A critical review on cancer vaccines based on tumor-associated antigens: 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. The development of cancer vaccines based on tumor-associated antigens is hurdled by lack of an efficient adjuvant and insufficient efficacy. To improve the efficacy of vaccines, a genetically engineered method was reviewed with the view to achieve 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 developments in the field of therapeutic, HPV and DNA cancer vaccines especially focusing on modelling 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 lymphocytes
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

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 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 very expensive disease i.e., annual costs for treating cancer in the UK alone are about £5 million, but the cost to society, including the cost for loss of productivity could be to the tune of £18.3 billion. The development of vaccines to either prevent or treat cancer is hampered by the complexity of the situation which is 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 (Sipuleucel-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 advancement of vaccination technology and refocused public attention on cancer vaccines (LaFleur, Muroyama, Drake, & Sharpe, 2018). To stimulate patient's immune system, cancer vaccines primarily include tumour-associated antigens (TAAs) and tumour-specific antigens (TSAs). 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. Presently, 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.

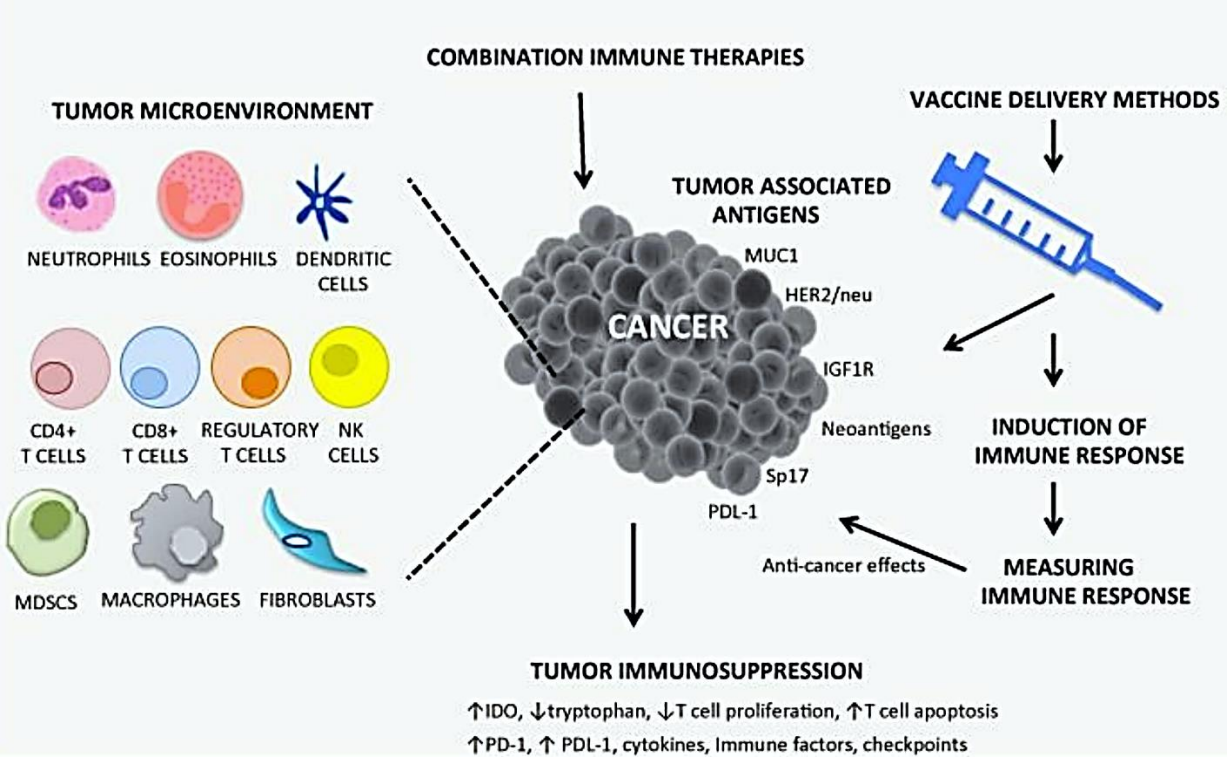


Fig. 1. Strategies for cancer vaccine development: Cancer immune therapeutics (Apostolopoulos, 2019).

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 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 both 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 PD-1 inhibitor nivolumab was mimicked by the vaccination, but it doesn't cause the innate and acquired resistance that drug and related treatments are known to cause. According to the study, PD1-Vaxx effectively slowed the growth of tumours. When combined with a second therapeutic peptide vaccine that specifically targets two HER-2 receptor sites on colon cancer cells, it was much more successful. In nine out of ten animals, the combined treatment resulted in full responses. The same scientific team also created the B-Vaxx vaccination earlier.

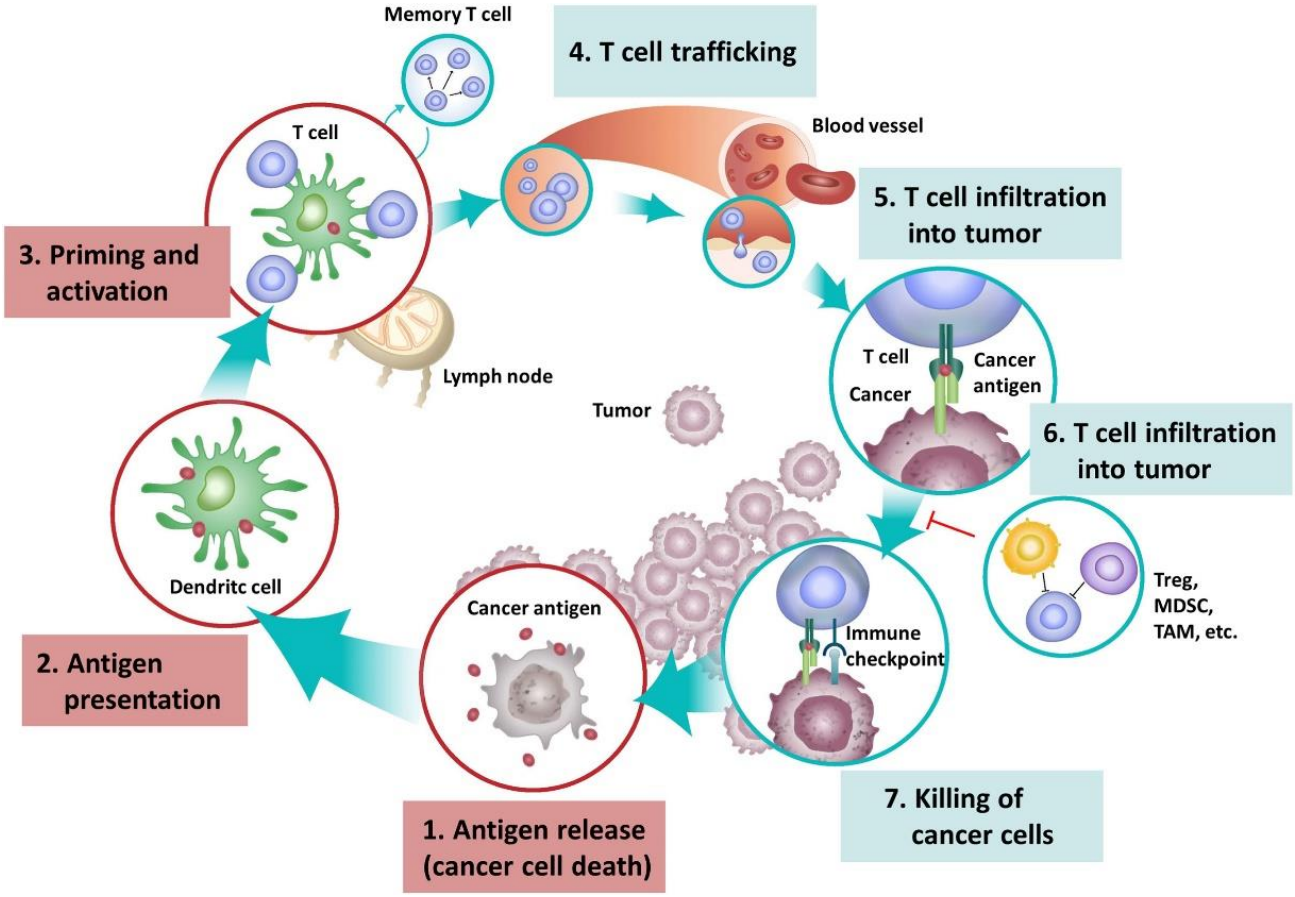


Fig. 2. Cancer-immunity cycle. This cycle is a self-sustaining multistep process that involves: (1) the release of cancer cell antigens; (2) cancer antigen presentation; (3) priming and activation; (4) the trafficking of T cells to the tumor; (5) the infiltration of T cells into tumors; (6) specifically 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-cell to encourage tumour elimination and (ii) the therapy aims to obstruct signalling pathways that are 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 along with an immunotherapy medicine. PD1-Vaxx is an immune checkpoint inhibitor, much as 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 cells 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). There are now many vaccination methods being tested in both preclinical and clinical settings. This review discusses preclinical and clinical trials using these therapeutic vaccines from various platforms or targets as well as HPV, DNA, 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

Several categories of cancer are brought on by viral infections. 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 cancer and cervical cancer. To guard against the development of HBV- and HPV-related malignancies, several vaccines have been developed that can prevent HBV and HPV infection (Hu et al., 2018). The U.S. Food and Drug Administration (FDA) has authorised four of these 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 prevention of HPV-related anal, head, cervical, neck, penile, vulvar and vaginal cancers 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 FDA in 2006 for prevention of HPV types 16, 103 18, 31, 33, 45, 52, and 58 as well as 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 differentiating cancer cells from healthy cells. Prostatic acid phosphatase (PAP), which is 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 was approved by the FDA in 2010 for treatment of individuals with advanced prostate cancer. Another interesting source of indicators that can be targeted by vaccines 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 immunotherapy of any kind 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' own activated dendritic cells. This was the first cancer treatment vaccine approved by the FDA, Sipuleucel-T is used for the treatment of asymptomatic or minimally symptomatic metastatic castrate-resistant (i.e., hormone-refractory) prostate cancer. Sipuleucel-T is an example of a personalized medicine, as it is manufactured using each patient’s own APCs that are activated via exposure to an antigen specific to prostate cancer. It contains autologous activated APCs that stimulate a response against PAP, an antigen expressed on 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 colony-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 normal-yet-overexpressed proteins like PAP. Neoantigens, also known as "new antigens," are molecules that are only expressed 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, double-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 as just a treatment due to the participants have tumours with HER2 present. Enrolled patients will have tumours expressing low or intermediate levels of the HER2 protein, and NeuVax vaccine is administered as adjuvant therapy. The primary endpoint of the study 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 modification 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 that are 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 the use of CARs seems promising, however, there are obstacles which we need to overcome before they can be used for a broad selection of cancer types, particularly 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 cells modification with CARs, including phase I and II studies on the treatment of 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 which is comprising of one or more genes encoding for the antigens of interest. The principle of utilising viruses to deliver the 'vaccine gene' is 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, there are several viruses that can target professional antigen-presenting cells that results in potent priming of the immune response. Additionally, a higher level of vaccine antigens can be attained in-vivo in those cases where viral vectors replication is used and therefore boost the immunogenicity of 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 in which can produce a high number of copies in bacterial cells, where one can replicate and purified to homogeneity by standard chromatographic methods. One great advantage of DNA vaccines are cost-effective, ease of production process and the ability to be repetitively administered due to the immune system not reacting against DNA vector.

Listeria monocytogenes *Technology:* The *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 the delivery of biomarkers introduced to the body. The uniqueness of Lm is due to its ability to induce strong responses to MHC I and II, then producing 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 as well as anti-PD-1 (anti-programmed-death receptor 1) antibody as a combination, which increased 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 antibody 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, a vaccine that target HPV-associated cancers and is at present 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 further immunotherapy vaccines at present 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. The ssRNA genome is surrounded by a protein envelope 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. Different 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 and 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 genes of the capsid and envelope proteins and for RNA self-replication (Strauss & Strauss, 1994). The engineered alphavirus vectors can produce replication-proficient and replication-deficient particles' recombinant appropriate for transgene expression in vivo and in 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 are implanted downstream 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 for the release of measles virus replication from cloned DNA expression forms (Radecke et al., 1995). The release of recombinant measles virus has been based in a helper cell line by reverse genetics. To produce measles virus' recombinant particles, the helper cell line is transfected with a plasmid comprising of the measles virus polymerase L gene and measles viruses' recombinant particles formed. The expression vectors carrying the structural protein of measles virus is downstream of T7 RNA polymerase promoter have been designed for the introduction of 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 required 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, an 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, for the negative stranded viruses, a reverse genetics and packaging cell lines is necessary to produce engineered replicons. On the other hand, in the case of positive stranded viruses, the intermediate DNA vectors along with in-vitro transcription method can sufficiently be used to produce self-replicating RNA.

To produce self-replicating RNA vectors from the above-mentioned viruses, the non-structural gene replicas remain untouched while the selected antigens or antigen are replaced for structural genes. The non-structural protein genes encoding the viral replicas complex are 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 the

production of synthetic self-replicating RNA replicons. In the DNA utilisation intermediate, the vaccine is used as a self-replicating RNA vector that is 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 produces immune responses as opposed to themselves, the viral replicon particles. Such method is not applicable due to the alteration of responses to various encoded antigens or/and obstruct 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 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 platform 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 treating the cancer. 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 key 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, epithelial-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 nucleotides (nt) and lncRNAs, the most abundant class, with >200 nt length (Carninci et al., 2005; Seal et al., 2020). In the past, ncRNAs were considered “evolutionary junk,” but 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) and 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, whereas the guide strand (mature miRNA) binds to AGO and additional proteins (Kawamata, Seitz, & Tomari, 2009) to form the microRNA-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.

In this section, an overview of the role of LncRNAs in modulating the immune response and the TME is provided since LncRNAs could be used as potential biomarkers or as 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 progression 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 possibility 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, 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 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).

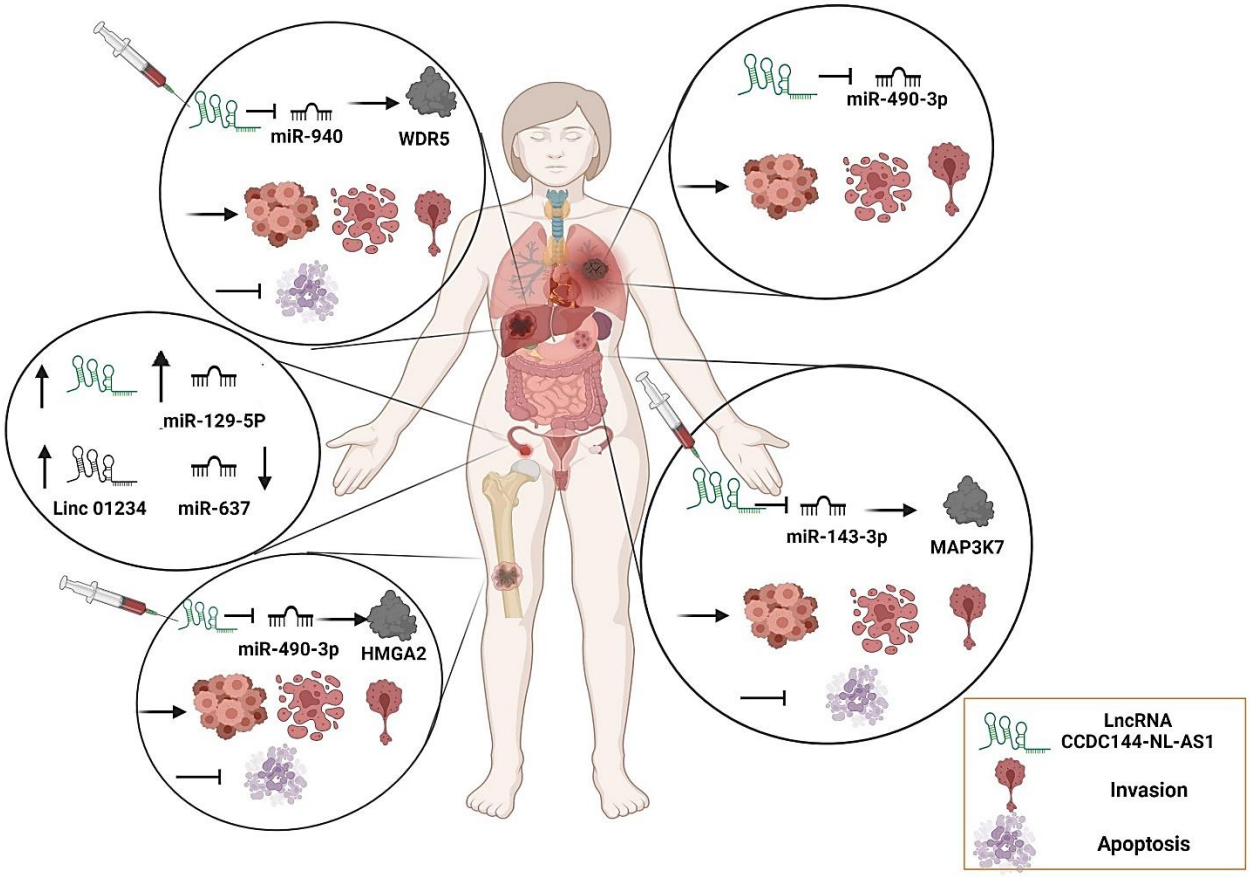


Fig. 3. Effect of lncRNA CCDC144NL-AS1 in multiple signaling pathways and possibility of being a therapeutic target for cancer. [lncRNA CCDC144NL-AS1 plays a significant role in the progression of different malignant tumors through ceRNA networks, competing with their target miRNAs; Non-small cell lung 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 act as 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, and 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, migration, and increases apoptosis, indicating that CCDC144NL-AS1 may be a therapeutic target for osteosarcoma] (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. The effectiveness of cancer vaccination depends heavily on the ability of T lymphocytes 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 is 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 ones are dendritic cells (DCs) because they serve as a vital link between innate immunity and adaptive immunity. Initial antigen presenters, DCs are capable of acquiring and cross-presenting 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 progressively lose their capacity to absorb antigens (Itano et al., 2003; West et al., 2004). The antigen loaded DCs move to the draining lymph nodes, which are where T cell priming occurs most frequently. To naive CD4+ and CD8+ T lymphocytes, 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, in order 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, effector tumor-specific T lymphocytes multiply and are transported to TME (Chudnovskiy, Pasqual, & Victora, 2019). Additionally, through complement-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).

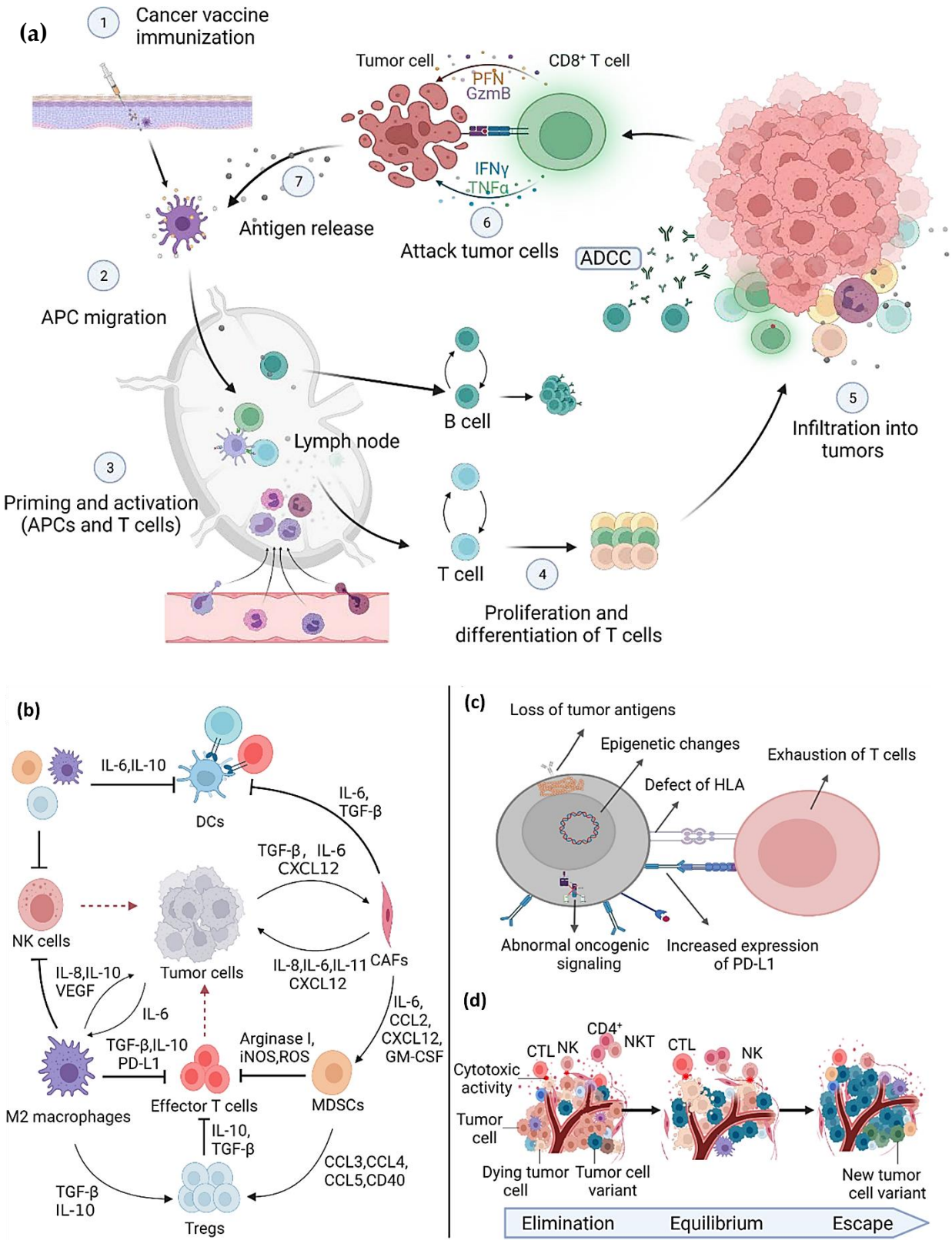


Fig. 4. (a) Cancer vaccinations activate the tumor-immune cycle. The tumor-immune cycle refers to the steps that enable repetition and expansion during the immune response that successfully destroys tumour cells. When the cancer vaccine is administered, DCs take in and analyze the tumour antigens before 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, respectively. 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 cells for killing tumour cells and present 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) peptides can be modified to increase their affinity and binding to the present MHC-I which can in turn improve the immunogenicity 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 an improvement of 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 with the corresponding wt peptide that stimulates a specific and better T cell response in vitro and in vivo. Clinical trials based on vaccination with 210 M antigen, alone or in combination 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 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 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 specific 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 stronger 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 as well as 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 different enrollment requirements. The terms "plasmid" and "tumour" led to the discovery of two further studies, NCT02531425 and NCT03502785. Two phase III studies (NCT03721978 and NCT03185013) employing VGX-3100 administered via IM EP against cervical cancer are of relevance. The trials continue to focus mostly 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).

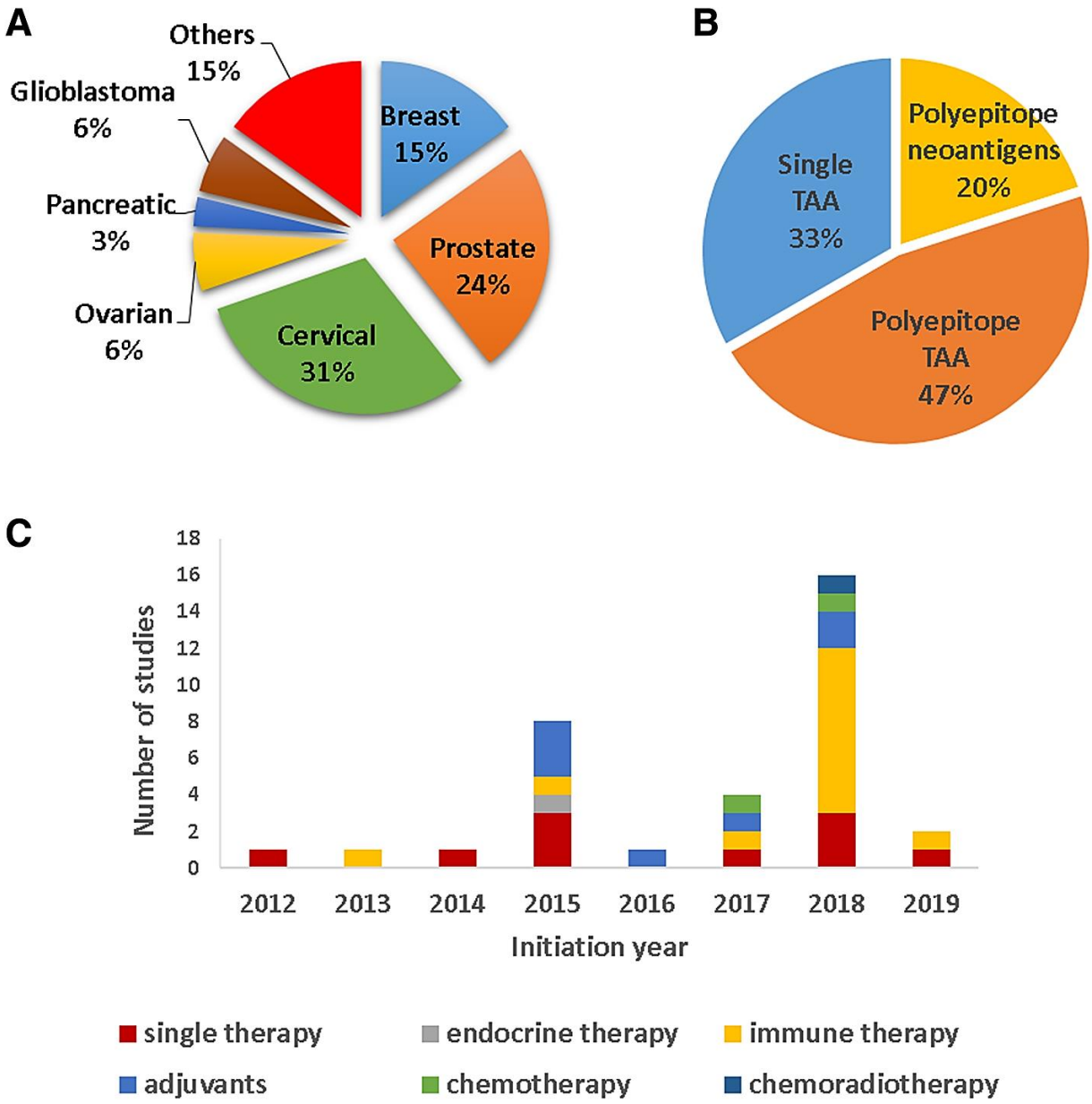


Fig. 5. ongoing clinical trials for the studies that were examined. cancer kinds that are testing cancer DNA vaccines. b The DNA vaccine's antigen type encoding. studies employing cancer DNA vaccines as a single therapy or in combination with other treatments (such as adjuvants, adjuvant chemotherapy, 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.), chemotherapy (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 individuals who have undergone less prior chemotherapy often respond better to vaccinations (Schlom, 2012). As a result, vaccination of individuals having incipient growth of 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, 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 length of time required to initiate the immune response, which is then 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, anticancer activity of vaccine-induced immune activation over a prolonged period leads in 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 metastatic 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). According to these findings, 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 categorise 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" have been shown to be 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 concentrate on finding and validating diagnostic biomarkers that respond to vaccination therapy. The clinical development of therapeutic cancer vaccines will be considerably aided by knowledge of the biomarkers of immunological and clinical responsiveness 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. Nowadays, technological 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). The aim of mRNA-based vaccination is 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 engineered 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 and 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 (eg, NCT04534205, NCT03313778, and NCT04503278) are enrolling patients for various mRNA-based cancer vaccine therapy studies with the aim of inducing an mRNA-based anti-tumour response (Table 1).

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

	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	With pembrolizumab	Intramuscular	Merck Sharp & Dohme

			colorectal neoplasms			
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)	Mela-noma	None	Intrave-nous	BioN-Tech
NCT04526899	2	BNT111 (NY-ESO-1, tyrosi-nase, MAGE-A3, and TPTE)	Mela-noma	With cemi-plimab	Intrave-nous	BioN-Tech
NCT04382898	½	BNT112 (PAP, PSA, and three undisclosed antigens)	Prostate	With cemi-plimab	Intrave-nous	BioN-Tech
NCT04534205	2	BNT113 (HPV16 E6 and E7 on-coproteins)	Head and neck squa-mous cell car-cinoma	With pem-broli-zumab	Intrave-nous	BioN-Tech
NCT03418480	½	BNT113 (HPV16 E6 and E7 on-coproteins)	HPV16-positive solid tu-mours	With anti-CD40 an-tibodies	Intrave-nous	Univer-sity of South-ampton

						(South-ampton, UK)
NCT05142189	1	BNT116 (non-small-cell lung cancer tu-mour-asso-ciated anti-gens)	Non-small-cell lung cancer	With cemi-plimab plus docet-axel	Intrave-nous	BioN-Tech
NCT04486378	2	BNT122 (personal-ised cancer vaccine en-coding indi-vidual tu-mour muta-tions)	Colo-recta	None	Intrave-nous	BioN-Tech
NCT02316457	1	BNT-114 plus BNT-122 (per-sonalised set of pre-manufac-tured non-mutated shared tu-mour-	Triple-negative breast cancer	None	Intrave-nous	BioN-Tech

		associated antigens plus a personalised cancer vaccine encoding individual tumour mutations)				
NCT04163094	1	BNT115 (ovarian cancer tumour-associated antigens)	Ovarian	With carboplatin plus paclitaxel	Intravenous	University Medical Center Groningen (Groningen, Netherlands)
NCT04161755	1	BNT122 (personalise2d 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

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 womaffected andut of 100,000 individuals are likely to be affected, and is one of the most difficult 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 used was to design a multi-epitope peptide vaccine to fight against TNBC through a method called 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 such as the adjuvant, the helper epitopes and the CD8+ cytotoxic T lymphocytes CTLs. These elements were united by proper linkers. The in-silico analyses consisted of MD simulation study for refinement of 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. For additional materials on the selection of CTL, CD8+, and CD4+ for sequences subjected to immunoinformatics analysis one can refer to the work elsewhere [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 analyzations performance (Oli et al., 2020). Based on the proposed vaccine, it is believed to have means of obtaining the responses of the immune that could potentially be used to target TNBC combination with other therapy or on its own. Fig.6 shows the TNBC 3D-structural model alongside its proposed vaccine.

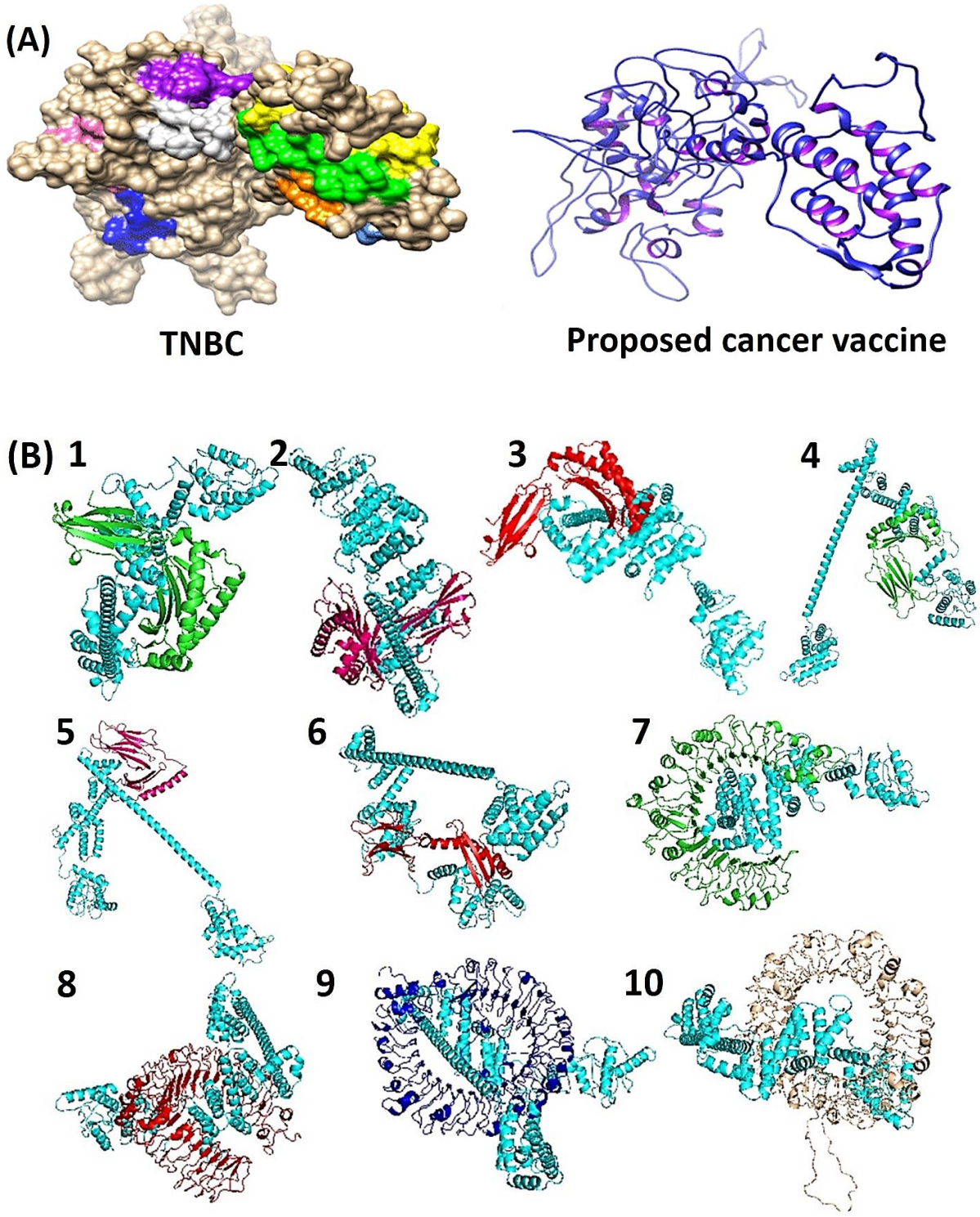


Fig. 6. illustrate the 3D-structure of the constructed epitope-based vaccine against triple-negative breast cancer (TNBC). (A) TNBC model with proposed cancer vaccine image inspired by Parvizpour et al and (B) Docking pose of vaccine build with targeted immune molecules where: 1. Shows the HLA-A allel, 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 recpetor. (Kumar et al., 2022; Parvizpour et al., 2019; Tomar & De, 2014).

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

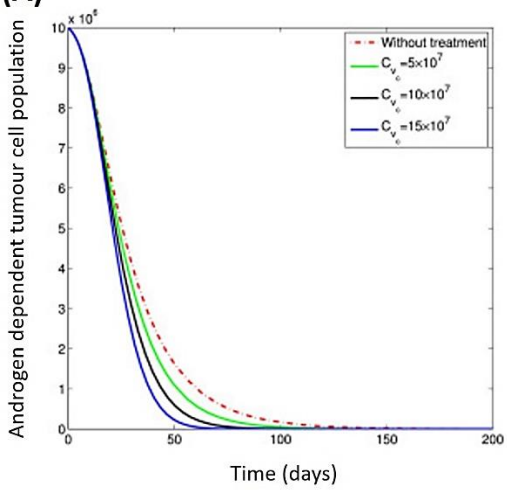
For many years, mathematical modelling has been used to assist scientists in understanding the dynamics and mechanisms behind experimental observations. Mathematical modelling facilitates an improved understanding about the systems as it can provide insights into complex processes implicated in biological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

In recent studies, mathematical modelling has been used as a technique to investigate the development of tumour vaccine. Wilson et al (Wilson & Levy, 2012) presented a mathematical model to investigate 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 to tumour free in comparison to tumour escape. To attain a precise analysis, the study was formed upon a previous experimental study conducted by Terabe et al (Terabe et al., 2009). 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).

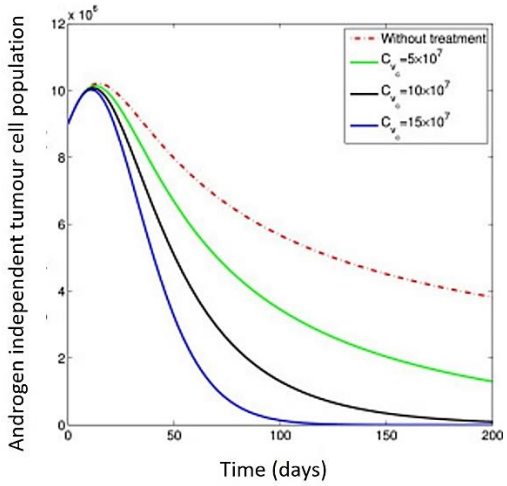
Salim et al. (Salim et al., 2021) looked at the treatment for prostate cancer using a curative vaccine that was created to establish the efficacy of constant drug infusion into the body tissue. The study developed a mathematical model to analyse the stability of the model and it showed a maximum carrying 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).

(A)

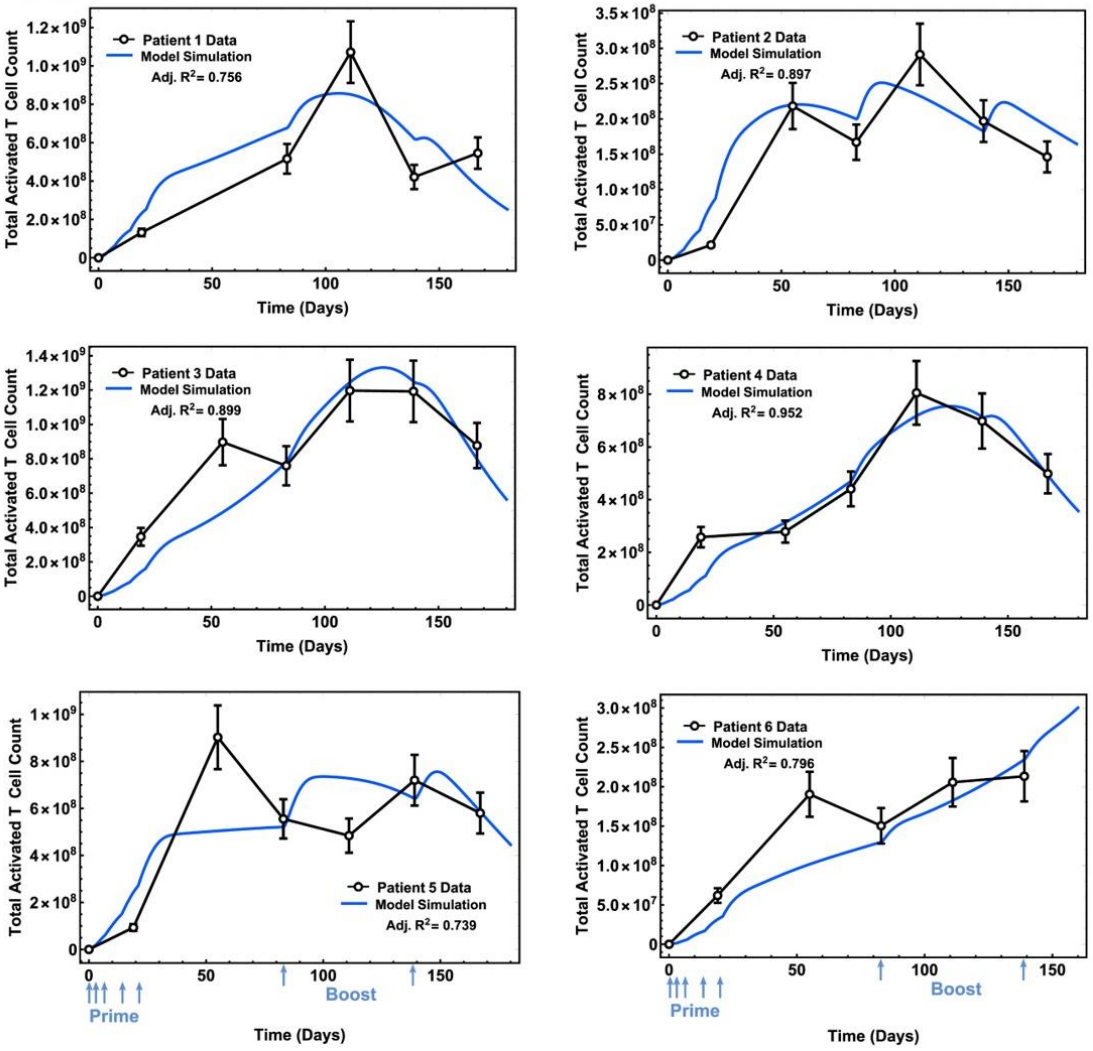


(a)



(b)

(B)


Patient 1 Data
Model Simulation
Adj. R^2= 0.756
Patient 2 Data
Model Simulation
Adj. R^2= 0.897
Patient 3 Data
Model Simulation
Adj. R^2= 0.899
Patient 4 Data
Model Simulation
Adj. R^2= 0.952
Patient 5 Data
Model Simulation
Adj. R^2= 0.739
Patient 6 Data
Model Simulation
Adj. R^2= 0.796
Total Activated T Cell Count
Time (Days)
Prime
Boost

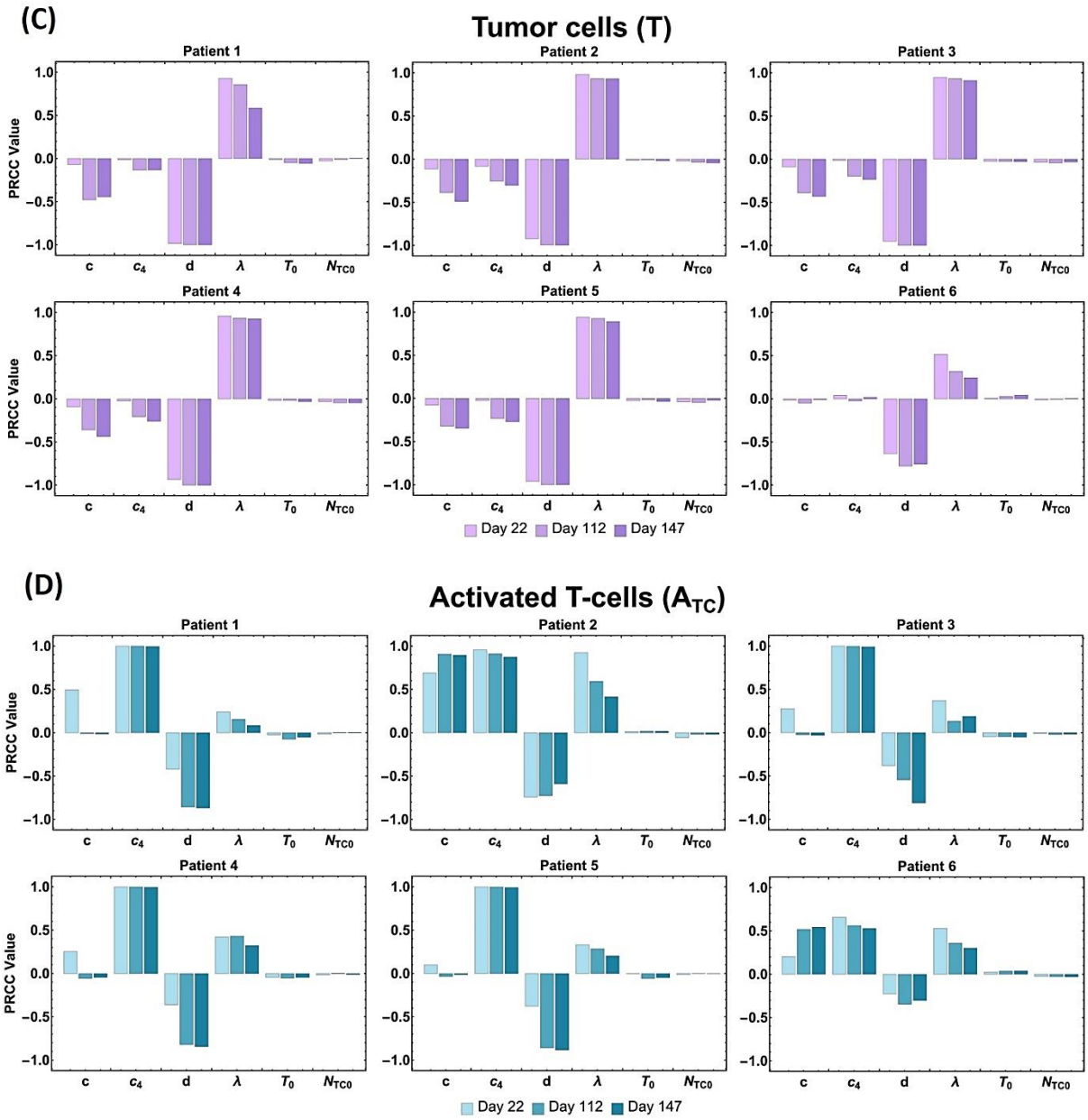


Fig. 7. Illustrates the mathematical model approach used to analyse the dynamics of prostate cancer with healing vaccine and customised neoantigen cancer vaccine based on specific patient's immune systems. (A) plotting effect of the healing vaccine on Androgen Independent (AI) and Androgen Dependent (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 output of interest. (Rodriguez Messan et al., 2021; Salim et al., 2021; Terabe et al., 2009).

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 enhancement of tumor vaccine efficacy by immunotherapy using mathematical modelling see study by Wilson et al (Wilson &

Levy, 2012) and for work on mathematical model describing vital interaction of customized neoantigen cancer vaccine using specific patient's immune system, see 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 problems that are overly complicated 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 opportunity for the modellers. The use of 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 extensive 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 in a short period of time 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, delivery of 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 opportunities in the pharmaceutical industry, however, it is vital for the healthcare paradigm, as healthcare depends on the exchange of web-based data by delivering services to connect devices of healthcare. It was reported by numerous studies (Rafique, Khan, Sarwar, & Dou, 2019).

There are 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 to medical devices. Hence, it is vital in the quantum-based healthcare paradigm to

have secure privacy and data protection protocols to avoid external users infiltrating the system and altering data or distributing illegal information. As such, incorporating 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 in near future 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) modifying the nanomaterials with targeting molecules, or (3) co-encapsulating with immunostimulators. 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 the integrated use of nanoparticle 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 continue to remain a hard to be treated disease. Conventional cancer therapies include surgeries, chemotherapies and radiation therapies. Inspite of this, the development of effective treatment of cancer continues to puzzle doctors around the globe. The therapeutic cancer vaccines appears to be promising method for inducing permanent antitumor immunity. 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 for ultimate translation of cancer vaccines into therapeutically usable drugs with broad uses. To create effective cancer vaccines, a deeper comprehension of host-tumour interactions and tumour immune escape mechanisms is needed. Finding specific tumour genes or protein products that turn normal

cells into tumour cells and accelerate the progression of cancer will also provide new possible targets for vaccination therapy. To identify patient populations that will most likely respond to and profit from vaccination therapies, "immune signatures" will also need to be developed and used. In 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.

Acknowledgments: SG acknowledges funding assistance from the UKRI through Grants Nos. EP/S036180/1 and EP/T024607/1, feasibility study awards from the UKRI National Interdisciplinary Circular Economy Hub to LSBU (EP/V029746/1) and Transforming the Foundation Industries: a Network+ (EP/V026402/1), Royal Academy of Engineering (TSP 1332) and Hubert Curien Alliance Award from the British Council.

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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Paavilainen-Mäntymäki, S. P. D. E., & Van Mumford, D. S. J. Subject International Business Date 30.5. 2022 Author Satu Hynninen Number of pages 89+ appendices.

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