REVIEW ARTICLE

A critical review on cancer vaccines: a promising immunotherapy

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Abstract: Cancer vaccines are a type of immunotherapy that can assist in educating the immune system about what cancer cells "look like" so that it can practively destroy them. A lack of an efficient adjuvant and insufficient efficacy hurdles the development of cancer vaccines based on tumor-associated antigens. To improve the efficacy of vaccines, a genetically engineered method was reviewed to achieving the codelivery of antigen and adjuvant to enhance immune responses. For more than 25 years, the development of cancer vaccines has been at the forefront of cancer research. The main emphasis has been on delivery strategies used to promote strong and long-lasting immune responses. Recent developments have made it possible to advance the engineering of therapeutic cancer vaccines. Target selection, vaccine development and techniques for overturning immunosuppressive systems used by malignancies have all made significant strides. To accelerate future developments and provide guidance to the prospective participants in this field, this commentary-style review provides an overview of recent devel-opments in therapeutic, HPV and DNA cancer vaccines especially focusing on modeling and simulation advances to date.

Keywords: Cancer vaccines; Immunotherapy; Immune suppression; Antigen delivery.

Abbreviations:

ADCC	Antibody-dependent cellular cytotoxicity
APCs	Antigen Presenting Cells
BCG	Bacillus Calmette-Guérin
CTLs	Cytotoxic T ly mphocytes
DCs	Dorsal Column Spinal cord stimulation
DNA	DeoxyriboNucleic Acid
FasL	Fas ligand
FDA	Food and Drug Administration
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HBV	Hepatitis B
HER-2	Human epidermal growth factor receptor 2
HPV	Human Papillomavirus
IFN-γ	Interferon-gamma
MHC	Major histocompatibility complex
PAP	Prostatic acid phosphatase
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
PSA	Prostate-specific antigen
RECIST	Response Evaluation Criteria in Solid Tumors
TAAs	Tumor-associated antigens
TME	Tumor microenvironment
TNF	Tumor necrosis factor
TSAs	Tumor-specific antigens

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1. INTRODUCTION

Vaccines are successful in preventing illnesses caused by viruses/germs. Since the creation of the first vaccine about 200 years ago, they have saved the lives of millions of people worldwide (Hu, Ott, & Wu, 2018; Igarashi & Sasada, 2020). A healthy individual is inoculated with attenuated/detoxified bacteria, viruses, or extracted toxins to artificially induce immune responses against infectious antigens, which serves as the method by which vaccinations protect against an illness (Apostolopoulos, 2019; Hall, Wodi, Hamborsky, Morelli, & Schillie, 2021). Cancer is a costly disease, i.e., annual costs for treating cancer in the UK alone are about £5 million, but the cost to society, including the price for loss of productivity, could be to the tune of £18.3 billion. The development of vaccines to prevent or treat cancer is hampered by the complexity of the situation described later. Cancer cells more closely resemble normal and healthy cells than bacteria and viruses. Therefore, our body perceives bacteria and viruses as foreign particles. Additionally, every person's tumour is distinctive in some way and includes distinct antigens. Therefore, more advanced methods are required to create efficient cancer vaccines. In the 1980s, the first cancer vaccination based on tumour cells and tumour lysates was created. Scientists treated colorectal cancer with autologous tumour cells (Jian Liu *et al.*, 2022; Singh, Bowne, & Snook, 2021). Melanoma-associated antigen 1, the first human tumour antigen discovered in the early 1990s (D. S. Chen & Mellman, 2013), opened the door to employing tumour antigens in cancer vaccines. The successful use of a dendritic cell-based vaccine (Sip-uleucel-T) to treat prostate cancer in 2010 propelled the subsequent wave of advancements in the field of cancer vaccines (Y. Yang, Nam, Kim, Kim, & Kim, 2019). The COVID-19 pandemic has prompted the improvement of vaccination technology and refocused public attention on cancer vaccines (LaFleur, Muroyama, Drake, & Sharpe, 2018). Cancer vaccines primarily include tumour-associated antigens (TAAs) and tumour-specific an-tigens (TSAs) to stimulate the patient's immune system. The vaccination may theoretically induce both a specific cellular immune response and a humoral immune response to stop the growth of tumours and ultimately eliminate malignant cells. Most cancer vaccines are still in the preclinical and clinical research stages (Verma, 2021). There is always a need to create more specialised antigens and vaccine development platforms. Fig. 1 illustrates current approaches to developing a cancer vaccine.

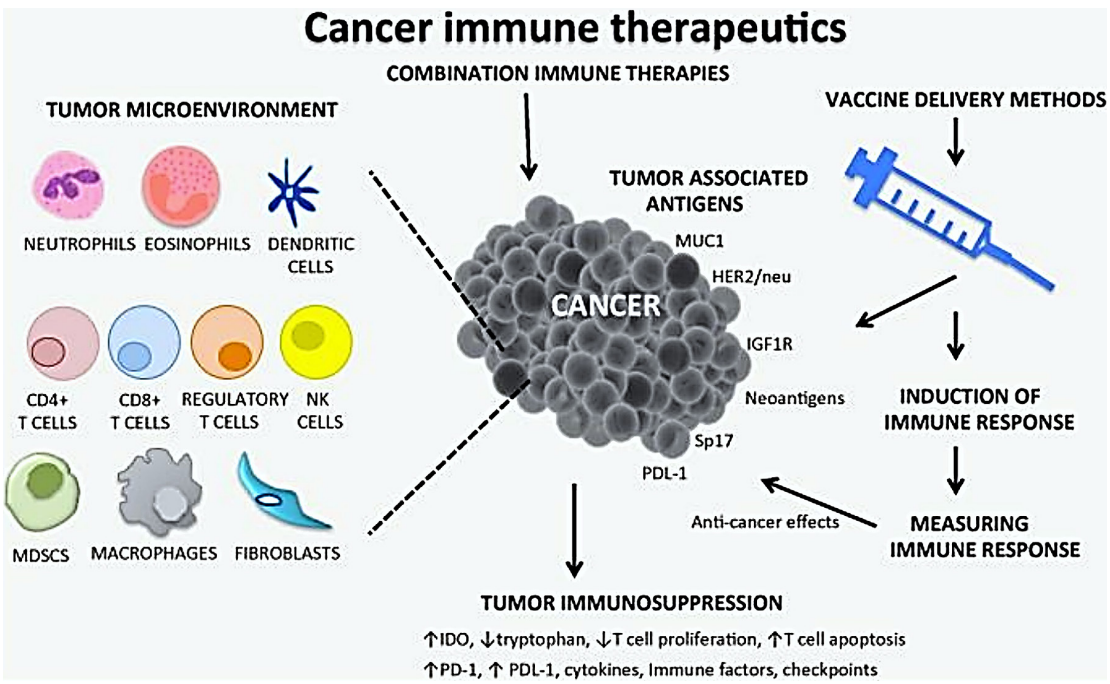


Figure 1. Strategies for cancer vaccine development: Cancer immune therapeutics (Apostolopoulos, 2019).

A study led by researchers at the Ohio State University Comprehensive Cancer Center - Arthur G. James Cancer Hospital and Richard J. Solove Research Institute (OSUCCC - James) described the potential of the therapeutic anticancer vaccine. The results released on October 1, 2020 (Y. Yang *et al.*, 2019) demonstrated that the peptide known as PD1-Vaxx, a first checkpoint inhibitor vaccination, was safe and efficacious in an animal model of colon cancer. The vaccine generated polyclonal antibodies that prevent cancer cells from expressing the PD-1 programmed cell death receptor. The vaccinationvaccination mimicked the PD-1 inhibitor nivolumab mimicked the PD-1 inhibitor nivolumab, but it did not cause the innate and acquired resistance that the drug and related treatments are known to cause. According to the study, PD1-Vaxx effectively slowed the growth of tumours. It was much more successful when combined with a second therapeutic peptide vaccine that specifically tar-getstargets explicitly two HER-2 receptor sites on colon cancer cells. The combined treatment resulted in full response in nine out of ten animals. The same scientific team also created the B-Vaxx vaccination earlier.

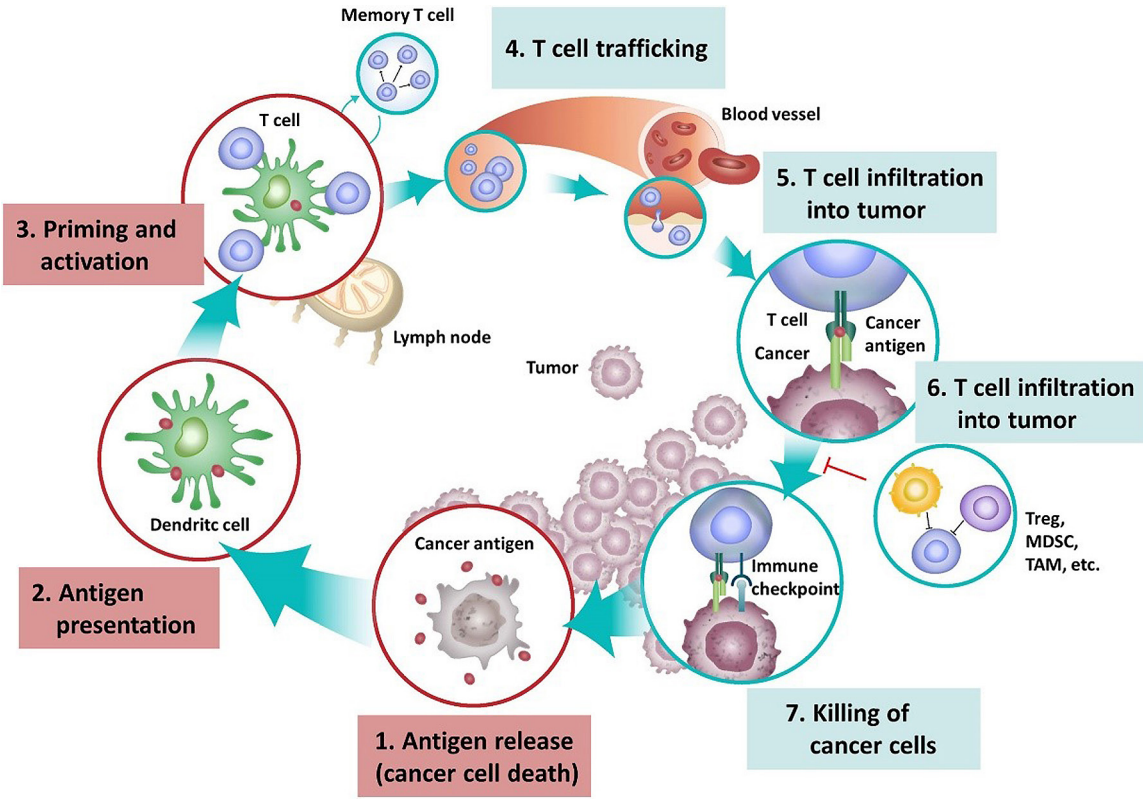


Figure 2. Cancer-immunity cycle. This cycle is a self-sustaining multistep process that involves: (1) the release of cancer cell antigens; (2) cancer antigen presentation; (3) priming and activation; (4) the trafficking of T cells to the tumor; (5) the infiltration of T cells into tumors; (6) specifically recognize and bind to cancer cells through the interaction between its T cell receptor (TCR) and its cognate antigen bound to MHCI; and (7) the killing of cancer target cells (Y. Yang *et al.*, 2019).

The two methods by which this vaccine acts are: (i) PD1-Vaxx activates both B- and T-cells to encourage tumour elimination, and (ii) the therapy aims to obstruct signalling pathways essential for tumour maintenance and growth. Researchers are essentially supercharging and precisely directing the immune system to target and kill cancer cells by administering this vaccination and immunotherapy medicine. PD1-Vaxx is an immune checkpoint inhibitor, much like the immunotherapy medication nivolumab. Proteins, known as immunological checkpoints, prevent immune cells from attacking healthy bodily cells. On killer T cells, the checkpoint protein PD-1 is present. Another checkpoint protein seen on both normal and many cancer cells is PD-L1. The T-cell is suppressed and unable to

kill the cell when PD-1 on the T cells connects with PD-L1 on a cancer cell (Fig. 2) (D. S. Chen & Mellman, 2013; Y. Yang *et al.*, 2019). Many vaccination methods are being tested in preclinical and clinical settings. This review discusses preclinical and clinical trials using these therapeutic vaccines from various platforms or targets and HPV, DNA, and mRNA vaccines. We also considered potential methods to block tumor-induced immune suppression, which reduces the effectiveness of therapeutic vaccinations, to promote more powerful anticancer immune responses.

2. CLASSIFICATION OF CANCER VACCINES

2.1. Preventive Cancer Vaccines

Viral infections bring on several categories of cancer. The use of preventative vaccines is crucial in lowering these risks. Hepatitis B viruses (HBV) can cause liver cancer, whereas the human papilloma viruses (HPV) can cause head and neck and cervical cancer. Several vaccines have been developed to guard against the development of HBV- and HPV-related malignancies that can prevent HBV and HPV infection (Hu *et al.*, 2018). The U.S. Food and Drug Administration (FDA) has authorised four preventative cancer vaccinations (FDA). Currently, two FDA-approved vaccinations for the treatment of cancer and four vaccines that can help prevent cancer have received FDA approval:

— **Cervarix®:** a vaccine for preventing HPV-related anal, head, cervical, neck, penile, vulvar and vaginal can-cers authorized for protection against HPV types 16 and 18 strains, the two HPV strains most likely to cause cervical cancer.
— **Gardasil®:** a vaccine approved by the FDA in 2006 for the prevention of HPV types 16, 103 18, 31, 33, 45, 52, and 58 as well as the prevention of HPV 6 and 11 induced genital warts; it can contribute to the prevention of cervical, neck, head, penile, vulvar, throat and vaginal cancers.
— **Gardasil-9®:** a vaccine that has been licenced for the prevention of HPV types 16, 18, 31, 33, 45, 52, and 58 infections as well as the prevention of genital warts brought on by types 6 or 11 of the virus, it can aid in the prevention of cervical, neck, head, penile, vulvar, throat and vaginal cancers.
— **Hepatitis B (HBV) vaccine (HEPLISAV-B®):** a vaccine for protection against HBV infection and contribution to regression of growth of liver cancer associated with HBV.

2.2. Therapeutic Cancer Vaccines

Every tumour is different and contains distinctive antigens. Therefore, more advanced cancer vaccination strategies are required. Fortunately, doctors can now locate targets on tumours in patients that can aid in dif-ferentiating cancer cells from healthy cells. Prostatic acid phosphatase (PAP), frequently overexpressed by prostate cancer cells, is an example of a normal protein that cancer cells manufacture at abnormally high levels. This realisation led to the development of the sipuleucel-T vaccine, which the FDA approved in 2010 for the treatment of individuals with advanced prostate cancer. Another interesting source of indicators that vaccines can target is virus-derived proteins generated by cancer cells that have been infected by viruses. BCG, a tuberculosis vaccination that also serves as an immunological stimulant, is an additional exception. BCG was the first im-munotherapy to receive FDA approval in 1990 and is still utilised to treat bladder cancer in its early stages (LaFleur *et al.*, 2018; Verma, 2021). These two immunizations are still considered to be safe

— **Bacillus Calmette-Guérin (BCG):** a vaccine allowed for people with early-stage bladder cancer that stimulates the immune system using weakened microorganisms.
— **Sipuleucel-T (Provenge®):** a prostate cancer-approved vaccination made from patients' activated dendritic cells. This was the first cancer treatment vaccine approved by the FDA. Sipuleucel-T is used to treat asymptomatic or minimally symptomatic metastatic castrate-resistant (i.e., hormone-refractory) prostate cancer. Sipuleucel-T is an example of personalized medicine, as it is manufactured using each patient's APCs that are activated via exposure to an antigen specific to prostate cancer. It contains autologous activated APCs that stimulate a re-sponse against PAP, an antigen expressed in most prostate cancer tissues. Once leukapheresis is completed, peripheral blood mononuclear cells are isolated, from which APC precursors, including DCs, are activated in vitro with a recombinant human fusion protein, PAP-GM-CSF

(i.e., PAP linked to granulocyte-macrophage colo-ny-stimulating factor). Once reinfused into the patient, PAP-GM-CSF targets APCs and directs the T cells to PAP, eventually destroying PAP-expressing prostate cancer cells (Singh *et al.*, 2021).

— **Neoantigen Vaccines:** Tumours have distinct targets that develop because of mutations, in contrast to nor-mal-yet-overexpressed proteins like PAP. Neoantigens, also known as "new antigens," are molecules only ex-pressed by tumour cells and never by the patient's healthy counterparts. Neoantigen vaccines have the potential to precisely target tumour cells in patients while sparing their healthy cells from immune attack, thus preventing side effects. In addition to the vaccines already listed, several neoantigen vaccines are currently being tested in clinical trials for a range of cancer types, both alone and in conjunction with other therapies (Fucikova *et al.*, 2020; Saxena, van der Burg, Melief, & Bhardwaj, 2021).

— **NeuVax HER2 Vaccine:** There is currently an ongoing multicenter, global, prospective, randomized, dou-ble-blind, controlled phase III trial (PRESENT) studying the efficacy of the nelipepimut-S (NeuVax) vaccine for the prevention of breast cancer recurrence in early-stage for node-positive breast cancer patients who have low-to-intermediate human epidermal growth factor receptor 2 (HER2) expression gene. Though this vaccine avoids reappearance, it is still deemed a treatment because the participants have tumours with HER2 present. Enrolled patients will have tumours expressing low or intermediate levels of the HER2 protein, and the NeuVax vaccine is administered as adjuvant therapy. The study's primary endpoint is a consecutive 3-year disease-free survival (DFS) (Mittendorf *et al.*, 2019).

  NeuVax is an immunodominant nonapeptide derived from the extracellular domain of the HER2 protein. The fragmented antigens from the vaccine activate the adaptive immunity, which causes Cytotoxic T lymphocytes (CTLs) to migrate to the target HER2 protein on malignant T cells and, subsequently, eradicate the tumour cells. Due to the success of the phase II trial, the FDA granted NeuVax a Special Protocol Assessment (SPA) for the PRESENT phase III trial (Mittendorf *et al.*, 2012).

— **Chimeric Antigen Receptors (CARs):** A novel and promising approach to immunotherapy is the genetic modi-fication of T cells with CARs. The discovery of CARs arose from the use of adoptive cellular therapy. CD8+ and CD4+ T lymphocytes are potent components of adaptive immunity vital in tumour removal. T cells have become attractive candidates for cancer-specific immunotherapy. First-generation CARs consist of a binding moiety that particularly recognizes a lymphocyte-activating signaling chain and tumour cell surface antigen. The CAR-mediated recognition induces cytokine production and tumour-directed cytotoxicity of T cells. Second- and third-generation CARs include signal sequences from various costimulatory molecules resulting in enhanced T-cell persistence and sustained antitumor reaction. Clinical trials have revealed that the adoptive transfer of T cells engineered with the first-generation CARs represents a feasible concept for the induction of clinical responses in some tumour patients. Further modifications, however, are required, which may be achieved by second- or third-generation CAR-engrafted T cells (Beavis *et al.*, 2016; Cartellieri *et al.*, 2010).

Though there are obstacles whichCARs seem promising. There are obstacles we need to overcome before they can be used for a broad selection of cancer types, mainly due to the differences in tumour microenvironments that could potentially impact the efficacy of therapy. Clinical trials and research are currently investigating the benefits and use of T cell modification with CARs, including phase I and II studies on treating refractory or lymphoma or relapsed leukemia (Hay & Turtle, 2017).

2.3. Viral Vectors and DNA Vaccines

Viral Vector: The composition of viral particles for viral vectored vaccines consists of modifying the genome comprising one or more genes encoding for the antigens of interest. The principle of utilising viruses to deliver the 'vaccine gene' is a number of folds. Primarily, the evolvement of viruses was to infect mammalian cells and to express encoded genes with high efficiency, hence solving the issue of poor in-vivo transduction of nucleic acids. Most significantly, several viruses can target professional antigen-presenting cells that result in potent priming of the immune response. Additionally, a higher

level of vaccine antigens can be attained in-vivo in those cases where viral vector replication is used and therefore boost the immunogenicity of the vaccine.

DNA Vaccines: The composition of DNA vaccines is circular or linear (plasmid) DNA molecules consisting of the translational regulatory sequences and the coding sequence for the antigen of interest under the control of potent mammalian transcriptional. Plasmid, the most frequent form of DNA vaccines that can produce many copies in bacterial cells, where one can replicate and purify, can produce many copies in bacterial cells, where one can replicate and purify to homogeneity by standard chromatographic methods. One great advantage of DNA vaccines is that they are cost-effective, have an easy of production process and can be repetitively administered due to the immune system not reacting against DNA vector.

Listeria monocytogenes *Technology: Listeria monocytogenes* (Lm) is another example of a therapeutic cancer vaccine that integrates the usage of Lm to produce an immune response to T cells directed at tumour cells. The technology of Lm uses live, attenuated strains of Lm as a vector for delivering biomarkers introduced to the body. The uniqueness of Lm is due to its ability to induce strong responses to MHC I and II, produce a potent CD8+ and CD4+ response. The protein of Lm, specifically the listeriolysin-O (LLO), is the most virulence factor that could stimulate the production of proinflammatory cytokines and exhibit a pathogen-associated molecular pattern (PAMP). Researchers can combine genetic biomarkers to a non-functional truncated form of LLO and enhance immunogenicity to antigens (Mkrtichyan *et al.*, 2013; Wallecha *et al.*, 2013).

Mkrtichyan *et al.* (Mkrtichyan *et al.*, 2013) used Lm Technology and an anti-PD-1 (anti-programmed-death receptor 1) antibody as a combination, which increased the therapeutic efficacy of LLO immunotherapy, and this was demonstrated in their preclinical study. The study demonstrated a substantial reduction in myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg). The use of anti-PD-1 antibodies showed an increase in CD8 T cell infiltration into the tumor and antigen-specific immune response peripherally (Wallecha *et al.*, 2013). Axalimogene filolisbac, previously known as ADXS-HPV, is a therapy that uses Lm Technology immunotherapy. Axalimogene filolisbac, is a vaccine that targets HPV-associated cancers and is currently undergoing clinical trials as an FDA-designated orphan drug for invasive cervical, neck and head, and anal cancers (Maciag, Radulovic, & Rothman, 2009). There are also two other immunotherapy vaccines currently under investigation, including ADXS-HER2 in HER2+ solid tumours and ADXS-PSA for use in prostate cancer.

2.4. Application of self-replicating RNA viruses and self-replicating RNA for cancer vaccine development

A usual feature of RNA self-replication in viruses is strongly related to their single-stranded RNA, also known as ssRNA. A protein envelope surrounds the ssRNA genome with an exterior structure of a capsid core. The purpose of ssRNA is to utilise its genome as a messenger RNA (mRNA) to precisely translate viral proteins that can draw in microRNAs (miRNAs) transcribed by the virus or host to interact with their genome and adjust the viral life cycle. Virus types have different genomes (Hannan *et al.*, 2012; Shahabi, Seavey, Maciag, Rivera, & Wallecha, 2011). As such, in the flavivirus and alphavirus, the genome possesses a positive polarity; for rhabdovirus and measles virus, their genome possesses a negative ssRNA. It has been observed in alphavirus that the genome consisting of four non-structural genes (nsP1-4) is responsible for the capsid and envelope proteins' genes and RNA self-replication (Strauss & Strauss, 1994). The engineered alphavirus vectors can produce replication-proficient and replication-deficient particles' recombinants appropriate for transgene expression in vivo and cell lines. Hence, because of these, other alphavirus vectors design, it can carry out study's recombinant viral particles, naked RNA replicons and layered DNA-RNA vectors (Lundstrom, 2018b). In flavivirus, the RNA self-replication is constructed differently as opposed to alphaviruses. In alphaviruses, the interested gene is implanted down-stream of non-structural genes. However, for flaviviruses, it is between the last 60 nucleotides of the 22 codons of the E22 envelope protein in frame with the viral polyprotein and the first 60 nucleotides of the C20 core proteins (Abd El Fattah, Abulsoud, AbdelHamid, & Hamdy, 2022; Hashemi *et al.*, 2022).

ssRNA genome possessing a negative polarity, such as the measles viruses, the packaging systems needed to engineer to release measles virus replication from cloned DNA expression forms (Radecke *et al.*, 1995). The recombinant measles virus release

has been based on a helper cell line by reverse genetics. To produce the measles virus' recombinant particles, the helper cell line is transfected with a plasmid comprising the measles virus pol-ymerase L gene and the measles viruses' recombinant particles formed. The expression vectors carrying the measles virus's structural protein downstream of T7 RNA polymerase promoter have been designed to introduce foreign genes between the large protein L and the hemagglutinin HA or otherwise between the matrix protein M and phosphoprotein P. When reaching about 80-90% effect of their cytopathic, the measles virus' recombinant is harvested three days after transfection. In rhabdoviruses, also a genome possessing a negative ssRNA, the re-quired application of reverse genetics is based upon a recombinant vaccinia virus vector-based an efficient transgene expression, as for measles viruses. Where both vesicular stomatitis virus and rabies virus have been subjected to expression vector engineered. When the vesicular stomatitis virus P, L and N genes were implanted downstream of an internal ribosome entry site and T7 promoter, effective retrieval of vesicular stomatitis virus was acquired from the transfected DNA in a vaccinia virus-free system (Dorange *et al.*, 2004). In similarity to rabies virus, the vectors have been engineered to introduce the gene of interest between P and rabies virus N genes. A retravel of rabies virus from cloned cDNA has been attained in a vaccinia virus-free reverse genetic system (Ito *et al.*, 2003). In summary, reverse genetics and packaging cell lines are necessary for the negative stranded viruses to produce engineered replicons. On the other hand, in the case of positive-stranded viruses, the intermediate DNA vectors and the in-vitro transcription method, can sufficiently be used to produce self-replicating RNA.

To produce self-replicating RNA vectors from the viruses mentioned above, the non-structural gene replicas remain untouched. At the same time, the selected antigens are replaced with structural genes. The non-structural protein genes encoding the viral replicas complex are the containment of these replicons. The production of self-replicating RNA vaccines can be created in three ways. DNA utilisation intermediate, the production of viral replicon particles and synthetic self-replicating RNA replicons. In the DNA utilisation intermediate, the vaccine is used as a self-replicating RNA vector encoded into a DNA construct. However, few successes are achieved in such form due to the incapability to efficiently transduce cells with DNA in vivo, as observed in the study by Geall *et al.* (Blakney, McKay, Yus, Aldon, & Shattock, 2019; Geall *et al.*, 2012; Lambeck *et al.*, 2010; Lundstrom, 2018a; Ying *et al.*, 1999). In the production of viral replicon particles, the transduction is optimised to produce viral replicon particles. However, this method immune responses as opposed to the viral replicon particles. Such method is not applicable due to the alteration of responses to various encoded antigens or/and obstructs with future usage of a specific self-replicating RNA viral replicon particles vaccine. Lastly, the production of synthetic self-replicating RNA replicons. A completely cell-free in-vitro method that is highly efficient, highly scalable, and can provide the benefit of not producing immunity as opposed to the structural viral replicon particles antigens (Colmenero, Chen, Castaños-Velez, Liljeström, & Jondal, 2002; Crosby *et al.*, 2019; Maine *et al.*, 2021; Ni *et al.*, 2004; Osada *et al.*, 2012). The production of synthetic self-replicating RNA replicons is an approach that is still being currently researched. The following list of published pre-clinical studies used various designs for self-replicating RNA vaccine platforms in the treatment of cancers (Avogadri *et al.*, 2010; Daemen, Regts, Holtrop, & Wilschut, 2002; Lambeck *et al.*, 2010; Leitner, Bergmann-Leitner, Hwang, & Restifo, 2006).

2.5. LncRNA CDC144NL-AS1 as a potential target for cancer therapy

Immunotherapy is one of the most promising areas of investigation and development for cancer treatment. While immune checkpoint-blocking monoclonal antibodies and chimeric antigen receptor (CAR) T-cell-based therapy have selectively provided valuable therapeutic options, the goal of cure has not yet been achieved for most malignancies. Further efforts are required on this front. Noncoding RNAs (ncRNA), including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) regulate several biological processes via selective targeting of crucial molecular signaling pathways. Recently, the critical roles of miRNA and lncRNAs as regulators of the immune-response in cancer have progressively emerged, since they may act (i) by shaping the intrinsic tumor cell and microenvironment (TME) properties; (ii) by regulating angiogenesis, immune-escape, epitheli-al-to-mesenchymal transition, invasion and drug resistance;

and (iii) by acting as potential biomarkers for prognostic assessment and prediction of response to immunotherapy (Di Martino *et al.*, 2021).

Genome-wide transcriptome analysis indicates that about 98% of eukaryotic genomes are transcribed as ncRNAs while a small fraction (≈2%) translates into proteins (Abulwerdi *et al.*, 2019; Kapranov, Willingham, & Gingeras, 2007). NcRNAs are a class of functional RNA molecules without protein-coding abilities. They include "house-keeping" RNAs such as ribosomal RNA (rRNA) and transfer RNA (tRNA), as well as regulatory RNAs. Based on transcript length, regulatory RNAs are divided into two groups: small ncRNAs with <200 nu-cleotides (nt) and lncRNAs, the most abundant class, with >200 nt lengths (Carninci *et al.*, 2005; Seal *et al.*, 2020). In the past, ncRNAs were considered "evolutionary junk." Still, growing evidence suggests that this dark matter of the genome can regulate several biological processes via selective targeting of crucial molecular pathways (Hüttenhofer, Schattner, & Polacek, 2005). MiRNAs, the widely explored group of small ncRNAs, are encoded at various locations as autonomous or clustered transcriptional units (Saini, Griffiths-Jones, & Enright, 2007). They are transcribed by RNA polymerase II (Pol II) in primary miRNA transcripts (pri-mRNAs) and then converted by the endonuclease DROSHA and its cofactor DGCR8 in pre-miRNA transcripts (Carthew & Sontheimer, 2009). Pre-miRNAs are generated in the nucleus from introns through the splicing machinery (Ruby, Jan, & Bartel, 2007). They are exported by exportin 5 into the cytosol (Bohnsack, Czaplinski, & Görlich, 2004), where they are processed by the RNAse III enzyme DICER and its partner binding protein TRBP (Hutvagner *et al.*, 2001). The result is the formation of mature miRNA/miRNA duplexes, which are rapidly unwinded by an argonaute protein (AGO). The passenger strand (miRNA) is degraded. In contrast, the guide strand (mature miRNA) binds to AGO and additional proteins (Kawamata, Seitz, & Tomari, 2009) to form the mi-croRNA-induced silencing complex (miRISC) (Kawamata *et al.*, 2009). The main function of miRNAs is the repression of gene expression by binding to the 3′-untranslated regions of target mRNAs (Hibio, Hino, Shimizu, Nagata, & Ui-Tei, 2012). Gene silencing can occur through mRNA destabilization or inhibition of translation (Eulalio, Huntzinger, & Izaurralde, 2008). However, in addition to the conventional role in posttranscriptional gene regulation, miRNAs can upregulate target translation by recruiting ribonucleoprotein complexes (Vasudevan, Tong, & Steitz, 2007). MiRNAs are also present in body fluids such as blood, plasma, and urine, where they are associated with carriers or incorporated into vesicles and microparticles (Gupta, Bang, & Thum, 2010). Circulating miRNAs act as signaling molecules transferring their cargo between cells or tissues (Viereck, Bang, Foinquinos, & Thum, 2014). Compared to miRNAs, lncRNAs can regulate gene expression at multiple levels in the cell.

This section provides an overview of the role of LncRNAs in modulating the immune response and the TME since LncRNAs could be used as potential biomarkers or targets for the development of new therapeutics for the clinical treatment of human cancer.

LncRNAs, in general, and CCDC144NL-AS1 contribute to the progression and metastasis of numerous cancers. CCDC144NL-AS1 is a novel upregulated oncogene being investigated in a few types of human cancers and plays a significant role in the advancement of these malignant tumors through ceRNA networks, competing with their target miRNAs (to be identified via bioinformatics tools) to affect multiple signaling pathways, as presented in Fig. 3 (Abd El Fattah *et al.*, 2022). In addition, its inhibition significantly repressed the migration, proliferation and invasion of various cancer cells, pointing to the possibility of developing competitive inhibitors toward CCDC144NL-AS1 as a possible therapeutic target for cancer. Studies about CCDC144NL-AS1 in cancer provide the opportunity of being a target for cancer therapy. In GC, inhibition of CCDC144NL-AS1 in vivo enhances cell apoptosis and reduces metastasis and growth of GC tumors, indicating that CCDC144NL-AS1 may be a target for GC treatment (Fan *et al.*, 2020). In vitro studies revealed that CCDC144NL-AS1 knockdown suppresses the proliferation of osteosarcoma cells, invasion, and migration and increases apoptosis rate. In tumor xenograft mice models, downregulation of CCDC144NL-AS1 significantly reduces osteosarcoma tumor growth (He *et al.*, 2021). Upon using the mice model, Zhang *et al.* noticed that targeting CCDC144NL-AS1/WDR5 or upregulating miR-940 could all inhibit the proliferation of HCC and enhance HCC prognosis in mice, signifying CCDC144NL-AS1/miR-940/WDR5 axis could act as a potential therapeutic target for HCC (Zhang, Zhang, & Wu, 2021).

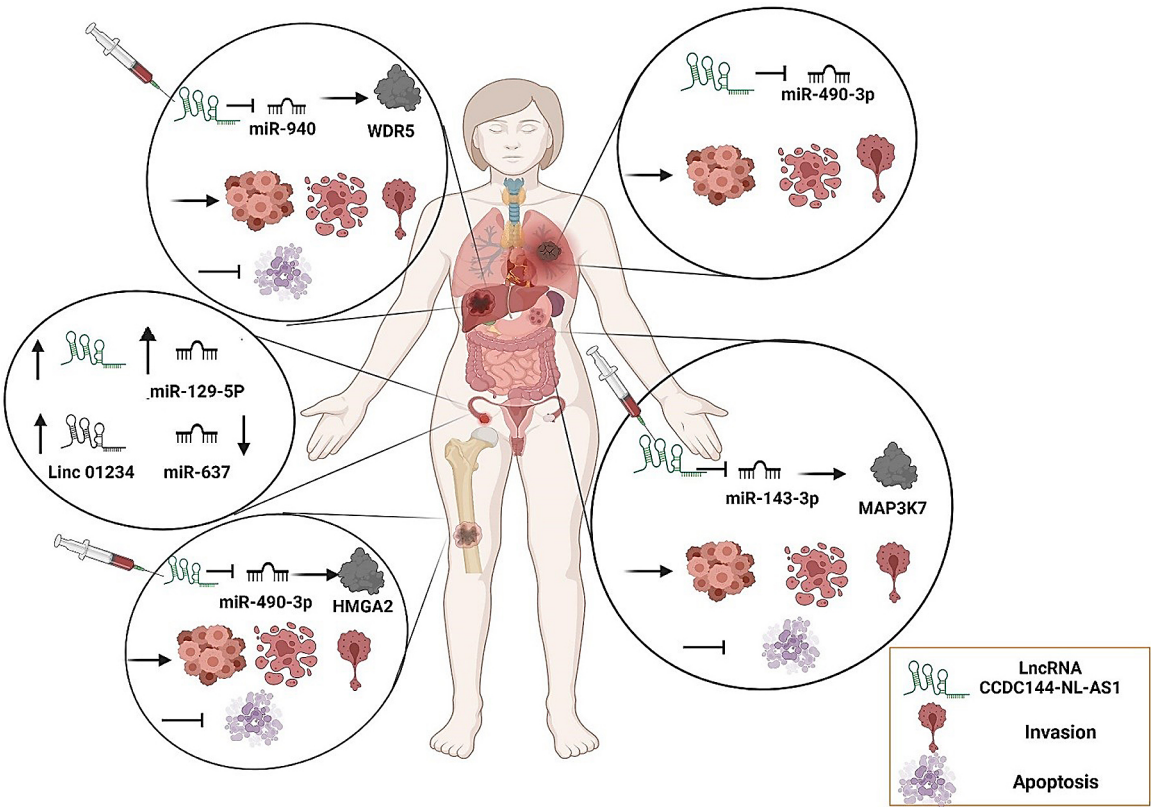
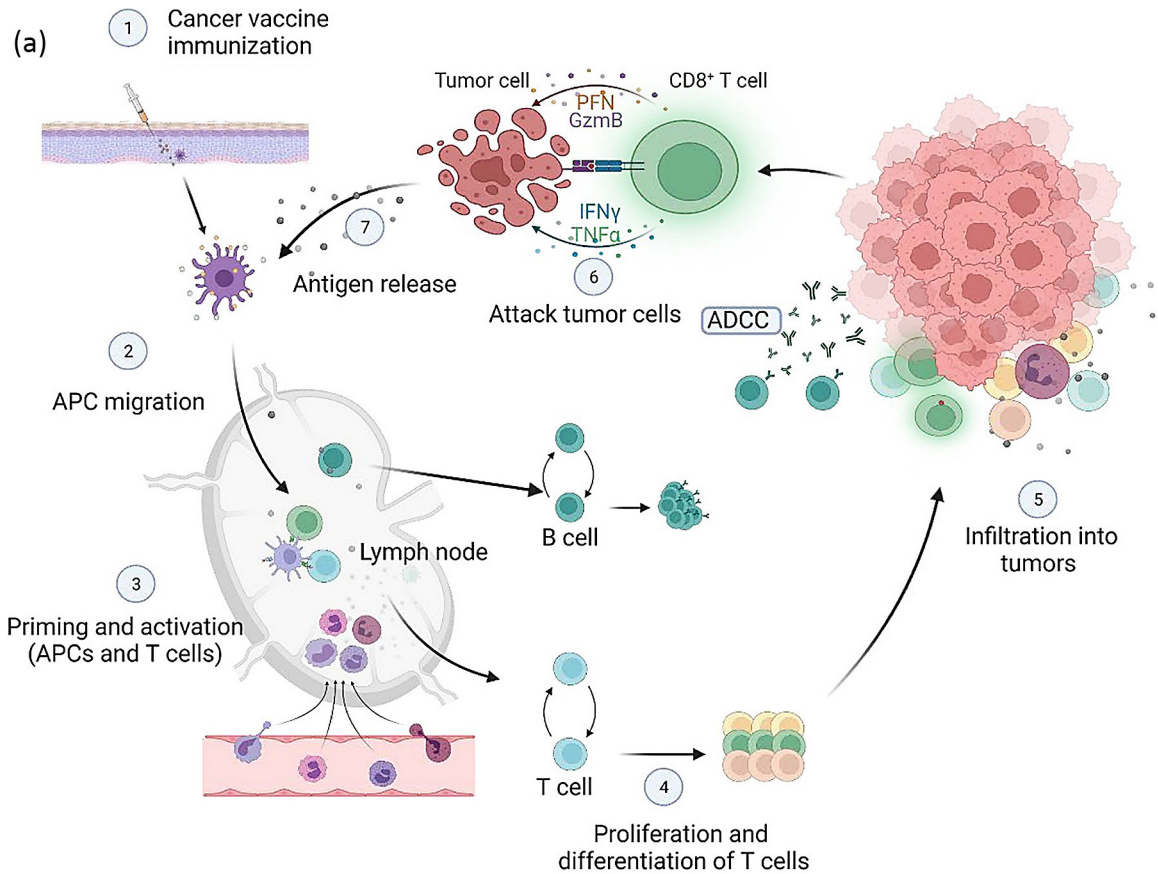


Figure 3. Effect of lncRNA CCDC144NL-AS1 in multiple signaling pathways and the possibility of being a thera-peutic target for cancer. (lncRNA CCDC144NL-AS1 plays a significant role in the progression of different ma-lignant tumors through ceRNA networks, competing with their target miRNAs; Non-small cell lung cancer: CCDC144NL-AS1 sponge miR-490-3p, resulting in proliferation, migration and invasion of tumor, Ovarian cancer: miR-637 is downregulated, while CCDC144NL-AS1 and LINC01234, as well as miR-129-5p, were found to be upregulated, Hepatocellular Carcinoma: sponge miR-940, resulting in upregulated expression of its target WDR5 promoting proliferation, migration, invasion, and inhibiting apoptosis. CCDC144NL-AS1/miR-940/WDR5 axis could be a potential therapeutic target for HCC, Gastric cancer: sponge miR-143-3p in CCDC144NL-AS1/miR-143-3p/ MAP3K7 axis, resulting in proliferation, migration, invasion, and inhibiting apoptosis. Inhibition of CCDC144NL-AS1 in vivo enhances cell apoptosis. It reduces metastasis and growth of GC tumors, indicating that CCDC144NL-AS1 may be a target for GC therapy, Osteosarcoma: sponge miR-490-3p in CCDC144NL-AS1/miR-490-3p/HMGA2 axis, promoting proliferation, migration, invasion, and inhibiting apoptosis. CCDC144NL-AS1 knockdown suppresses the proliferation of osteosarcoma cells, invasion, and migration, and increases apoptosis, indicating that CCDC144NL-AS1 may be a therapeutic target for os-teosarcoma) (Abd El Fattah *et al.,* 2022).

3. ANTIGEN SELECTION FOR CANCER VACCINES DESIGN

For the creation of cancer vaccines, antigen selection is a crucial step. Cancer vaccination's effectiveness depends heavily on T lymphocytes' ability to identify tumour antigens (Giaccone *et al.*, 2015; Jian Liu *et al.*, 2022). A cancer vaccine's ideal antigen should be highly immunogenic, explicitly expressed in all cancer cells (but not in normal cells) and essential for cancer cells to survive (Coulie, Van den Eynde, Van Der Bruggen, & Boon, 2014). TAAs and TSAs are two categories of tumour antigens. Tumor-shared antigens are another name for TAAs. Differentiated antigens, overexpressed antigens, cancer-testicular antigens and viral-derived "non-self" antigens are examples of "self-antigens" that are included in TAAs (Hollingsworth & Jansen, 2019). The most crucial are dendritic cells (DCs) because they are a vital link between innate

immunity and adaptive immunity. Initial antigen presenters, DCs can acquire and cross-present antigens on MHC I molecules (Saxena *et al.*, 2021). Immature DCs are very good at recognising and phagocytosing antigens via micropinocytosis and phagocytosis. Toll-like receptor ligands may temporarily promote antigen-specific micropinocytosis in the tumour microenvironment (TME), which may improve the capacity of DCs to capture antigens with toll-like receptor ligand adjuvants. MHC I, MHC II, and costimulatory molecules on the surface of DCs can be elevated after antigen uptake, and they progres-sively lose their capacity to absorb antigens (Itano *et al.*, 2003; West *et al.*, 2004). The antigen-loaded DCs move to the draining lymph nodes, where T cell priming occurs most frequently. To naive CD4+ and CD8+ T lym-phocytes, mature DCs deliver the antigen epitopes on MHC I and MHC II molecules that have been processed (Roberts *et al.*, 2016; Sallusto, Cella, Danieli, & Lanzavecchia, 1995). Additionally, to boost the synthesis of costimulatory factors, DCs secrete IL-12 and interferon (IFN) (Wculek *et al.*, 2020). By interacting with the MHC-peptide complex-T cell receptor and costimulatory "signal 2," tumor-specific T cells are activated. Then, activated T cells undergo differentiation to become effectors and long-lasting memory T cells. To stimulate tumour destruction by cytotoxicity and the generation of effector cytokines, tumor-specific T lymphocytes multiply and are transported to TME (Chudnovskiy, Pasqual, & Victora, 2019). Additionally, through comple-ment-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC), activated B cells encourage tumour death (Sautès-Fridman, Petitprez, Calderaro, & Fridman, 2019). Additionally, tumour antigens and damage-related molecular patterns are released by immunogenic cell death (Fucikova *et al.*, 2020). To increase the antigenic breadth of anti-tumor-immune responses, the tumour antigens released by lysed tumour cells can then be collected, processed, and re-presented by antigen-presenting cells (APCs) to trigger polyclonal T cell responses (Ott *et al.*, 2020). The cycle of cancer and immunity refers to these processes (D. S. Chen & Mellman, 2013).



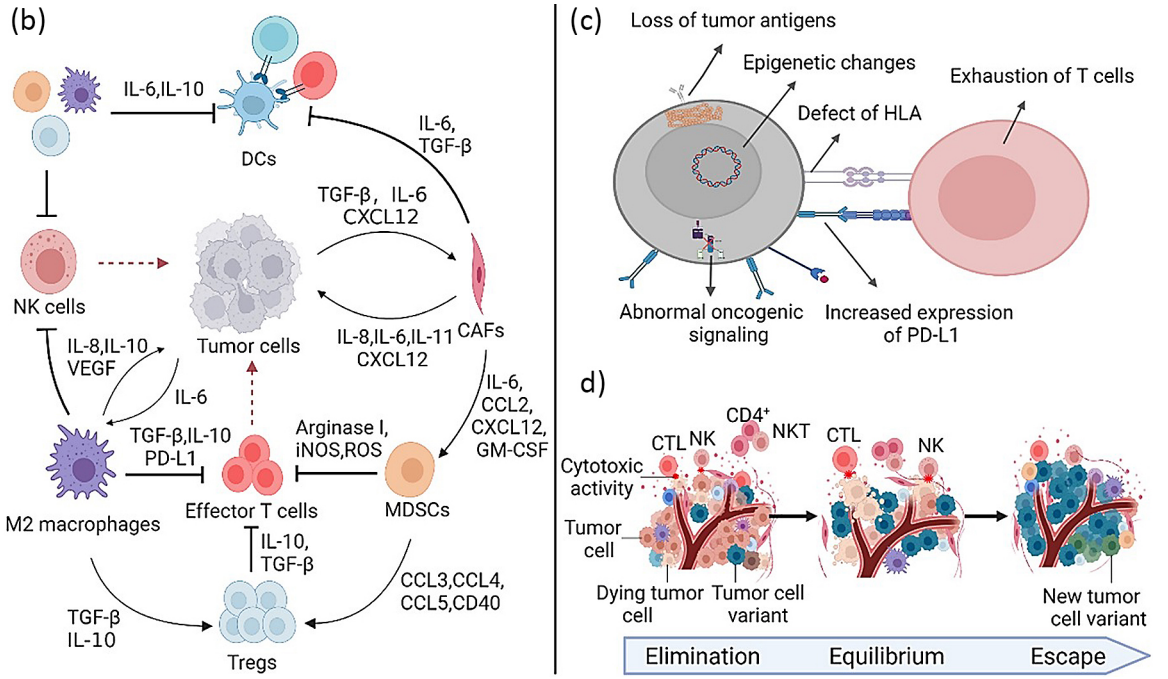


Figure 4. (a) Cancer vaccinations activate the tumor-immune cycle. The tumor-immune cycle is the steps that enable repetition and expansion during the immune response that successfully destroys tumour cells. When the cancer vaccine is administered, DCs analyze the tumour antigens before presenting them to MHC II or MHC I. (through cross-presentation). DCs carrying antigens move to lymph nodes to attract and stimulate immune cells. Memory B cells and plasma cells are generated more quickly thanks to follicular DCs. Through ADCC, activated B lymphocytes support tumour death. Activated T cells multiply and develop into effector and memory T cells. Traveling to the TME, effector T cells either directly destroy tumour cells or cause tumour cell death. The release of TAAs and danger signalling molecules by immunogenic dead tumour cells can broaden and deepen the response in succeeding cycles and overcome the resistance to cancer vaccines. (b) External tumour resistance. Anti-immunoglobulin cells (c) Resistance inherent to the tumour (d) Immune selection: from tumour escape to immune surveillance (Jian Liu *et al.,* 2022).

CD4+ T cells work in coordination with various immune cells. CD4+ T cells trigger continuous T cell initiation, expansion and antigen spread, thus expanding the anti-tumor T cell repertoire (Melief, 2015; Sahin & Türeci, 2018). IFN-γ secreted by T1 CD4+ T cells upregulates MHC I on tumor cells, improving the killing effector of CD8+ T cells. Furthermore, T1 CD4+ T cells promote the inflammatory microenvironment by acting on various immune cells in tumours. CD4+ T cells also control the differentiation of CD8+ T effector cells. Cytotoxic T lymphocytes (CTLs) are crucial for killing tumour cells and presenting their cognate antigen (Halle, Halle, & Förster, 2017). After antigen receptor-mediated activation, CD8+ T cells proliferate and differentiate into effector cells called CTLs. Activated CTLs will penetrate the core of the tumour or infiltrate the site to kill tumour cells. The number of CTLs in TME is a critical prognostic marker of cancer. CTLs detect tumour cells presenting target antigens and attack target cells through different mechanisms (Thomas & Massagué, 2005). First, CTLs could kill cancer cells by producing and releasing cytotoxic particles such as perforin and granzymes. Furthermore, CTLs induce apoptosis of target cells through Fas ligand (FasL)-mediated interactions (Borst, Ahrends, Bąbała, Melief, & Kastenmüller, 2018). In addition, the release of IFN-γ and tumour necrosis factor α (TNF-α) by CTLs induces cytotoxicity of cancer cells [41]. IFN-γ could inhibit the angiogenesis of cancer cells and cause macrophage polarity to M1 cells. IFN-γ produced by CTLs supports their further differentiation into effector CTLs (van der Burg, 2018). In summary, cancer vaccines eradicate tumour cells mainly by activating cellular immunity, and cancer vaccines start the cancer-immunity cycle to play a persistent anti-tumour role (Fig. 4).

3.1. Optimizing Antigenic Targets

In this section, a summary of work on optimizing antigen targets in the development of therapeutic cancer vaccine strategies has been discussed. According to Buonaguro *et al.,* (Buonaguro & Tagliamonte, 2020) pep-tides can be modified to increase their affinity and binding to the present MHC-I, improving the immunogenic-ity of tumor antigens, mainly the TAAs. Such modified peptides (heteroclitic peptides) have been shown to break the immunological tolerance, inducing a more potent CD8+ T cell response that can recognise the native peptide expressed on the tumor cells and kill them. The low affinity between the T cell receptor (TCR) and the peptide-major histocompatibility complex (pMHC) would allow the TCR to cross-react with multiple pMHCs (Buonaguro & Tagliamonte, 2020).

3.1.1. Heteroclitic Peptides Improving Binding to MHC-I

Most of the studies have described improving the CD8+ T cell response by modifying the amino acid residues in the anchor positions interacting with the HLA molecule (Dao *et al.,* 2017; Dyson, 2015; Madura *et al.,* 2015).

In the study conducted by Buonaguro *et al.* (Buonaguro & Tagliamonte, 2020), a peptide derived from gp100, a lineage differentiation antigen identified in melanoma, was modified (heteroclitic) to optimise its bind to the MHC complex. This modified peptide, gp100:209–217(210 M), binds with a higher affinity to HLA-A2 and the corresponding wt peptide that stimulates a specific and better T cell response in vitro in vivo. Clinical trials based on vaccination with 210 M antigen, alone or combined with interleukin-2 (IL-2), have demonstrated the induction of peptide- and tumor-specific cytotoxic T-lymphocyte responses in peripheral blood (Sosman *et al.,* 2008). In particular, a randomized phase III clinical trial, based on the 210 M peptide vaccine, showed that in the group treated with gp100 peptide vaccine followed by high-dose interleukin-2, the response rate was higher and progression-free survival longer than in the group treated with interleukin-2 alone (Schwartzentruber *et al.,* 2011).

Another modified peptide, CAP1-6D, an epitope of CEA, was modified to improve the binding to the MHC-I complex and has been shown to trigger a more potent CTL response, and T cells activated are cross-reactive with wild-type CAP1 and to recognize CEA+ HLA-A2+ tumor cells (Tsang *et al.,* 1997).

3.1.2. Heteroclitic Peptides Improving Binding to TCR

An alternative approach for improving the immunogenicity of natural TAAs is to generate heteroclitic peptides with mutations in the TCR-binding residues to break the immunological tolerance and induce a more potent CD8+ T cell response (Binkowski, Marino, & Joachimiak, 2012). Heteroclitic peptides modified in the TCR-binding residues of melanoma specific Trp2 TAA have been shown to improve the control of tumor growth (Capasso *et al.,* 2017). Preliminary results from Buonaguro *et al.* (Buonaguro & Tagliamonte, 2020) showed that the recognition of wild-type (WT) epitope by Peripheral blood mononuclear cells (PBMCs) can be significantly improved by modifying the TCR-facing amino acids, in particular at the P4 residue, of the HPV E7 WT epitope expressed on TC1 mouse lung tumor cell lines. Bioinformatics prediction algorithms identified spe-cific amino acid substitutions at the P3 and P4 residues of the epitope, resulting in an increased affinity of the WT peptide to the H-2-Db allele. Moreover, heteroclitic peptides with amino acid changes in one of the TCR-facing and anchor position residues elicit an even more robust immune response, cross-reacting with the parental wild-type peptide. CTL elicited by the heteroclitic peptides shows potent lytic activity on target cells expressing the WT peptide and control of tumor growth in vivo (Buonaguro & Tagliamonte, 2020).

4. ONGOING CLINICAL TRIALS

4.1. DNA-based vaccines

DNA vaccines are typically provided following the standard of care for each form of cancer, including surgical ablation, radiotherapy, and/or chemotherapy (C. Guo *et al.,* 2013; Lopes, Vandermeulen, & Préat, 2019; Ott *et al.,* 2020; Sahin *et al.,* 2017; Schlom, 2012). In the past ten years, a different study with the search terms “DNA electroporation” and “cancer” generated 3 further studies (NCT03499795, NCT03491683, and NCT02301754), each with varying requirements of enrollment. The terms “plasmid” and “tumour” led to the discovery of two further studies,

NCT02531425 and NCT03502785. Two phases III studies (NCT03721978 and NCT03185013) employing VGX-3100 administered via IM EP against cervical cancer are of relevance. The trials continue to focus primarily on breast, prostate, and cervical cancer (Fig. 5a). Most vaccinations contain well-known TAAs, such as the prostatic acid phosphatase (PAP) for prostate cancer and the Mam-A or HER2 protein for breast cancer (G. Chen *et al.*, 2022). According to Fig. 5b, only 17% of clinical studies (including NCT02348320 and NCT03122106) employed personalized/neoantigen vaccinations. Since 80% of the neoantigen studies began in 2018–2019, this number has climbed recently. In both TAA and neoantigen vaccinations, the DNA vaccines typically encode more than one epitope (Lambricht *et al.*, 2016; Obara *et al.*, 2018; von Mehren *et al.*, 2001; Wang *et al.*, 2021).

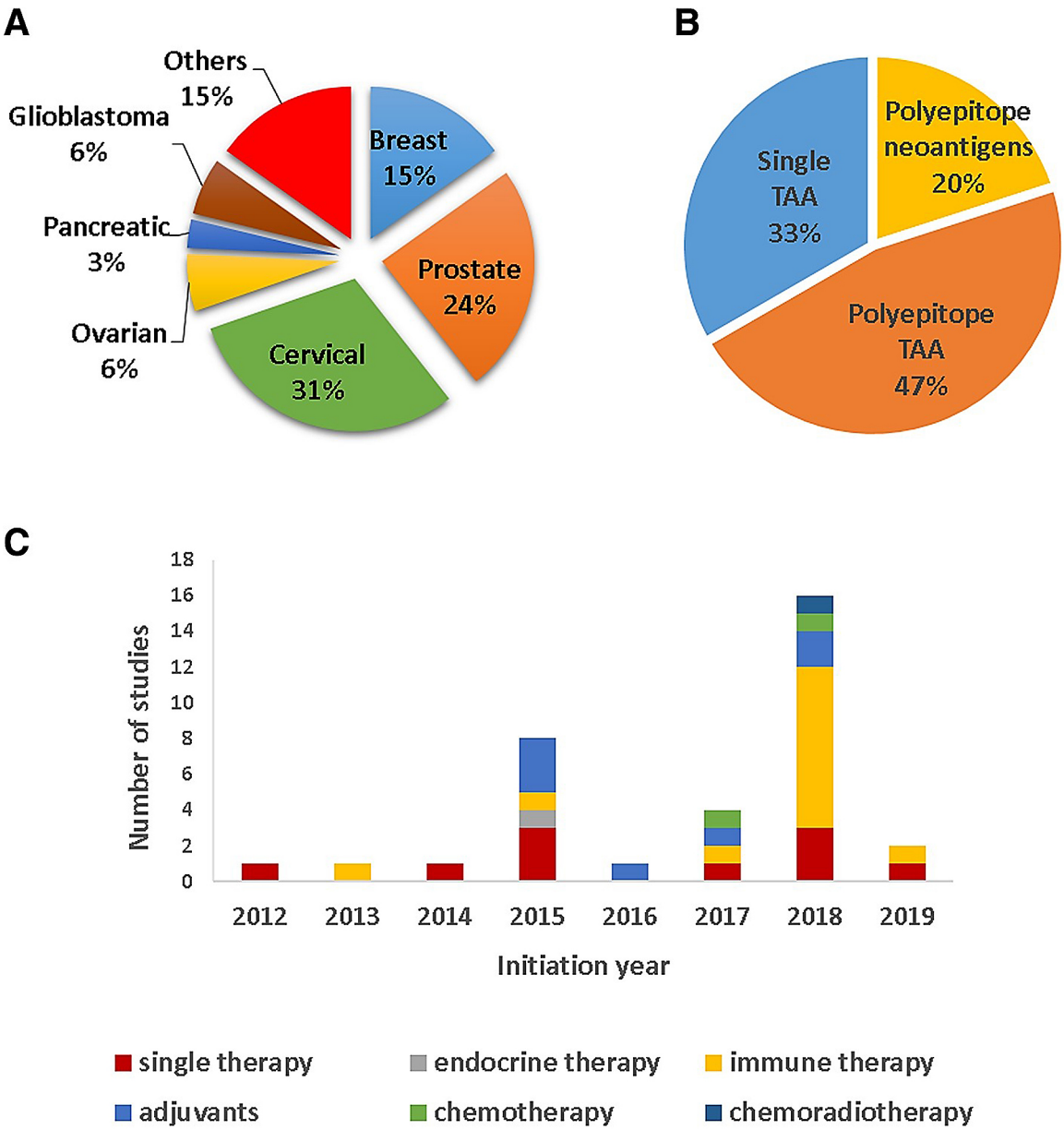


Figure 5. ongoing clinical trials for the studies that were examined. Cancer kinds that are testing cancer DNA vaccines. b The DNA vaccine's antigen type encoding. Studies employing cancer DNA vaccines as a single therapy or in combination with other treatments (such as adjuvants, adjuvant chemotherapy, adjuvant immunotherapy, or adjuvant endocrine therapy) (Lopes *et al.*, 2019).

DNA vaccines are typically used in combination with other therapies, such as immunotherapies (antibodies against HER2, CTLA4, PD1, PD-L1, and cell vaccines), immune adjuvants (GM-CSF, hIL-12, etc.), chemo-therapy (carboplatin, paclitaxel, cyclophosphamide), and endocrine therapies (anastrozole, letrozole, tamoxifen, exemestane, and goserelin). Studies combining DNA vaccines and other medicines have become more prevalent in recent years (Fig. 5c). DNA vaccines are often injected intramuscularly (IM) or intradermally (ID), seldom SC, and rarely in the lesion or tumour, and then electroporated. 100 g to a few mg can be given as a dosage. The delivery schedule varies depending on the vaccination type. Clinical research has now demonstrated that indi-viduals undergoing less prior chemotherapy often respond better to vaccinations (Schlom, 2012). As a result, vaccinating individuals with incipient growth of the tumour may lead to noticeably better outcomes (Gulley, Madan, & Schlom, 2011), emphasizing the significance of choosing the right patient populations for inclusion in randomised vaccine trials. Surprisingly, the vaccine therapy mechanism of action and the timing of clinical responses seem to be very different from chemotherapy (Stein *et al.*, 2011). It might be accounted for by the time required to initiate the immune response, followed by ongoing tumour cell eradication and cross-priming of Teff reactive with other TAAs. Therefore, even while patients do not exhibit significant decreases in tumour burden or an increase in relapse-free survival, the anticancer activity of vaccine-induced immune activation over a pro-longed period leads to a slower tumour growth rate and improved OS (Madan, Gulley, Fojo, & Dahut, 2010). Similar results have been reported in clinical trials investigating the use of ipilimumab for the treatment of met-astatic melanoma, where those who received the drug had a statistically significant improvement in OS without a statistically significant change in time to progression (Hodi *et al.*, 2003). These findings suggest clinical responses to vaccination treatment or immunotherapy may not be adequately assessed using established response criteria. The original purpose of the RECIST criteria, or classic response evaluation criteria in solid tumours, was to track patients receiving cytotoxic chemotherapies (Therasse, Eisenhauer, & Verweij, 2006). To more accurately cat-egorise and assess clinical activity, new standards or "immune response criteria" for immunotherapeutic activity in solid tumours have been devised (Wolchok, Yang, & Weber, 2010). The study of immune infiltrates in cancer biopsies and the "immune signature" is independent predictors of survival in numerous studies (Ascierto *et al.*, 2012; Camus *et al.*, 2009; Galon *et al.*, 2006; Grimmett *et al.*, 2022). Future work should focus on finding and validating diagnostic biomarkers responding to vaccination therapy. The clinical development of therapeutic cancer vaccines will be considerably aided by knowledge of the biomarkers of immunological and clinical re-sponsiveness to effective treatment (Z. S. Guo *et al.*, 2019; Paavilainen-Mäntymäki & Van Mumford).

4.2. mRNA-based cancer vaccine trials

In 1996, an in-vitro study tested dendritic cells pulsed with RNA as a first effort towards the mRNA-based cancer vaccine. echnological advances have led to optimised mRNA structure, stability and delivery methods, and multiple clinical trials are now enrolling patients with cancer for mRNA-based vaccine treatments (Table 1). MRNA-based vaccination aims to induce or boost an effective anti-tumour immune response. Synthetic mRNA encoding tumour-associated or tumour-specific antigens are delivered through autologous dendritic cells engi-neered with mRNA ex vivo or through formulated or non-formulated mRNA injections (Lorentzen, Haanen, Met, & Svane, 2022). After vaccination and cellular uptake by antigen-presenting cells, mRNA is transported to the cytoplasm, undergoes antigen processing, and enters the MHC presentation cascade. Thus, antigen-presenting cells present tumour-associated antigens on MHC class I and MHC class II to activate CD8+ and CD4+ T cells. In addition, CD4+ T cells can coactivate antigen-specific B cells and induce a humoral immune response. B cells that function as antigen-presenting cells can conversely activate CD4+ T cells after internalization of extracellular proteins and presentation on B cells' MHC class II (Miao, Zhang, & Huang, 2021; Mirjalili & Feig, 2013).

Several clinical trials (e.g., NCT04534205, NCT03313778, and NCT04503278) are enrolling patients for various mRNA-based cancer vaccine therapy studies to induce an mRNA-based anti-tumour response (Table 1).

	Trial phase	Target antigen	Cancer type	Combination	Vaccine route of administration	Sponsor
Lipid nanoparticle formulation						
NCT03948763	1	mRNA-5671 (KRAS gene driver mutations)	Non-small-cell lung, pancreatic, and colorectal neoplasms	With pembrolizumab	Intramuscular	Merck Sharp & Dohme
NCT03313778	1	mRNA-4157 (personalised cancer vaccine encoding several neoantigens)	Solid tumours (resected)	With pembrolizumab	Intramuscular	Moderna
NCT03897881	2	mRNA-4157 (personalised cancer vaccine encoding 20 different mutated neoepitopes)	Melanoma	With pembrolizumab	Intramuscular	Moderna
NCT04573140	1	Formulation with pp65 LAMP and tumour mRNA	Glioblastoma	None	Intravenous	University of Florida (Gainesville, FL, USA)
Lipoplex formulation						
NCT02410733	1	BNT111 (NY-ESO-1 [CTAG1A], tyrosinase, MAGE-A3, and TPTE)	Melanoma	None	Intravenous	BioNTech
NCT04526899	2	BNT111 (NY-ESO-1, tyrosinase, MAGE-A3, and TPTE)	Melanoma	With cemiplimab	Intravenous	BioNTech
NCT04382898	½	BNT112 (PAP, PSA, and three undisclosed antigens)	Prostate	With cemiplimab	Intravenous	BioNTech
NCT04534205	2	BNT113 (HPV16 E6 and E7 oncoproteins)	Head and neck squamous cell carcinoma	With pembrolizumab	Intravenous	BioNTech
NCT03418480	½	BNT113 (HPV16 E6 and E7 oncoproteins)	HPV16-positive solid tumours	With anti-CD40 antibodies	Intravenous	University of Southampton (Southampton, UK)
NCT05142189	1	BNT116 (non-small-cell lung cancer tumour-associated antigens)	Non-small-cell lung cancer	With cemiplimab plus docetaxel	Intravenous	BioNTech
NCT04486378	2	BNT122 (personalised cancer vaccine encoding individual tumour mutations)	Colorecta	None	Intravenous	BioNTech

	Trial phase	Target antigen	Cancer type	Combination	Vaccine route of administration	Sponsor
NCT02316457	1	BNT-114 plus BNT-122 (personalised set of premanufactured non-mutated shared tumour-associated antigens plus a personalised cancer vaccine encoding individual tumour mutations)	Triple-negative breast cancer	None	Intravenous	BioNTech
NCT04163094	1	BNT115 (ovarian cancer tumour-associated antigens)	Ovarian	With carboplatin plus paclitaxel	Intravenous	University Medical Center Groningen (Groningen, Netherlands)
NCT04161755	1	BNT122 (personalised cancer vaccine encoding individual tumour mutations)	Pancreatic	With oxaliplatin, irinotecan, fluorouracil, leucovorin, and atezolizumab	Intravenous	Memorial Sloan Kettering Cancer Center (New York, NY, USA)
NCT03815058	2	BNT122 (personalised cancer vaccine encoding individual tumour mutations)	Advanced melanoma W	With pembrolizumab	Intravenous	Genentech
NCT03289962	1	BNT122 (personalised cancer vaccine encoding individual tumour mutations)	Solid tumours	With atezolizumab	Intravenous	Genentech
NCT04503278	½	CARVac (CLDN6)	Solid tumours	With chimeric antigen receptor therapy	Intravenous	BioNTech and Gene Therapies

Table 1. ClinicalTrials.gov-registered mRNA-based cancer vaccine trials by type of formulation (Miao *et al.,* 2021)

https://doi.org/10.37819/bpb.008.03.0300

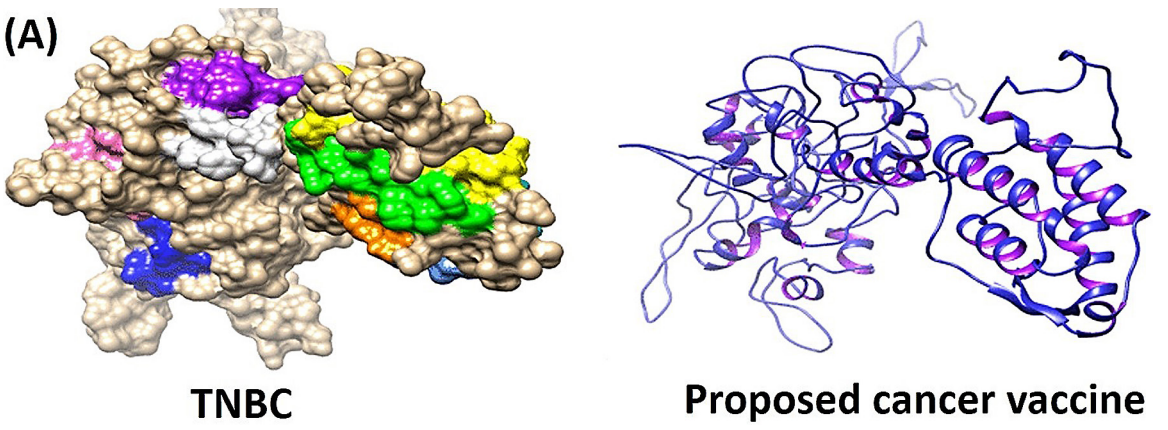
5. MATHEMATICAL AND SIMULATION-BASED STUDIES ON VACCINES FOR CANCER

Molecular Dynamics simulations, abbreviated as MD simulations, are widely used to construct or enhance structural models formed on experimental structural biology data (Mirjalili & Feig, 2013). MD simulations can give insight into the conformational changes of a molecule based on their time-depending non-local and local (McCammon, Gelin, & Karplus, 1977) phases. These conformational changes are used to elucidate biological processes at a molecular scale, for instance, the modelling of thermodynamics energies, analysis of binding interfaces, identification of vital binding epitopes and amino acids residues, and the design of novel molecules of immunological significance comprising of vaccines and drugs.

In recent years, a vaccine proposal was developed by Sepideh *et al.* (Parvizpour, Razmara, Pourseif, & Omidi, 2019) to combat the immunotherapy of TNB. TNBC, known as Triple Negative Breast Cancer, is one of the rarest cancers found in women and out of 100,000 individuals are likely to be affected. It is one of the most challenging breast cancers to treat. TNBC develops due to the absence of progesterone, estrogen and HER-2 receptors. Although in recent studies, it is believed that the TNBC can potentially be a cancer-testis antigen (CTA) positive tumour, suggesting that a treatment alternative is possible for patient-bearing through a cancer vaccine. In their proposed study, the approach was to design a multi-epitope peptide vaccine to fight against TNBC through immunoinformatics. Immunoinformatics is a method that combines experimental immunology and computer science to create computational immunology (Tomar & De, 2014). The construction of the vaccine peptide consisted of three important elements: the adjuvant, the helper epitopes and the CD8+ cytotoxic T lymphocytes CTLs. Proper linkers united these elements. The in-silico analyses consisted of an MD simulation study to refine the vaccine structure. The modelling approach used to predict the homology 3D-structure model of the vaccine peptide MODELLER v9.17 program was used, and based upon this analysis. The proposed vaccine can be treated for the immunotherapy of TNBC. One can refer to the work elsewhere for additional materials on the selection of CTL, CD8+, and CD4+ for sequences subjected to immunoinformatics analysis [93].

Furthermore, Kumar *et al.* (Kumar *et al.*, 2022) constructed a multi-epitope vaccine to combat TNBC where the cancer vaccine constituted of helper T-lymphocytes antigenic and the cytotoxic epitopes identified from the proteins test, selected for analysation, together fused with suitable linkers and an adjuvant. MD simulations and molecular docking were performed in the study, along with other analyzis performances (Oli *et al.*, 2020). Based on the proposed vaccine, it is believed to have means of obtaining the immune responses that could potentially be used to target TNBC in combination with other therapy or on its own. Fig.6 shows the TNBC 3D-structural model alongside its proposed vaccine.

For an overview on immunoinformatics and vaccine development, see work by Oli *et al.* (Oli *et al.*, 2020) and for a review that highlights the current efforts to determine the safety and efficacy of immunotherapeutic ap-proaches, see work by [95]. For a study on constructing a novel SOX9-based multi-epitope vaccine for TNBC using an immunoinformatics approach, see work by Rajendran *et*



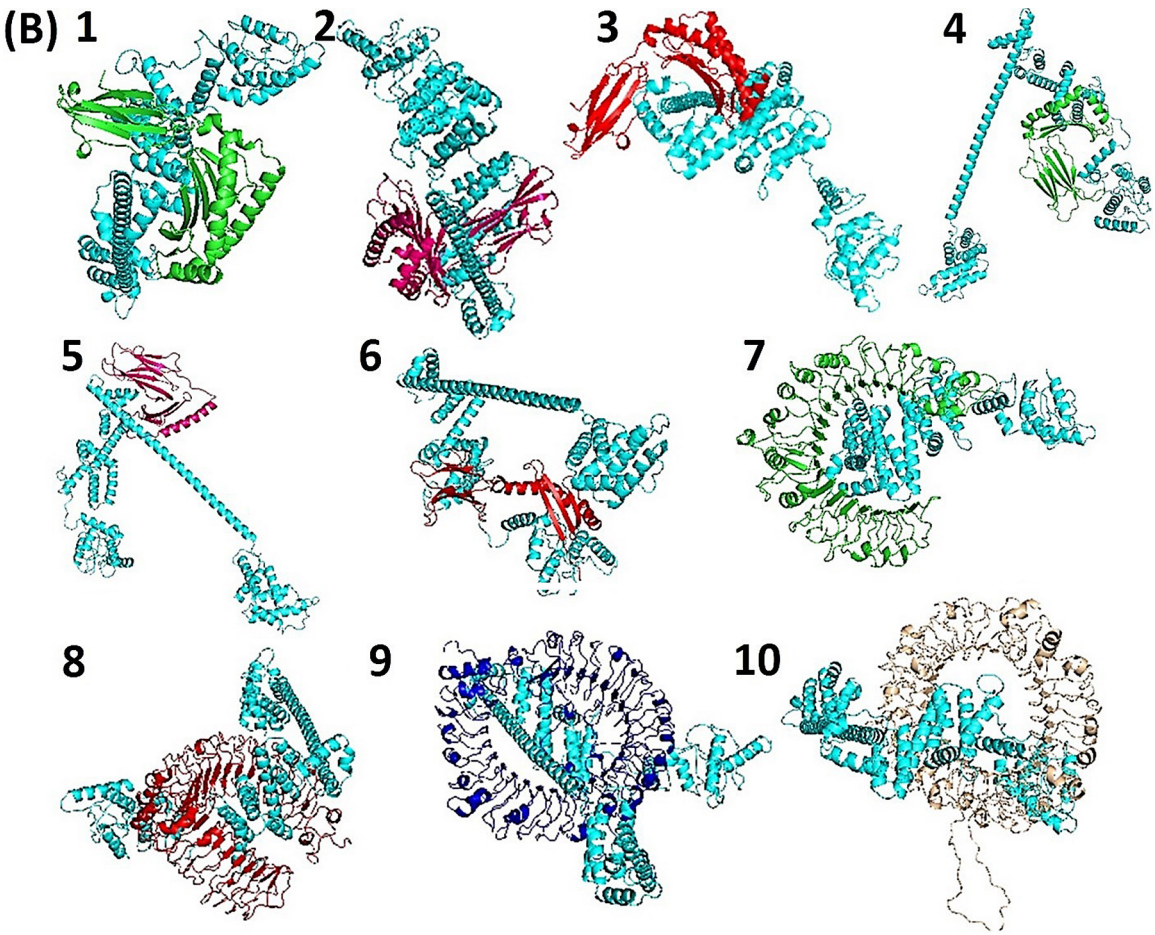


Figure 6. illustrate the 3D-structure of the constructed epitope-based vaccine against triple-negative breast cancer (TNBC). (A) TNBC model with proposed cancer vaccine image inspired by Parvizpour *et al.* and (B) Docking pose of vaccine build with targeted immune molecules where: 1. Shows the HLA-A allel, 2. HLA-B allel, 3. HLA-C allel, 4. HLA-DQB1, 5. HLA-DQA1, 6. HLA-DBR1, 7. TLR2 receptor, 8. TLR4 receptor, 9. TLR7 receptor and 10. TLR9 receptor. (Kumar *et al.,* 2022; Parvizpour *et al.,* 2019; Tomar & De, 2014).

al. (Abdou *et al*., 2022), and for a study on con-structing a multi-epitope vaccine against BLV virus using an immune and molecular dynamics simulation ap-proaches see work by Samad *et al.* (Rajendran Krishnamoorthy & Karuppasamy, 2022).

For many years, mathematical modelling has assisted scientists in understanding the dynamics and mechanisms behind experimental observations. Mathematical modelling facilitates an improved understanding of the systems as it can provide insights into complex processes implicated in biological systems by retrieving vital information. It also permits examining the effect of changes in its elements and the environmental conditions of systems be-haviour (Fischer, 2008; Samad, Meghla, Nain, Karpiński, & Rahman, 2022; Torres & Santos, 2015).

In recent studies, mathematical modelling has been used to investigate the tumour vaccine development. Wilson *et al.* (Wilson & Levy, 2012) presented a mathematical model to examine the influence of anti-TGF- β treatment – TGF- β a numerous functional cytokine that performs in a cell and system-like as a tumour suppressor or tumour promoter - when used in concurrence with a vaccine as treatments for tumour growth. The researchers were interested in quantifying the impact of both anti-TGF-β and vaccine treatments to achieve the stability of the tumour-immune dynamic and to analyse how this joint 'treatment' could promote tumour free in comparison to tumour escape. The study was formed upon a previous experimental study conducted by Terabe et al (Terabe *et al*., 2009) to attain a precise analysis. The researchers believe

the work presented to be perceived as a move toward creating a structure in which experimentalists could test treatment procedures before performing experimental studies (Salim, Mureithi, Shaban, & Malinzi, 2021). To view the mathematical model graph plot by Wilson *et al.* (Wilson & Levy, 2012) against experimental data from Terabe *et al.* (Terabe *et al.*, 2009) on the dynamics of tumour size in the 4 treatments control including no treatment, vaccine treatment, TGF-β inhibitor treatment and combined TGF-β inhibitor and vaccine shown in fig. 6 (Abdou *et al.*, 2022; Kumar *et al.*, 2022; McCammon *et al.*, 1977; Oli *et al.*, 2020; Parvizpour *et al.*, 2019; Tomar & De, 2014).

Salim *et al.* (Salim *et al.*, 2021) looked at the treatment for prostate cancer using a curative vaccine that was created to establish the efficacy of constant drug infusion into the body tissue. The study developed a mathe-matical model to analyse the model's stability, showing a maximum carrying capacity of the prostate tumour cells growth when treatment was not introduced. Additionally, the analysis showed that the vaccine could potentially remove the prostate tumour cells if the efficacy of the curative vaccine is lower than the ratio of the product of death of 'dendritic cells' and the activation rate to the decaying rate of the therapy. To review the model, mathematical equations and the mathematical modelling proof were developed by Salim *et al.* (Salim *et al.*, 2021). Fig. 7 illustrates the plotting effect of the curative vaccine on Androgen Independent (AI) and Androgen Dependent (AD) tumour cells (Fischer, 2008; Rajendran Krishnamoorthy & Karuppasamy, 2022; Samad *et al.*, 2022; Terabe *et al.*, 2009; Torres & Santos, 2015; Wilson & Levy, 2012).

Additionally, to see the numerous mathematical studies addressing the dynamics of prostate tumors and their treatments, see work by Baez *et al.* (Baez & Kuang, 2016), Hirata *et al.* (Hirata, Akakura, Higano, Bruchovsky, & Aihara, 2012; Hirata, Bruchovsky, & Aihara, 2010), Guo *et al.* (Wilson & Levy, 2012), Jain *et al.* (Jain, Clinton, Bhinder, & Friedman, 2011) and Yang *et al.* (J. Yang, Zhao, Yuan, Xie, & Hao, 2016). For work on the en-hancement of tumor vaccine efficacy by immunotherapy using mathematical modelling, see the study by Wilson *et al.* (Wilson & Levy, 2012), and for work on a mathematical model describing the vital interaction of customized neoantigen cancer vaccine using specific patient's immune system, see the study by Rodriguez *et al.* (Rodriguez Messan *et al.*, 2021).

6. FUTURE DIRECTIONS

Quantum computing, abbreviated as QC, is an emerging technology that uses the laws of quantum mechanics to solve overly complicated problems for classical/traditional computers.

In terms of healthcare, a significant improvement in computational power was made through QC, which is expected to provide an avalanche of newer opportunities for the modellers. QC will offer wider benefits, such as rapid analysis of events using simulations to propose new drugs, personalized treatment with DNA sequencing, silico diagnostic testing through virtual humans and the development of advanced therapy and drugs with ex-tensive modelling. Not only does QC offer these benefits, but it can tackle complex optimized problems, such as effective plans to annihilate the selected cancer cells while preventing further damage to healthy body parts and organs (Chugh *et al.*, 2020; Newman-Toker *et al.*, 2021; Niedermaier *et al.*, 2021; Rasool, Ahmad, Rafique, Qayyum, & Qadir, 2022). The analysis of genome, sequencing and atomic-level molecular interaction using qubits are achieved quickly, and it allows the development of drugs and medical research. Furthermore, migrating the infrastructure of hospitals to the cloud provides an advance in securing medical records and predicting chronic medical conditions faster through qubit processing, also known as quantum bits. The exponential benefit of introducing QC in healthcare paradigms offers numerous advantages, including promoting medical professional experiences, improving patient management, delivering improved patient treatment and lowering treatment costs (Rasool *et al.*, 2022).

The quantum-based innovation in healthcare applications consists of molecular simulation, diagnosis analysis costs, drug development and recovery, medical precision, diagnosis assistance, radiotherapy, medical imaging, and clinical trials. Although, over the years, the growth of QC has been beneficial in providing innovative op-portunities in the pharmaceutical industry, it is vital for the healthcare paradigm, as healthcare depends on the exchange of web-based data by delivering services to connect devices healthcare. It was reported by numerous studies (Rafique, Khan, Sarwar, & Dou, 2019).

There are a few cautions as well. For example, a potential attack could lead data breach. As such, by leveraging the QC, it is possible to design a safe, end-to-end, and private protocol to provide services

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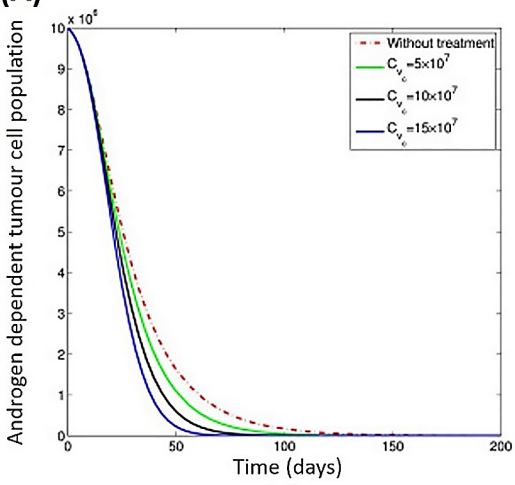

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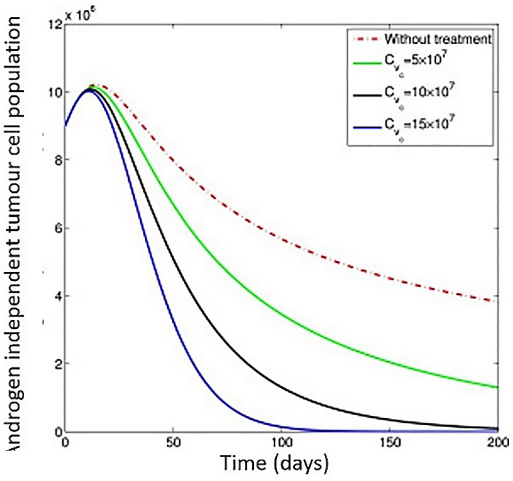
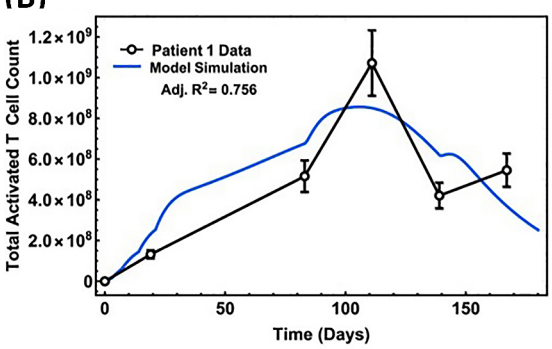
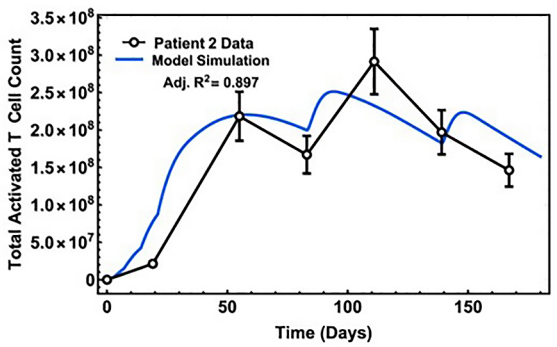
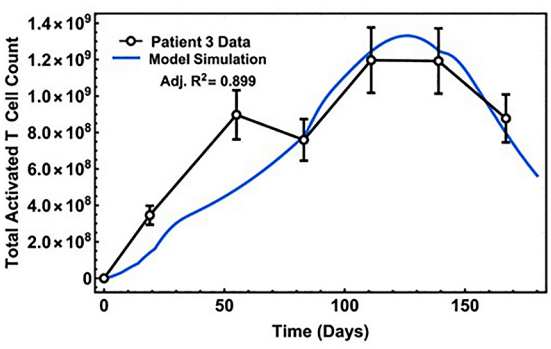


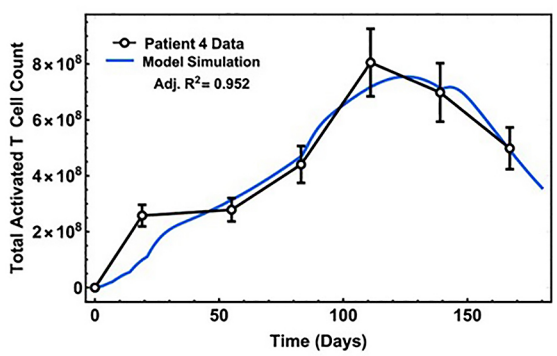
(b)

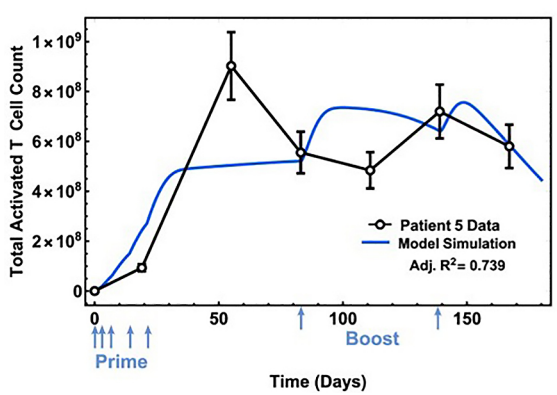
(B)

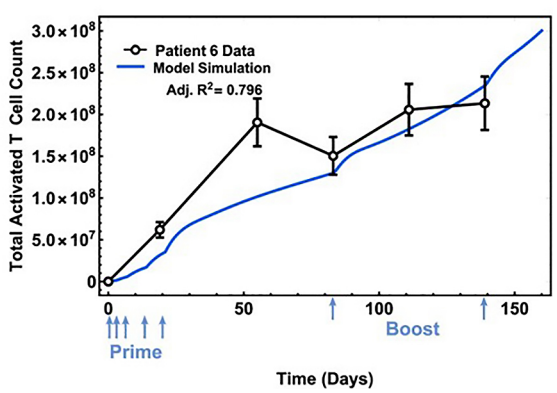












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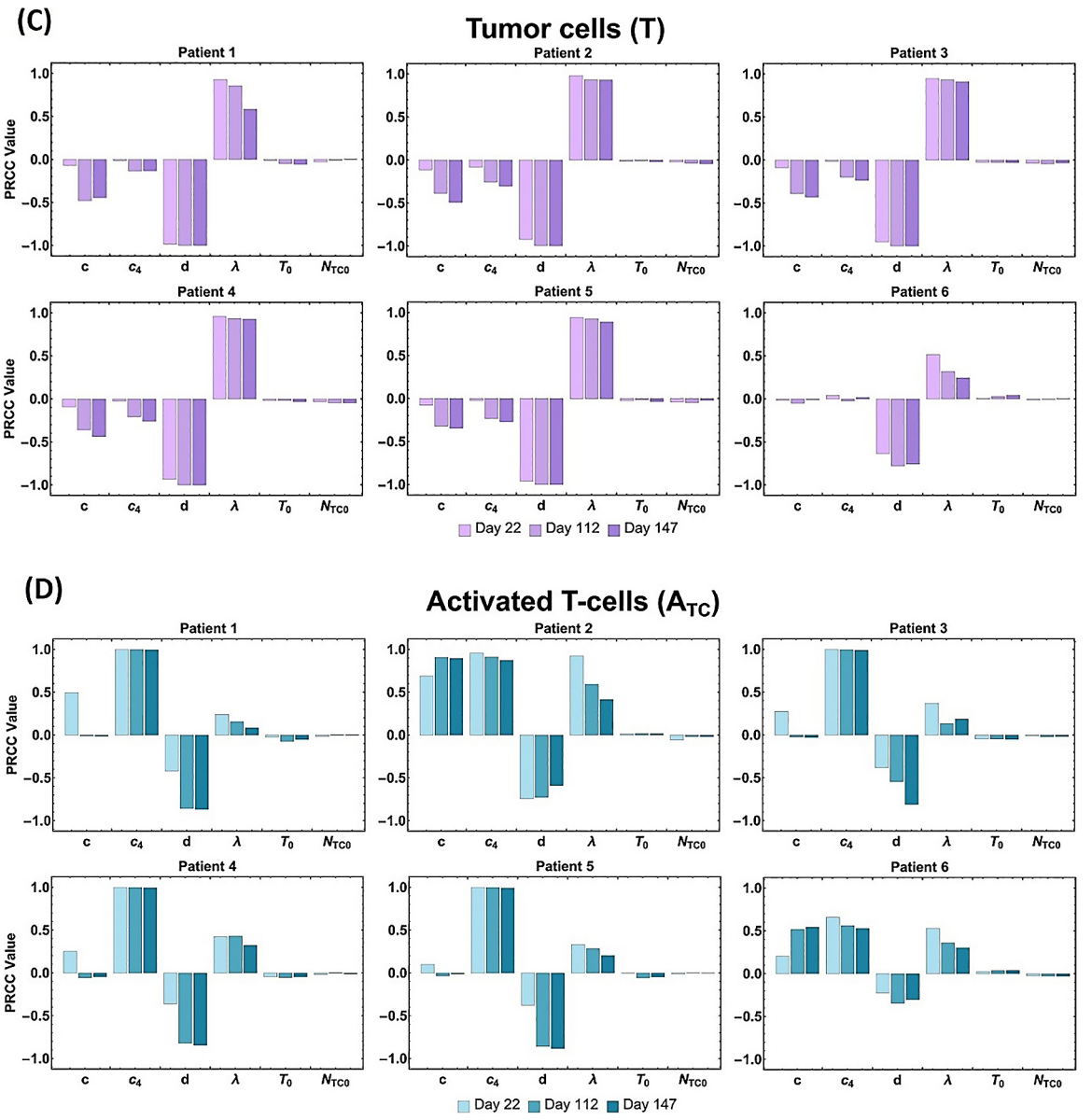

Figure 7. Illustrates the mathematical model approach used to analyse the dynamics of prostate cancer with a healing vaccine and customised neoantigen cancer vaccine based on specific patient's immune systems. (A) plotting effect of the healing vaccine on Androgen Independent (AI) and Androgen Dependent (AD) tumour cells, (B) shows the time profile of 6 different patients' data T cell responses, (C) shows the active T cell population as the output of interest and (D) shows the tumour cell population as an output of interest (Rodriguez Messan *et al.,* 2021; Salim *et al.,* 2021; Terabe *et al.,* 2009).

to medical devices. Hence, in the quantum-based healthcare paradigm, it is vital to have secure privacy and data protection protocols to avoid external users infiltrating the system and altering data or distributing illegal information. As such, incor-porating healthcare 4.0 leverages the Internet of things, abbreviated as IoT, and cloud services to gain access remotely to medical data regarding the healthcare 4.0 element (Rafique, Khan, Zhao, Sarwar, & Dou, 2019).

Nanomaterials (NPs) could also be a good candidate shortly for delivering cancer vaccines due to their safety and versatility. Compared to traditional vaccines, cancer vaccines delivered by nanomaterials can be tuned towards desired immune profiles by (1) optimising the physicochemical properties of the

nanomaterial carriers, (2) mod-ifying the nanomaterials with targeting molecules, or (3) co-encapsulating with immunostimulators (Verma *et al.*, 2023). Due to the extensive suppressive immune microenvironment, cancer vaccines alone are difficult to prevent disease recurrence, which requires further tuning of the suppressive tumor microenvironment to improve T cell penetration and activation *in situ*. Therefore, hybrid modes of therapy and the integrated use of nano-particle-mediated delivery can provide newer horizons in this area (Jingjing Liu, Miao, Sui, Hao, & Huang, 2020; Vermaa *et al*.).

7. CONCLUSION AND REMARKS

Numerous studies have shown various cell signaling pathways to control cancer, yet, it continues to remain a hard-to-be-treated disease. Conventional cancer therapies include surgeries, chemotherapies and radiation therapies. In spite of this, the development of effective cancer treatment continues to puzzle doctors around the globe. Therapeutic cancer vaccines appear to be a promising method for inducing permanent antitumor im-munity. The first therapeutic cancer vaccine's recent approval will open the door for creating cutting-edge, next-generation vaccinations with improved anticancer potency. Therapeutic vaccines will likely be used in the adjuvant or neoadjuvant setting for treating patients with minimal residual disease or more sluggish metastatic disease or those patients with a high risk of recurrence, based on the most recent data from clinical trials and the safety profiles of therapeutic vaccines. Overcoming the immune tolerance/suppression pathways in the TME will be necessary to translate cancer vaccines into therapeutically usable drugs with broad uses. A deeper, more profound comprehension of host-tumour interactions and tumour immune escape mechanisms is needed to create effective cancer vaccines. Finding specific tumour genes or protein products that turn normal cells into tumour cells and accelerate cancer progression will also provide new targets for vaccination therapy. To identify patient populations that will most likely respond to and profit from vaccination therapies, "immune signatures" must be developed and used. In the near time, improved clinical outcomes should also result from strategically combining vaccine strategies with other drugs or methods that work in concert to boost antitumor immunity and/or activate complementing antitumor responses.

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Declaration of Competing Interest

The author (s) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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