced drug delivery systems are also under clinical development for effective targeted cancer treatment. The retention time enhancement property of PEGylation protects therapeutic agents such as enzymes, liposomes, small molecular drugs, proteins and nanoparticles from degradation in biological fluids. Additionally, PEGylation is proven to alter the pharmacokinetics of anticancer drug to enable adjustment to target site pathophysiological environment without any toxic reactions [132]. Li et al. [133] revealed via various cell and animal studies that combinations of the cancer stem cell targeting agents and chemotherapy are highly beneficial targeted cancer treatments. These targeting agents, along with chemotherapy, possess the ability to block signaling pathways of self-renewal, reduce drug and ATP-binding cassette transporter efflux expression, promote cancerous stem cell differentiation and modulate epigenetic aberrations.

Improvements in the pharmacokinetics, efficacy and safety of combinatorial therapy are the objectives of ongoing research in this area [133]. Belfiore et al. [134] discussed that ligand-functionalized liposomes can be used as a targeted cancer agent. It is noteworthy that ligand-directed liposomes can be engineered to possess active targeting ability toward cell receptors of the tumor cell surface, facilitating uptake of anticancer drugs into the tumor or tumor-associated stromal cells with enhanced selectivity [134]. However, translation from preclinical to clinical studies faces several hurdles, including cytotoxicity, ineffective characterization of active anticancer drugs, and the nonexistence of in vivo preclinical tumor models to evaluate the performance of anticancer drugs.

In spite of various hurdles from lab to preclinical and clinical studies, there are few targeted cancer treatments and prevention methods that are currently available on the market. Cancer vaccines such as T cell vaccines (TPIV200) from Marker Therapeutics, Inc. are currently available on the market to prevent ovarian and breast cancers. This vaccine consists of five peptide antigens of folate receptor alpha (FRα), which is overexpressed in the surface of ovarian and triple-negative breast cancer cells [135]. Similarly, Panacea Pharmaceuticals and 3 M Drug Delivery Systems recently developed a cancer vaccine, which can prevent cancers via the hollow microstructure transdermal system. This microstructure system is proven to possess enhanced ability to deliver cancer vaccines into highly vascularized dermis with enhanced reproducibility of direct delivery [136]. Hill et al. [137] reported that imatinib, lapatinib, erlotinib and sorafenib are the four types of tyrosine kinase inhibitors available on the market for the targeted treatment of cancer. However, economic barriers affect the mass production of these drugs [137]. Moreover, antibody–drug conjugates such as Polatuzumab Vedotin-piiq, Brentuximab Vedotin, Ado-trastuzumab emtansine, Inotuzumab ozogamicin, and Gemtuzumab ozogamicin are approved by FDA and are available on the market for targeted inhibition of cancer cells [138]. Additionally, Herceptin® for targeting human epidermal growth factor receptor-2 protein for breast and stomach cancer treatment [139], Zelboraf® for targeting BRAF protein for metastatic melanoma treatment [140], and Gleevec® for targeting BCR-ABL protein for promoting leukemia cell growth [141] are anticancer drugs that are currently available on the market for effective targeted cancer treatment.

Future Outlook

It is speculated that the combination of genomic, proteomics, bioinformatics, and MD simulations, along with advancements in the field of nanomedicine and genetic engineering, will be beneficial in developing the next-generation targeted treatment modalities for cancer [142]. Bioinformatic tools are currently used to probe the genomics as well as proteomics of cancer patients and will be essential in designing and evaluating the molecular efficiency of patient-specific targeted treatment strategies. The combinatorial efforts of bioinformatics and MD simulations form a novel computational oncological field, which reduces the number of experiments and ease the optimization process via in silico approaches [143, 144]. The development in these computational oncological approaches has led to the emergence of machine learning methods to facilitate precise and controlled delivery of cancer drugs at the target site. Ding et al. [145] suggested that the combination of omics data and machine learning approaches can be useful in precision oncology to identify specific tumor-treating drugs and prescribe optimal regimens for clinical treatment. They also reported that machine or deep learning approaches are beneficial in extracting genomic information and train classifiers to predict optimal cancer drugs for improved therapy [145]. Bibault et al. [146] recommended that big data and machine learning will be beneficial in combining electronic health record and genomic phenotypic profiles to generate high-quality precision medicine in radiation oncology [146]. Similarly, Majumder et al. [147] showed that machine learning methods can help in the chemical screening of cancer-cell-specific lethality via a network of degrading specific protein target. In addition, they discussed that machine learning-based computational chemical screening approaches are useful in recognizing pathways of protein-targeted networks, the anticancer potency of specific compounds with enhanced cell-specific activity, and drug combinations for targeted inter- or intra-type heterogenous cancer treatment [147].

The introduction of nanotechnology-based approaches has shifted the conventional therapeutic approaches of cancer into the molecular and genetic level, targeting cancer cells without altering the metabolism of normal healthy cells. Nano-aptameric sensors have been developed for early and rapid diagnosis of cancer [148, 149]. Dehghani et al. [150] discussed the ability of aptamer-based biosensors and nanosensors in monitoring vascular endothelial growth factor (VEGF) for the effective cancer diagnosis [150]. Further, Hao et al. [151] showed that aptameric graphene-based nanosensors are beneficial for high-sensitivity detection of biomarkers of lung cancer with improved stability [151]. Liu et al. [152] revealed that multifunctional aptamer-based nanoparticles are highly potent for targeted delivery of cancer drugs against circumvent resistant cancer cells [152]. Ouyang et al. [153] recently demonstrated via in vitro and in vivo studies that DNA nanostructures with guidance control and warhead can be highly beneficial in the aptamer-mediated delivery of anticancer drugs into targeted cancer cells via precision-guided missile approach [153]. Thus, nanoparticles with aptamers that are designed

via computational approaches are proposed to a promising next-generation strategy for cancer theranostic applications.

In recent times, nanorobots are recommended for targeted cancer theranostic applications. Nanorobots designed with nanoparticles, anticancer drugs, and biosensors can detect cancer biomarkers released by specific cancer cells, navigate to the target cell, internalize, and release the drug payload to inhibit cancer cell growth [154]. Li et al. [155] reported a novel DNA nanorobot that can function as a self-assembled cancer treatment agent via in vivo molecular trigger. The nanorobots are fabricated using a DNA origami method with nucleolin-targeting DNA aptamer and thrombin protein for programmed delivery of the drug payload at the tumor-associated endothelial cells. The nanorobots can deliver the thrombin in tumor-affected blood vessels after intravenous administration to cause necrosis and inhibit tumor growth by inducing intravascular thrombosis. Further, the nanorobots proved to be immunologically safe in mice and Bama miniature pigs, showing the efficacy of these nanosized molecular robots in targeted cancer therapy [58]. Iron-palladium-based mobile magnetic nanocatalysts [156], intelligent DNA nanorobot with enhanced protein lysosomal HER2 degradation [157], and magnetically actuated multifunctional nanoplatforms [158] are the other nanorobots that are under extensive studies for targeted cancer cell inhibition. Correspondingly, multicompartmental nanoformulations are introduced as a mechanism to encapsulate multiple anticancer drugs in a single platform for controlled release programs. Such a system may contain anticancer phytochemicals, nanoparticles, nanosized conventional enzyme inhibitors and genes in a single polymer formulation such as dendrimers and coated with aptamers for programmed targeting and release of drug payloads [159–161]. This novel nanoformulation concept along with nanorobotics and nanomedicine approaches can be a highly efficient, targeted cancer treatment opportunity to improve existing cancer treatment methods.

Conclusion

Cancer continues to be a major life-threatening disease among humans. The complex process of identifying the type of cancer, delivering anticancer drugs to target cancer cells, avoiding toxicity toward healthy cells, and the prevalence of lateral cancer emergence are some of the challenges hindering complete eradication of the disease. The discovery and development of oncotarget-based drugs could offer promising opportunities that would catalyze the treatment of cancer. Computational in silico techniques such as genomics, proteomics, and bioinformatics as well as MD simulation tools for probing ligand-target interactions play a key role in identifying and designing targeting strategies and smart cancer drug formulations and their interactions with cancer and tumor cells. Additionally, the combination of in silico methods with treatment methods such as chemotherapy, nanomedicine, or gene therapy would offer improved treatment modalities. However, challenges including chronic toxicity to healthy cells and chances for cancer re-emergence via a minimal residual disease (MRD) must be addressed to create more promising oncotarget-mediated treatment approaches.

Acknowledgements The author (Dr. Jaison Jeevanandam) acknowledge the support of FCT-Fundação para a Ciência e a Tecnologia (Base Fund UIDB/00674/2020 and Programmatic Fund UIDP/00674/2020, Portuguese Government Funds), ARDITI-Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação through the project M1420-01-0145-FEDER-000005-CQM+ (Madeira 14-20 Program).

Author Contributions JJ, GS, and KXT prepared the manuscript. JJ finalized the manuscript format and structure. MKD reviewed and revised the final manuscript. All authors read and approved the final version of the manuscript.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Callari, M., Gandellini, P., Skvortsova, I., & Span, P. N. (2018). Predicting and understanding cancer response to treatment. *Disease Markers*. https://doi.org/10.1155/2018/6159214.
2. Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D. M., Piñeros, M., et al. (2019). Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International Journal of Cancer, 144,* 1941–1953.
3. Hinck, L., & Näthke, I. (2014). Changes in cell and tissue organization in cancer of the breast and colon. *Current Opinion in Cell Biology, 26,* 87–95.
4. Cooper, G. (2000). *The cell: A molecular approach* (2nd ed.). Sinauer Associates: Sunderland.
5. American Society of Cancer. (2015). *Cancer facts and figures 2015*. Atlanta: American Society of Cancer.
6. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians, 68,* 394–424.
7. Kim, R. (2017). Anesthetic technique and cancer recurrence in oncologic surgery: unraveling the puzzle. *Cancer and Metastasis Reviews, 36,* 159–177.
8. Liu, J., Yang, Y., Zhu, W., Yi, X., Dong, Z., Xu, X., et al. (2016). Nanoscale metal−organic frameworks for combined photodynamic & radiation therapy in cancer treatment. *Biomaterials, 97,* 1–9.

9. Van Driel, W. J., Koole, S. N., Sikorska, K., Schagen van Leeuwen, J. H., Schreuder, H. W. R., Hermans, R. H. M., et al. (2018). Hyperthermic intraperitoneal chemotherapy in ovarian cancer. *New England Journal of Medicine, 378,* 230–240.
10. Vertosick, E. A., Assel, M., Tokita, H. K., Zafirova, Z., Vickers, A. J., Simon, B. A., & Twersky, R. (2019). Suitability of outpatient or ambulatory extended recovery cancer surgeries for obese patients. *Journal of Clinical Anesthesia, 58,* 111–116.
11. Martin, O. A., Anderson, R. L., Narayan, K., & MacManus, M. P. (2017). Does the mobilization of circulating tumour cells during cancer therapy cause metastasis? *Nature Reviews Clinical Oncology, 14,* 32.
12. Morris, D., Tu, D., Tehfe, M. A., Nicholas, G. A., Goffin, J. R., Gregg, R. W., et al. (2016). *A Randomized phase II study of reolysin in patients with previously treated advanced or metatstatic non small cell lung cancer (NSCLC) receiving standard salvage chemotherapy-canadian cancer trials group IND 211*. Atlanda: American Society of Clinical Oncology.
13. Shamsi, M., Sedaghatkish, A., Dejam, M., Saghafian, M., Mohammadi, M., & Sanati-Nezhad, A. (2018). Magnetically assisted intraperitoneal drug delivery for cancer chemotherapy. *Drug Delivery, 25,* 846–861.
14. Lorusso, D., Bria, E., Costantini, A., Di Maio, M., Rosti, G., & Mancuso, A. (2017). Patients' perception of chemotherapy side effects: Expectations, doctor–patient communication and impact on quality of life–An Italian survey. *European Journal of Cancer Care, 26,* e12618.
15. Yin, L., Lu, S., Zhu, J., Zhang, W., & Ke, G. (2019). Ovarian transposition before radiotherapy in cervical cancer patients: Functional outcome and the adequate dose constraint. *Radiation Oncology, 14,* 100.
16. Anghileri, L. J., & Robert, J. (2019). *Hyperthermia in cancer treatment*. Boca Raton: CRC Press.
17. He, Z., Liu, P., Zhang, S., Yan, J., Wang, M., Cai, Z., et al. (2019). A freezing-induced turn-on imaging modality for real-time monitoring of cancer cells in cryosurgery. *Angewandte Chemie International Edition, 58,* 3834–3837.
18. Qiu, W., Zhang, H., Chen, X., Song, L., Cui, W., Ren, S., et al. (2019). A glypican-1-targeted and gmcitabine-loaded biocompatible nanoplatform for pancreatic cancer near-infrared fluorescence/magnetic resonance multimodal imaging and therapy. *Magnetic Resonance Multimodal Imaging and Therapy*. https://doi.org/10.2139/ssrn.3328649.
19. Chang, D., Lim, M., Goos, J. A. C. M., Qiao, R., Ng, Y. Y., Mansfeld, F. M., et al. (2018). Biologically targeted magnetic hyperthermia: Potential and limitations. *Frontiers in Pharmacology, 9,* 831.
20. Mucciardi, G., Magno, C., Inferrera, A., & Lugnani, F. (2016). Cryosurgery and irreversible electroporation: The state of the art, advantages, and limitations. *Handbook of Electroporation*. https://doi.org/10.1007/978-3-319-32886-7_110.
21. Charmsaz, S., & Boyd, A. W. (2017). Eph receptors as oncotargets. *Oncotarget, 8,* 81727.
22. Jiao, Y., Wang, Y., Guo, S., & Wang, G. (2017). Glutathione peroxidases as oncotargets. *Oncotarget, 8,* 80093.
23. Chen, X., Zheng, Q., Li, W., Lu, Y., Ni, Y., Ma, L., & Fu, Y. (2018). SOX5 induces lung adenocarcinoma angiogenesis by inducing the expression of VEGF through STAT3 signaling. *OncoTargets and Therapy, 11,* 5733.
24. Chen, Y.-H., Lin, T.-T., Wu, Y.-P., Li, X.-D., Chen, S.-H., Xue, X.-Y., et al. (2019). Identification of key genes and pathways in seminoma by bioinformatics analysis. *OncoTargets and Therapy, 12,* 3683.
25. Wang, S., Yu, Z.-H., & Chai, K.-Q. (2019). Identification of EGFR as a novel key gene in clear cell renal cell carcinoma (ccRCC) through bioinformatics analysis and meta-analysis. *BioMed Research International*. https://doi.org/10.1155/2019/6480865.
26. American Cancer Society. (2019). *Cancer facts and figures 2019*. Atlanta: American Cancer Society.
27. Dela Cruz, C. S., Tanoue, L. T., & Matthay, R. A. (2011). Lung cancer: epidemiology, etiology, and prevention. *Clinics in Chest Medicine, 32,* 605–644.
28. Zhang, Q., Zeng, L., Chen, Y., Lian, G., Qian, C., Chen, S., et al. (2016). Pancreatic cancer epidemiology, detection, and management. *Gastroenterology Research and Practice, 2016,* 8962321.
29. Akram, M., Iqbal, M., Daniyal, M., & Khan, A. U. (2017). Awareness and current knowledge of breast cancer. *Biological Research, 50,* 33–33.
30. Hoffman, A. M., & Cairns, P. (2011). Epigenetics of kidney cancer and bladder cancer. *Epigenomics, 3,* 19–34.
31. De Minicis, S., Kisseleva, T., Francis, H., Baroni, G. S., Benedetti, A., Brenner, D., et al. (2013). Liver carcinogenesis: Rodent models of hepatocarcinoma and cholangiocarcinoma. *Digestive and Liver Disease, 45,* 450–459.
32. Cuzick, J., Thorat, M. A., Andriole, G., Brawley, O. W., Brown, P. H., Culig, Z., et al. (2014). Prevention and early detection of prostate cancer. *The Lancet Oncology, 15,* e484–e492.
33. Terwilliger, T., & Abdul-Hay, M. (2017). Acute lymphoblastic leukemia: A comprehensive review and 2017 update. *Blood Cancer Journal, 7,* e577–e577.
34. Shanbhag, S., & Ambinder, R. F. (2018). Hodgkin lymphoma: A review and update on recent progress. *CA: A Cancer Journal for Clinicians, 68,* 116–132.
35. Nabors, L. B., Ammirati, M., Bierman, P. J., Brem, H., Butowski, N., Chamberlain, M. C., et al. (2013). Central nervous system cancers. *Journal of the National Comprehensive Cancer Network, 11,* 1114–1151.
36. Fisher, R., Pusztai, L., & Swanton, C. (2013). Cancer heterogeneity: Implications for targeted therapeutics. *British Journal of Cancer, 108,* 479–485.
37. Meacham, C. E., & Morrison, S. J. (2013). Tumour heterogeneity and cancer cell plasticity. *Nature, 501,* 328–337.
38. Joensuu, H., & Dimitrijevic, S. (2001). Tyrosine kinase inhibitor imatinib (STIS71) as an anticancer agent for solid tumours. *Annals of Medicine, 33,* 451–455.
39. Li, M., Chen, W.-D., Papadopoulos, N., Goodman, S. N., Bjerregaard, N. C., Laurberg, S., et al. (2009). Sensitive digital quantification of DNA methylation in clinical samples. *Nature Biotechnology, 27,* 858–863.
40. Doi, A., Park, I.-H., Wen, B., Murakami, P., Aryee, M. J., Irizarry, R., et al. (2009). Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nature Genetics, 41,* 1350–1353.
41. Esteller, M. (2007). Epigenetic gene silencing in cancer: The DNA hypermethylome. *Human Molecular Genetics, 16,* R50–R59.
42. Whitwell, H. J., Worthington, J., Blyuss, O., Gentry-Maharaj, A., Ryan, A., Gunu, R., et al. (2020). Improved early detection of ovarian cancer using longitudinal multimarker models. *British Journal of Cancer*. https://doi.org/10.1038/s41416-019-0718-9.
43. Eftimie, R., & Hassanein, E. (2018). Improving cancer detection through combinations of cancer and immune biomarkers: A modelling approach. *Journal of Translational Medicine, 16,* 73.
44. Afonso, J., Santos, L. L., Longatto-Filho, A., & Baltazar, F. (2020). Competitive glucose metabolism as a target to boost bladder cancer immunotherapy. *Nature Reviews Urology*. https://doi.org/10.1038/s41585-019-0263-6.
45. Padma, V. V. (2015). An overview of targeted cancer therapy. *Biomedicine (Taipei), 5,* 19–19.

46. Yap, T. A., & Workman, P. (2012). Exploiting the cancer genome: Strategies for the discovery and clinical development of targeted molecular therapeutics. *Annual Review of Pharmacology and Toxicology, 52,* 549–573.
47. Kwak, E. L., Bang, Y. J., Camidge, D. R., Shaw, A. T., Solomon, B., Maki, R. G., et al. (2010). Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *New England Journal of Medicine, 363,* 1693–1703.
48. Chapman, P. B., Hauschild, A., Robert, C., Haanen, J. B., Ascierto, P., Larkin, J., et al. (2011). Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *New England Journal of Medicine, 364,* 2507–2516.
49. Tian, X., Yan, L., Zhang, D., Guan, X., Dong, B., Zhao, M., & Hao, C. (2016). PTK7 overexpression in colorectal tumors: Clinicopathological correlation and prognosis relevance. *Oncology Reports, 36,* 1829–1836.
50. Kim, J. H., Kwon, J., Lee, H. W., Kang, M. C., Yoon, H. J., Lee, S. T., & Park, J. H. (2014). Protein tyrosine kinase 7 plays a tumor suppressor role by inhibiting ERK and AKT phosphorylation in lung cancer. *Oncology Reports, 31,* 2708–2712.
51. Ahn, T., Roberts, M. J., Abduljabar, A., Joshi, A., Perera, M., Rhee, H., et al. (2019). A review of prostate-specific membrane antigen (PSMA) positron emission tomography (PET) in renal cell carcinoma (RCC). *Molecular Imaging and Biology, 21,* 799–807.
52. Sun, H., Zhu, X., Lu, P. Y., Rosato, R. R., Tan, W., & Zu, Y. (2014). Oligonucleotide aptamers: New tools for targeted cancer therapy. *Molecular Therapy: Nucleic Acids, 3,* e182.
53. Aravind, A., Jeyamohan, P., Nair, R., Veeranarayanan, S., Nagaoka, Y., Yoshida, Y., et al. (2012). AS1411 aptamer tagged PLGA-lecithin-PEG nanoparticles for tumor cell targeting and drug delivery. *Biotechnology and Bioengineering, 109,* 2920–2931.
54. Zhang, J., Chen, R., Fang, X., Chen, F., Wang, Y., & Chen, M. (2015). Nucleolin targeting AS1411 aptamer modified pH-sensitive micelles for enhanced delivery and antitumor efficacy of paclitaxel. *Nano Research, 8,* 201–218.
55. Meng, Y., Xu, B.-Q., Fu, Z.-G., Wu, B., Xu, B., Chen, Z.-N., & Li, L. (2015). Cytoplasmic EpCAM over-expression is associated with favorable clinical outcomes in pancreatic cancer patients with Hepatitis B virus negative infection. *International Journal of Clinical and Experimental Medicine, 8,* 22204–22216.
56. Tayama, S., Motohara, T., Narantuya, D., Li, C., Fujimoto, K., Sakaguchi, I., et al. (2017). The impact of EpCAM expression on response to chemotherapy and clinical outcomes in patients with epithelial ovarian cancer. *Oncotarget, 8,* 44312–44325.
57. Fourcade, J., Sun, Z., Chauvin, J.-M., Ka, M., Davar, D., Pagliano, O., et al. (2018). CD226 opposes TIGIT to disrupt Tregs in melanoma. *JCI Insight, 3,* e121157.
58. Bottcher, J. P., Bonavita, E., Chakravarty, P., Blees, H., Cabeza-Cabrerizo, M., Sammicheli, S., et al. (2018). NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell, 172,* 1022–1037.
59. Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design, 7,* 146–157.
60. Thomas, R. K., Baker, A. C., DeBiasi, R. M., Winckler, W., LaFramboise, T., Lin, W. M., et al. (2007). High-throughput oncogene mutation profiling in human cancer. *Nature Genetics, 39,* 347–351.
61. Ding, L., Ley, T. J., Larson, D. E., Miller, C. A., Koboldt, D. C., Welch, J. S., et al. (2012). Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature, 481,* 506–510.
62. Gerlinger, M., Rowan, A. J., Horswell, S., Math, M., Larkin, J., Endesfelder, D., et al. (2012). Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *New England Journal of Medicine, 366,* 883–892.
63. Vishwakarma, M., & Piddini, E. (2020). Outcompeting cancer. *Nature Review Cancer*. https://doi.org/10.1038/s41568-020-0261-2.
64. Baker, N. E. (2017). Mechanisms of cell competition emerging from Drosophila studies. *Current Opinion in Cell Biology, 48,* 40–46.
65. Di Gregorio, A., Bowling, S., & Rodriguez, T. A. (2016). Cell competition and its role in the regulation of cell fitness from development to cancer. *Developmental Cell, 38,* 621–634.
66. Claveria, C., & Torres, M. (2016). Cell competition: Mechanisms and physiological roles. *Annual Review of Cell and Developmental Biology, 32,* 411–439.
67. Wagstaff, L., Goschorska, M., Kozyrska, K., Duclos, G., Kucinski, I., Chessel, A., et al. (2016). Mechanical cell competition kills cells via induction of lethal p53 levels. *Nature Communications, 7,* 11373.
68. Levayer, R., Dupont, C., & Moreno, E. (2016). Tissue crowding induces caspase-dependent competition for space. *Current Biology: CB, 26,* 670–677.
69. Vincent, J. P., Kolahgar, G., Gagliardi, M., & Piddini, E. (2011). Steep differences in wingless signaling trigger Myc-independent competitive cell interactions. *Developmental Cell, 21,* 366–374.
70. Moreno, E., Basler, K., & Morata, G. (2002). Cells compete for decapentaplegic survival factor to prevent apoptosis in Drosophila wing development. *Nature, 416,* 755–759.
71. Rhiner, C., Lopez-Gay, J. M., Soldini, D., Casas-Tinto, S., Martin, F. A., Lombardia, L., & Moreno, E. (2010). Flower forms an extracellular code that reveals the fitness of a cell to its neighbors in Drosophila. *Developmental Cell, 18,* 985–998.
72. Madan, E., Pelham, C. J., Nagane, M., Parker, T. M., Canas-Marques, R., Fazio, K., et al. (2019). Flower isoforms promote competitive growth in cancer. *Nature, 572,* 260–264.
73. Ma, J., Wang, J., Ghoraie, L. S., Men, X., Liu, L., & Dai, P. (2019). Network-based method for drug target discovery at the isoform level. *Scientific Reports, 9,* 13868.
74. Maley, C. C., Aktipis, A., Graham, T. A., Sottoriva, A., Boddy, A. M., Janiszewska, M., et al. (2017). Classifying the evolutionary and ecological features of neoplasms. *Nature Reviews Cancer, 17,* 605–619.
75. Yang, B., Zhang, J., Yin, Y., & Zhang, Y. (2013). Network-based inference framework for identifying cancer genes from gene expression data. *BioMed Research International*. https://doi.org/10.1155/2013/401649.
76. Hori, S. S., Lutz, A. M., Paulmurugan, R., & Gambhir, S. S. (2017). A Model-based personalized cancer screening strategy for detecting early-stage tumors using blood-borne biomarkers. *Cancer Research, 77,* 2570–2584.
77. Hori, S. S., & Gambhir, S. S. (2011). Mathematical model identifies blood biomarker-based early cancer detection strategies and limitations. *Science Translational Medicine, 3,* 109–116.
78. Kwong, G. A., Dudani, J. S., Carrodeguas, E., Mazumdar, E. V., Zekavat, S. M., & Bhatia, S. N. (2015). Mathematical framework for activity-based cancer biomarkers. *Proceedings of the National Academy of Sciences of the USA , 112,* 12627–12632.
79. Wang, Y.-C., & Chen, B.-S. (2011). A network-based biomarker approach for molecular investigation and diagnosis of lung cancer. *BMC Medical Genomics, 4,* 2.
80. Zeng, T., Sun, S. Y., Wang, Y., Zhu, H., & Chen, L. (2013). Network biomarkers reveal dysfunctional gene regulations during disease progression. *FEBS Journal, 280,* 5682–5695.
81. Vilar, S., Gonzalez-Diaz, H., Santana, L., & Uriarte, E. (2009). A network-QSAR model for prediction of genetic-component biomarkers in human colorectal cancer. *Journal of Theoretical Biology, 261,* 449–458.

82. von Loga, K., Woolston, A., Punta, M., Barber, L. J., Griffiths, B., Semiannikova, M., et al. (2020). Extreme intratumour heterogeneity and driver evolution in mismatch repair deficient gastro-oesophageal cancer. *Nature Communications, 11,* 139.
83. Ludwig, J. A., & Weinstein, J. N. (2005). Biomarkers in cancer staging, prognosis and treatment selection. *Nature Reviews Cancer, 5,* 845.
84. Chin, L., Andersen, J. N., & Futreal, P. A. (2011). Cancer genomics: From discovery science to personalized medicine. *Nature medicine, 17,* 297.
85. Simpson, R. J., Bernhard, O. K., Greening, D. W., & Moritz, R. L. (2008). Proteomics-driven cancer biomarker discovery: Looking to the future. *Current Opinion in Chemical Biology, 12,* 72–77.
86. Gal, S., Fidler, C., Lo, Y. M. D., Taylor, M., Han, C., Moore, J., et al. (2004). Quantitation of circulating DNA in the serum of breast cancer patients by real-time PCR. *British Journal of Cancer, 90,* 1211.
87. Sozzi, G., Conte, D., Mariani, L., Vullo, S. L., Roz, L., Lombardo, C., et al. (2001). Analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients. *Cancer Research, 61,* 4675–4678.
88. Lehninger, A. L., Nelson, D. L., Cox, M. M., & Cox, M. M. (2005). *Lehninger principles of biochemistry*. New York: Macmillan.
89. Liu, P., Wang, Y., & Li, X. (2019). Targeting the untargetable KRAS in cancer therapy. *Acta Pharmaceutica Sinica B*. https://doi.org/10.1016/j.apsb.2019.03.002.
90. Pantsar, T. (2019). The current understanding of KRAS protein structure and dynamics. *Computational and Structural Biotechnology Journal*. https://doi.org/10.1016/j.csbj.2019.12.004.
91. Xi, X., Li, T., Huang, Y., Sun, J., Zhu, Y., Yang, Y., & Lu, Z. (2017). RNA biomarkers: Frontier of precision medicine for cancer. *Non-coding RNA, 3,* 9.
92. Berindan-Neagoe, I., Monroig, P. D. C., Pasculli, B., & Calin, G. A. (2014). MicroRNAome genome: A treasure for cancer diagnosis and therapy. *CA: A Cancer Journal for Clinicians, 64*(311), 336.
93. Sand, M., Bechara, F. G., Gambichler, T., Sand, D., Bromba, M., Hahn, S. A., et al. (2016). Circular RNA expression in cutaneous squamous cell carcinoma. *Journal of Dermatological Science, 83,* 210–218.
94. Akao, Y., Nakagawa, Y., & Naoe, T. (2006). MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. *Oncology Reports, 16,* 845–850.
95. Link, A., Balaguer, F., Shen, Y., Nagasaka, T., Lozano, J. J., Boland, C. R., & Goel, A. (2010). Fecal MicroRNAs as novel biomarkers for colon cancer screening. *Cancer Epidemiology and Prevention Biomarkers, 19,* 1766–1774.
96. Srinivas, P. R., Verma, M., Zhao, Y., & Srivastava, S. (2002). Proteomics for cancer biomarker discovery. *Clinical chemistry, 48,* 1160–1169.
97. Núñez, C. (2019). Blood-based protein biomarkers in breast cancer. *Clinica Chimica Acta, 490,* 113–127.
98. Ohno, Y., Maehashi, K., & Matsumoto, K. (2010). Label-free biosensors based on aptamer-modified graphene field-effect transistors. *Journal of the American Chemical Society, 132,* 18012–18013.
99. Li, L., Lei, Q., Zhang, S., Kong, L., & Qin, B. (2017). Screening and identification of key biomarkers in hepatocellular carcinoma: Evidence from bioinformatic analysis. *Oncology Reports, 38,* 2607–2618.
100. Homer, N., Szelinger, S., Redman, M., Duggan, D., Tembe, W., Muehling, J., et al. (2008). Resolving individuals contributing trace amounts of DNA to highly complex mixtures using high-density SNP genotyping microarrays. *PLoS Genetics, 4,* e1000167.
101. Im, H. K., Gamazon, E. R., Nicolae, D. L., & Cox, N. J. (2012). On sharing quantitative trait GWAS results in an era of multiple-omics data and the limits of genomic privacy. *American Journal of Human Genetics, 90,* 591–598.
102. Schadt, E. E., Woo, S., & Hao, K. (2012). Bayesian method to predict individual SNP genotypes from gene expression data. *Nature Genetics, 44,* 603–608.
103. Hildebrand, P. W., Rose, A. S., & Tiemann, J. K. S. (2019). Bringing molecular dynamics simulation data into view. *Trends in Biochemical Sciences*. https://doi.org/10.1016/j.tibs.2019.06.004.
104. Karplus, M., & Petsko, G. A. (1990). Molecular dynamics simulations in biology. *Nature, 347,* 631.
105. Godwin, R. C., Melvin, R., & Salsbury, F. R. (2015). Molecular dynamics simulations and computer-aided drug discovery. In D. B. Singh (Ed.), *computer-aided drug discovery* (pp. 1–30). New York: Springer.
106. Klepeis, J. L., Lindorff-Larsen, K., Dror, R. O., & Shaw, D. E. (2009). Long-timescale molecular dynamics simulations of protein structure and function. *Current Opinion in Structural Biology, 19,* 120–127.
107. Phillips, J. C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., et al. (2005). Scalable molecular dynamics with NAMD. *Journal of Computational Chemistry, 26,* 1781–1802.
108. Brooks, B. R., Brooks Iii, C. L., Mackerell, A. D., Jr., Nilsson, L., Petrella, R. J., Roux, B., et al. (2009). CHARMM: The biomolecular simulation program. *Journal of Computational Chemistry, 30,* 1545–1614.
109. Case, D. A., Cheatham Iii, T. E., Darden, T., Gohlke, H., Luo, R., Merz, K. M., Jr., et al. (2005). The Amber biomolecular simulation programs. *Journal of Computational Chemistry, 26,* 1668–1688.
110. Hess, B., Kutzner, C., Van Der Spoel, D., & Lindahl, E. (2008). GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *Journal of Chemical Theory and Computation, 4,* 435–447.
111. Rampogu, S., Baek, A., Zeb, A., & Lee, K. W. (2018). Exploration for novel inhibitors showing back-to-front approach against VEGFR-2 kinase domain (4AG8) employing molecular docking mechanism and molecular dynamics simulations. *BMC Cancer, 18,* 264.
112. Jeevanandam, J., Tan, K. X., Danquah, M. K., Guo, H., & Turgeson, A. (2019). Advancing aptamers as molecular probes for cancer theranostic applications: The role of molecular dynamics simulation. *Biotechnology Journal*. https://doi.org/10.1002/biot.201900368.
113. Danquah, M. K., Guo, H.-B., Tan, K. X., & Bhakta, M. (2020). Atomistic probing of aptameric binding of CD19 outer membrane domain reveals an "aptamer walking" mechanism. *Biotechnology Progress, 36,* e2957.
114. Kumar, A., & Purohit, R. (2014). Use of long term molecular dynamics simulation in predicting cancer associated SNPs. *PLoS Computational Biology, 10,* e1003318.
115. Weako, J., Uba, A. I., Keskin, Ö., Gürsoy, A., & Yelekçi, K. (2020). Identification of potential inhibitors of human methionine aminopeptidase (type II) for cancer therapy: Structure-based virtual screening, ADMET prediction and molecular dynamics studies. *Computational Biology and Chemistry, 86,* 107244.
116. Reddy, P. S., & K. B. L., Shuchi Nagar, Vaddi Damodara Reddy, P. Sushma Murthy, K. Venkateswara Swamy, . (2018). Molecular Modeling, Docking, Dynamics and Simulation of Gefitinib and its Derivatives with EGFR in Non-small Cell Lung Cancer. *Current Computer-Aided Drug Design, 14,* 246–252.

117. Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry, 31,* 455–461.
118. Rich, R. L., & Myszka, D. G. (2004). Why you should be using more SPR biosensor technology. *Drug Discovery Today: Technologies, 1,* 301–308.
119. Suenaga, A., Ichikawa, M., Hatakeyama, M., Yu, X., Futatsugi, N., Narumi, T., et al. (2003). Molecular dynamics, free energy, and SPR analyses of the interactions between the SH2 domain of Grb2 and ErbB phosphotyrosyl peptides. *Biochemistry, 42,* 5195–5200.
120. Case, D. A., Darden, T. A., Cheatham, T. E., Simmerling, C. L., Wang, J., Duke, R. E., et al. (2019). *Amber 11*. San Francisco: University of California.
121. Amiri, M., Ajloo, D., Fazli, M., Mokhtarieh, A., Grivani, G., & Saboury, A. A. (2018). Spectroscopic, electrochemical, docking and molecular dynamics studies on the interaction of three oxovanadium (IV) Schiff base complexes with bovine serum albumin and their cytotoxicity against cancer. *Journal of Biomolecular Structure and Dynamics, 36,* 3753–3772.
122. Rao, N. V., Yoon, H. Y., Han, H. S., Ko, H., Son, S., Lee, M., et al. (2016). Recent developments in hyaluronic acid-based nanomedicine for targeted cancer treatment. *Expert Opinion on Drug Delivery, 13,* 239–252.
123. Bonifert, G., Folkes, L., Gmeiner, C., Dachs, G., & Spadiut, O. (2016). Recombinant horseradish peroxidase variants for targeted cancer treatment. *Cancer Medicine, 5,* 1194–1203.
124. Dong, K., Zhang, Y., Zhang, L., Wang, Z., Ren, J., & Qu, X. (2019). Facile preparation of metal−organic frameworks-based hydrophobic anticancer drug delivery nanoplatform for targeted and enhanced cancer treatment. *Talanta, 194,* 703–708.
125. Gavande, N. S., VanderVere-Carozza, P. S., Hinshaw, H. D., Jalal, S. I., Sears, C. R., Pawelczak, K. S., & Turchi, J. J. (2016). DNA repair targeted therapy: The past or future of cancer treatment? *Pharmacology and Therapeutics, 160,* 65–83.
126. Wang, H., & Mooney, D. J. (2018). Biomaterial-assisted targeted modulation of immune cells in cancer treatment. *Nature Materials, 17,* 761–772.
127. Jeevanandam, J., Pal, K., & Danquah, M. K. (2018). Virus-like nanoparticles as a novel delivery tool in gene therapy. *Biochimie, 157,* 38–47.
128. Jeevanandam, J., Sundaramurthy, A., Sharma, V., Murugan, C., Pal, K., Kodous, M. H. A., & Danquah, M. K. (2020). Sustainability of One-Dimensional Nanostructures: Fabrication and Industrial Applications. In G. Székely & A. G. Livingston (Eds.), *Sustainable nanoscale engineering* (pp. 83–113). New York: Elsevier.
129. Kuryk, L., Vassilev, L., Ranki, T., Hemminki, A., Karioja-Kallio, A., Levälampi, O., et al. (2017). Toxicological and bio-distribution profile of a GM-CSF-expressing, double-targeted, chimeric oncolytic adenovirus ONCOS-102: Support for clinical studies on advanced cancer treatment. *PLoS ONE, 12,* e0182715.
130. Massacesi, C., Di Tomaso, E., Urban, P., Germa, C., Quadt, C., Trandafir, L., et al. (2016). PI3K inhibitors as new cancer therapeutics: Implications for clinical trial design. *OncoTargets and Therapy, 9,* 203–210.
131. Mirzaei, H., Sahebkar, A., Salehi, R., Nahand, J., Karimi, E., Jaafari, M., & Mirzaei, H. (2016). Boron neutron capture therapy: Moving toward targeted cancer therapy. *Journal of Cancer Research and Therapeutics, 12,* 520–525.
132. Mishra, P., Nayak, B., & Dey, R. K. (2016). PEGylation in anticancer therapy: An overview. *Asian Journal of Pharmaceutical Sciences, 11,* 337–348.
133. Li, Y., Atkinson, K., & Zhang, T. (2017). Combination of chemotherapy and cancer stem cell targeting agents: Preclinical and clinical studies. *Cancer Letters, 396,* 103–109.
134. Belfiore, L., Saunders, D. N., Ranson, M., Thurecht, K. J., Storm, G., & Vine, K. L. (2018). Towards clinical translation of ligand-functionalized liposomes in targeted cancer therapy: Challenges and opportunities. *Journal of Controlled Release, 277,* 1–13.
135. Yeku, O., Zamarin, D., Gallagher, J., Aghajanian, C. A., & Konner, J. (2018). A phase II trial of TPIV200 (a polypeptide vaccine against folate receptor alpha) plus durvalumab (anti-PD-L1 antibody) in patients with platinum-resistant ovarian cancer. *Gynecologic Oncology, 149,* 56–57.
136. Fuller, S., Tomai, M., Sesay, M., Cunningham, D. & Price, J. (2017). Optimizing a unique cancer vaccine for intradermal delivery. *BioPharm International Development Strategies for Emerging Therapies eBook*.
137. Hill, A., Gotham, D., Fortunak, J., Meldrum, J., Erbacher, I., Martin, M., et al. (2016). Target prices for mass production of tyrosine kinase inhibitors for global cancer treatment. *British Medical Journal Open, 6,* e009586.
138. Meegan, M. J., & O'Boyle, N. M. (2019). *Special issue "anticancer drugs."* Basel: Multidisciplinary Digital Publishing Institute.
139. Peiris, D., Spector, A. F., Lomax-Browne, H., Azimi, T., Ramesh, B., Loizidou, M., et al. (2017). Cellular glycosylation affects herceptin binding and sensitivity of breast cancer cells to doxorubicin and growth factors. *Scientific Reports, 7,* 43006.
140. Corrie, P. G., Terheyden, P., Ten Tije, A. J., Herbst, R., Jansen, R., Marples, M., et al. (2019). A prospective observational safety study of patients with BRAFV 600-mutated unresectable or metastatic melanoma treated with vemurafenib (Zelboraf Safety Study). *British Journal of Dermatology, 180,* 1254–1255.
141. Somlyai, G., Collins, T. Q., Meuillet, E. J., Hitendra, P., D'Agostino, D. P., & Boros, L. G. (2017). Structural homologies between phenformin, lipitor and gleevec aim the same metabolic oncotarget in leukemia and melanoma. *Oncotarget, 8,* 50187.
142. Parvizpour, S., Razmara, J., & Omidi, Y. (2018). Breast cancer vaccination comes to age: Impacts of bioinformatics. *BioImpacts: BI, 8,* 223.
143. Barbolosi, D., Ciccolini, J., Lacarelle, B., Barlési, F., & André, N. (2016). Computational oncology: Mathematical modelling of drug regimens for precision medicine. *Nature Reviews Clinical Oncology, 13,* 242.
144. Nalley, C. (2019). Utilizing computational oncology to better understand AML and MDS patients. *Oncology Times, 41,* 13.
145. Ding, M. Q., Chen, L., Cooper, G. F., Young, J. D., & Lu, X. (2018). Precision oncology beyond targeted therapy: Combining omics data with machine learning matches the majority of cancer cells to effective therapeutics. *Molecular Cancer Research, 16,* 269–278.
146. Bibault, J.-E., Giraud, P., & Burgun, A. (2016). Big data and machine learning in radiation oncology: State of the art and future prospects. *Cancer Letters, 382,* 110–117.
147. Majumder, J., Fine, J. A., Lantz, T. C., Conder, C. J., & Chopra, G. (2018). Abstract LB-076: Cancer cell specific lethality by degrading specific protein target network identified using a chemical screening based machine learning method. *Cancer Research, 78,* 076.
148. Wu, D., Yu, Y., Jin, D., Xiao, M.-M., Zhang, Z.-Y., & Zhang, G.-J. (2020). Dual-aptamer modified graphene field-effect transistor nanosensor for label-free and specific detection of hepatocellular carcinoma-derived microvesicles. *Analytical Chemistry, 92,* 4006–4015.
149. Mehmood, S., Khan, A. Z., Bilal, M., Sohail, A., & Iqbal, H. M. N. (2019). Aptamer-based biosensors: A novel toolkit for early diagnosis of cancer. *Materials Today Chemistry, 12,* 353–360.

150. Dehghani, S., Nosrati, R., Yousefi, M., Nezami, A., Soltani, F., Taghdisi, S. M., et al. (2018). Aptamer-based biosensors and nanosensors for the detection of vascular endothelial growth factor (VEGF): A review. *Biosensors and Bioelectronics, 110,* 23–37.
151. Hao, Z., Pan, Y., Huang, C., Wang, Z., & Zhao, X. (2019). Sensitive detection of lung cancer biomarkers using an aptameric graphene-based nanosensor with enhanced stability. *Biomedical Microdevices, 21,* 65.
152. Liu, J., Wei, T., Zhao, J., Huang, Y., Deng, H., Kumar, A., et al. (2016). Multifunctional aptamer-based nanoparticles for targeted drug delivery to circumvent cancer resistance. *Biomaterials, 91,* 44–56.
153. Ouyang, C., Zhang, S., Xue, C., Yu, X., Xu, H., Wang, Z., et al. (2020). Precision guided missile-like DNA nanostructure containing warhead and guidance control for aptamer-based targeted drug delivery into cancer cells in vitro and in vivo. *Journal of the American Chemical Society*. https://doi.org/10.1021/jacs.9b09782.
154. da Silva Luz, G. V., Barros, K. V. G., de Araújo, F. V. C., da Silva, G. B., da Silva, P. A. F., Condori, R. C. I., & Mattos, L. (2016). Nanorobotics in drug delivery systems for treatment of cancer: A review. *Journal of Material Sciences and Engineering A, 6,* 167–180.
155. Li, S., Jiang, Q., Liu, S., Zhang, Y., Tian, Y., Song, C., et al. (2018). A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo. Nature Biotechnology, 36(3), 258
156. Hoop, M., Ribeiro, A. S., Rösch, D., Weinand, P., Mendes, N., Mushtaq, F., et al. (2018). Mobile magnetic nanocatalysts for bioorthogonal targeted cancer therapy. *Advanced Functional Materials, 28,* 1705920.
157. Ma, W., Zhan, Y., Zhang, Y., Shao, X., Xie, X., Mao, C., et al. (2019). An intelligent DNA nanorobot with in vitro enhanced protein lysosomal degradation of HER2. *Nano Letters*. https://doi.org/10.1021/acs.nanolett.9b01320.
158. Serrà, A., Vázquez-Mariño, G., García-Torres, J., Bosch, M., & Vallés, E. (2018). Magnetic actuation of multifunctional nanorobotic platforms to induce cancer cell death. *Advanced Biosystem, 2,* 1700220.
159. Jeevanandam, J., Aing, Y. S., Chan, Y. S., Pan, S., & Danquah, M. K. (2017). Nanoformulation and application of phytochemicals as antimicrobial agents. In A. Mehai (Ed.), *Antimicrobial nanoarchitectonics: From synthesis to applications* (Vol. 1, pp. 62–82). New York: Elsevier.
160. Jeevanandam, J., San Chan, Y., & Danquah, M. K. (2016). Nanoformulations of drugs: Recent developments, impact and challenges. *Biochimie, 128,* 99–112.
161. Jeevanandam, J., Chan, Y. S., Pan, S., & Danquah, M. K. (2019). Metal oxide nanocomposites: Cytotoxicity and targeted drug delivery applications. In K. Pal (Ed.), *Hybrid nanocomposites: Fundamentals, synthesis and applications* (pp. 111–147). Singapore: Pan Stanford Publishing.