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Novel treatment strategies in cancer immunotherapy

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Abstract: Immune system has gained importance in research especially in cancer treatment studies which is also regarded as cancer immunotherapy. Cancers are the leading causes of death in the world, yet there is no effective therapeutics found against them. Current treatment strategies are found to be insufficient and non-specific. Recent studies showed that, for an effective cancer therapy, host immune system needs to be activated. Research on cancer and tumor microenvironment provided more molecular information about cancer and immune cells. Immune based studies on cancer immunotherapy highlighted the importance of host immune system on cancer eradication. One of the first immunotherapeutic used against cancer cells are trastuzumab which is a monoclonal antibody targeting Epidermal Growth Factor Receptor (EGFR). Although, it is regarded as a huge milestone in cancer immunotherapy, it is found to be insufficient in treatment. In this review, recent advances in both active and passive immunotherapies are going to be discussed including the obstacles in the treatment strategies and studies carried out to overcome them.

Keywords: Immunotherapy, Cancer, Vaccine, mAb.

Introduction

Cancers are the leading causes of death across the world in 21st century. The proportions of death caused by cancers are expected to rise in the next decades. Current treatment strategies for cancer are chemotherapy and radiotherapy which are just extending the lives of patients. Apart from not being therapeutics, they are also non-specific and insufficient. This gave rise to a need for new treatment strategies and therapies for cancer. A lot of cancer research is now being carried out across the world to find a better way of treatment with higher specificity and longer remission (WHO, 2016). Cancer cells can be classified according to the body part they originate (Golub, 2004). Cancers that develop in epithelial cells are known as carcinomas that can be further subdivided into groups according to the cell type they start to develop. Cancers developing in non-hematopoietic mesenchymal cells are known as sarcomas, those that present in hematopoietic cells and maturing in bloodstream are known as Leukemia, whereas carcinomas developing in germ cells are regarded as Germinoma (Golub, 2004).

Tumors are regarded as complex tissues that are composed of stroma cells and neoplastic cells. Characterizations of proteins belonging to these complex tissues provided a new opportunity for targeted therapies. The main problem behind this logic is the heterogeneity of the tumors between different individuals. This problem is tried to be solved by using combinational therapies in recent studies (Yasri et al., 2014).

Scientists are currently working on different treatment strategies to find alternative ways of cancer immunotherapy as current strategies are not sufficient. In this review, recent data published about cancer immunotherapy is going to be discussed.

Method

This study was carried out by doing a literature research mainly from sciencedirect and pubmed search engines by searching the keywords: cancer immunotherapy, cancer vaccine, cancer immunotherapy strategies. Recently published most relevant and interesting articles were tried to be summarized in this review.

Table 1. Classification of immunotherapy strategies.

I. Passive immunotherapy	i. Growth factor blockers ii. Checkpoint inhibitors iii. Antagonistic mAbs iv. Tyrosine Kinase inhibitors
II. Active immunotherapy	i. Growth factor blockers ii. Checkpoint inhibitors iii. Peptide based vaccines iv. Therapies via using microorganisms v. Foreignizing tumor cells vi. Nucleic Acids in immunotherapy vii. Cytokines and Growth factors in immunotherapy
III. Combinatorial Therapeutic Approaches	Antibody-Drug Conjugates

Results

Immune system based cancer therapeutics gained a lot of importance in the past 10-15 years. Preliminary data obtained from results proved the ideal way for eliminating cancer is based on using host immune system via activating especially Cytotoxic T-Cell (CTL), T-helper 1 (Th1) and T-helper 2 (Th2) responses. Cancer immune-therapy is the use of host immune system against cancer cells, which is specific, systemic and durable. Research on cancer and tumor microenvironment provided more molecular information about tumor evasion from host immune cells. Currently tumor immunotherapy is merged on two main classified approaches that are passive immunotherapy and active immunotherapy. These classes are also subdivided within themselves according to the strategies used (Sathyanarayanan and Neelapu, 2015) (Table 1).

Passive immunotherapy (monoclonal antibody): Monoclonal antibody therapy is regarded as passive immunotherapy due to the fact that they do not induce any activation on the patient's immune system. There are a couple of US Food and Drug Administration (FDA) approved monoclonal antibody based therapies against various cancers (Hudis et al., 2007; Markovic et al., 2012). Current advances and clear establishment of molecular immunology and cancer lead to the development of checkpoint inhibitors and various monoclonal antibody based treatments (Brahmer et al., 2012; Hamid et al., 2013). The main and most popular targeted therapy is the use of monoclonal antibody therapies that are being investigated since the last decade and gave very promising results (Yasri et al., 2014). Antibodies used in cancer immunotherapy are highly specific and provide a very specific targeted response. Targeted therapies mainly aim to block angiogenesis, to induce apoptosis in tumor cells and to block tumor growth factors activity (Mimeault et al., 2010).

Growth factor blockers: Growth factors are important milestones for cancer cells as they are over activated in tumors when compared to normal cells. This is one of the main reasons of why they are important targets in cancer immunotherapies. Various monoclonal antibodies were produced against different growth factors. The most popular monoclonal antibody developed against cancer is trastuzumab and some others like cetuximab, bevacizumab (Hudis et al., 2007; Markovic et al., 2012). Trastuzumab and cetuximab have similar activities on tumor cells as they both target the EGFR receptor that is commonly over-expressed in various cancer types including breast cancer (Hudis et al., 2007; Hurvitz et al., 2013; Markovic et al., 2012). Trastuzumab is well-known for the first monoclonal antibody based immunotherapy approved for the treatment of HER2 positive breast cancer (Fig. 1) (Hudis et al., 2007; Hurvitz et al., 2013). Bevacizumab is another monoclonal antibody that targets different type of growth factor in various cancer types.

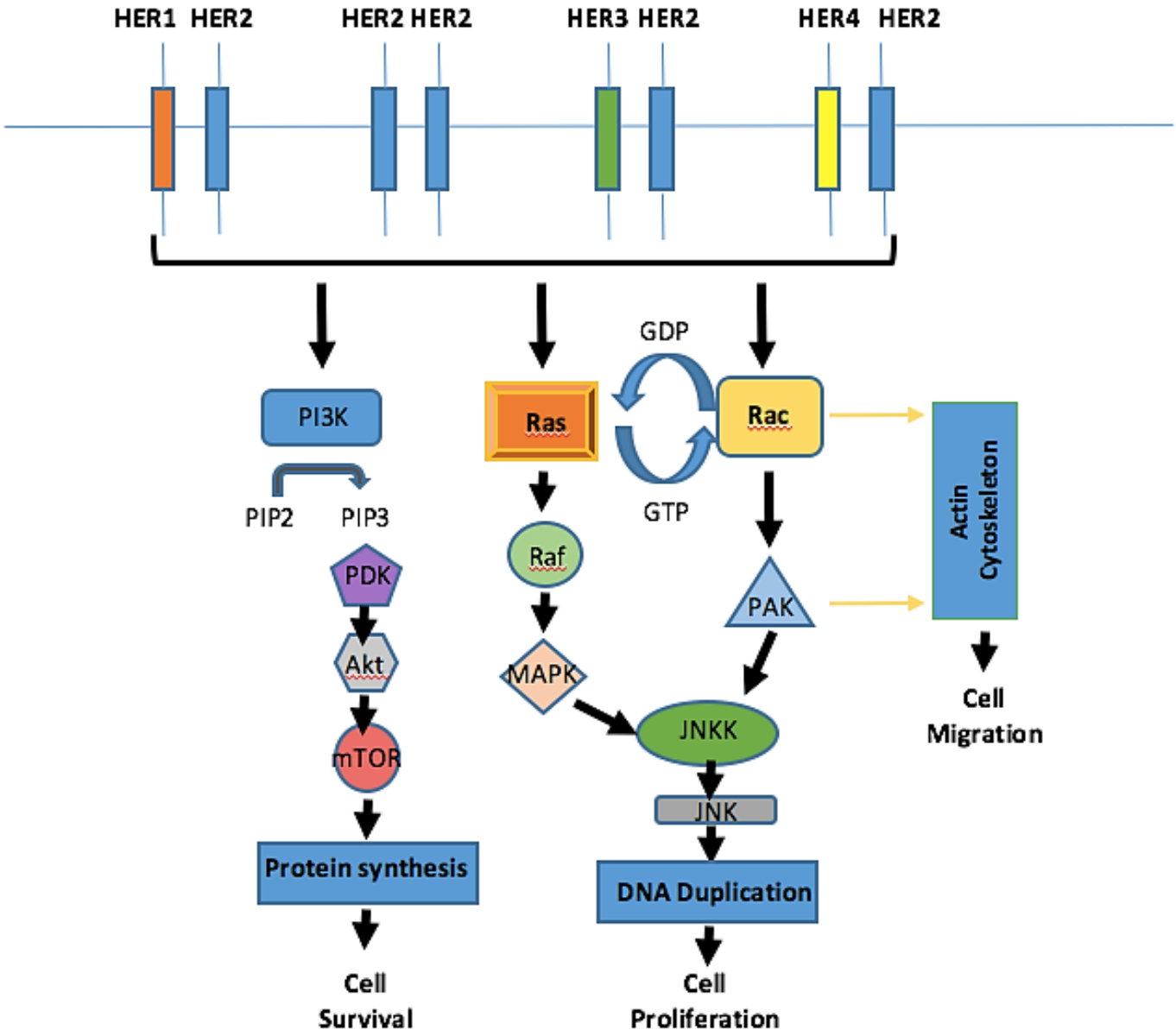

Figure 1. HER2 positive cancer downstream activation pathways targeted by HER2 specific mAb Trastuzumab that inhibits cell survival and proliferation of cancer cells.

The mode of action of this monoclonal antibody is to block angiogenesis via blocking vascular endothelial growth factor A (VEGF-A) (Lambrechts et al., 2007).

Checkpoint inhibitors: It is well-known that immune system needs to be in homeostasis in order not to damage healthy tissues and cause autoimmune disorders. Co-stimulatory and co-inhibitory receptors are crucial in the regulation of T-cell mediated immunity. In cancer immunotherapy, novel immunotherapeutic strategies mostly focus on monoclonal antibodies blocking co-inhibitory receptors in order to keep T-cells in active state against tumor cells (Sledzinska et al., 2015).

CTLA-4 is an inhibitory receptor found on T-cells, which is especially highly expressed in regulatory T-cells (Tregs) (Quandt et al., 2007). It is highly expressed especially soon after T-cell activation, and keeps its levels elevated 2-3 days post-activation (Walunas et al., 1994). One of the first attempts to block inhibitory activity of CTLA-4 was carried out in 1996 by using a monoclonal antibody causing a rejection in the formation of colon carcinoma in mice (Leach et al., 1996). Anti-CTLA-4 therapy was tried in several murine tumor models

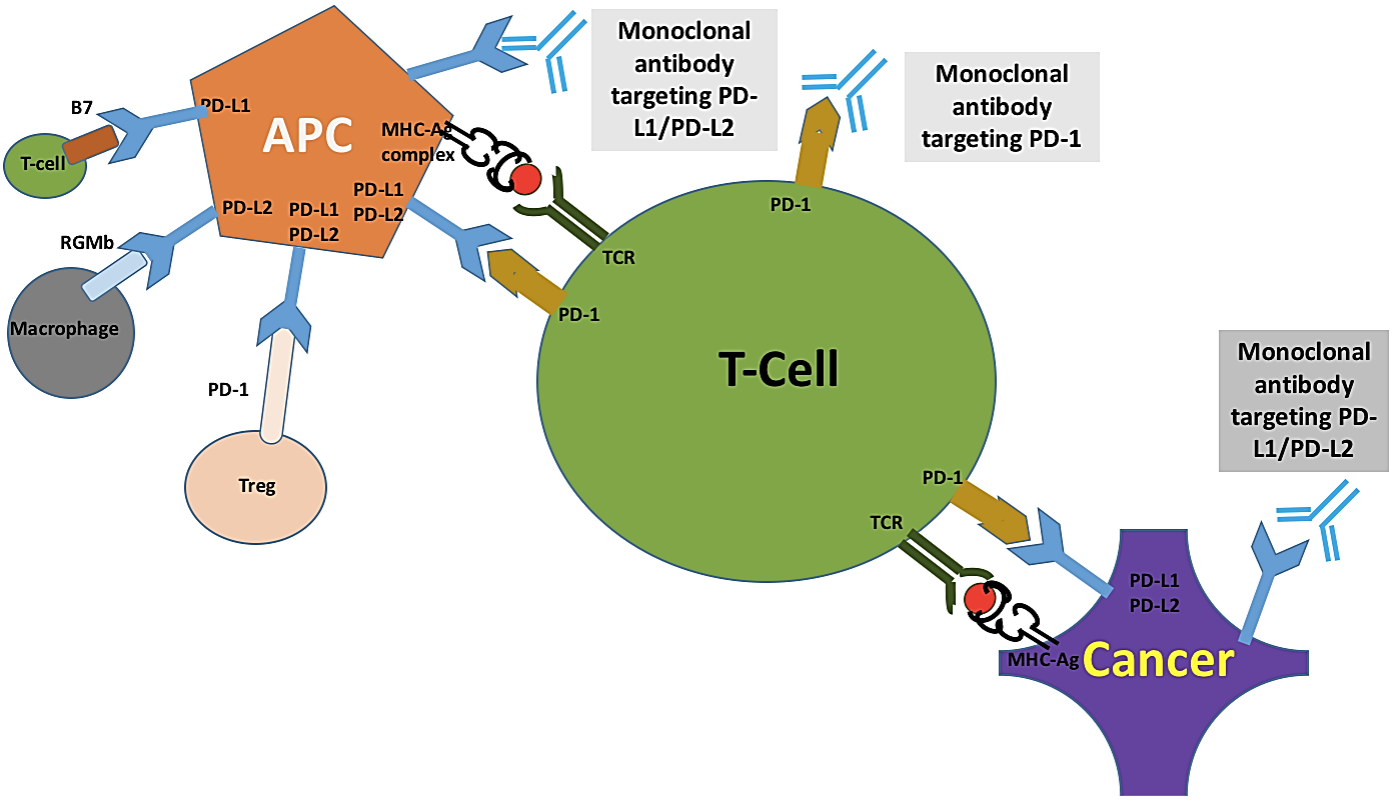

Figure 2. Strategies aimed by applying human anti-PD-1 and anti-PD-L1. Antigens released from tumors can be taken up by APCs for being processed and presented to T-cells. Cancer cells can also directly present antigens to T-cells to some extent. Up-on T-cell activation, T-cells express PD-1 receptors and PD-1/PD-L1 or PD-L2 interaction results with immune blockade. The strategy described in the figure aims to block PD-1/PD-L1 or PD-1/PD-L2 interaction by using a monoclonal antibody in order to enhance antitumor immunity. PD-L1 and PD-L2 can also bind to B7 and induce immune inhibition so applying a monoclonal antibody would prevent this pathway as well.

however it was poorly effective in the rejection of some various tumor types (Sledzinska et al., 2015). Ipilimumab and tremelimumab are the two only fully humanized monoclonal antibodies against CTLA-4 that have entered the clinical trials (Sledzinska et al., 2015). One of the first phase III study was carried out in 675 randomized melanoma patients. Ipilimumab activity was tested alone or in combination with gp100. Data obtained from the randomized study showed that ipilimumab significantly increased the lifespan of the melanoma patients (Hodi et al., 2010). In 2011, ipilimumab was approved by FDA (Sledzinska et al., 2015). Ipilimumab was also tried in combination with a conventional chemotherapy. The data obtained showed that, addition of CTLA-4 blockers in treatment increased the patients' survival by 15% (Robert et al., 2011).

PD-1 is an inhibitory molecule that is present on activated T- and B-cells (Good-Jacobson et al., 2010). PD-1 has two known ligands that are PDL-1 and PDL-2. PDL-1 and PDL-2 are both activated by various cancer types (Fig. 2) (Chen et al., 2012).

Like CTLA-4, PD-1 activation is important for immune system homeostasis, peripheral tolerance and prevention of autoimmune diseases (Okazaki et al., 2003). Tumor cells induce PD-L's in order to suppress T-cell activation and promote Tregs activation for evading host immune cells (Crespo et al., 2013; Wang et al., 2008). Tumors not only induce T-cell suppression and Treg induction by ligating PD-1, but also protect themselves from cytotoxic T-cell attack and apoptosis (Azuma et al., 2008). Understanding this mechanism gave rise to the idea of PD-1 blocking by using monoclonal antibody therapy (Fig. 3) (Strome et al., 2003). Nivolumab and pembrolizumab are two FDA approved PD-1 inhibitor monoclonal antibodies (Robert et al., 2015). In a clinical trial, non-small cell lung cancer, renal cell cancer and melanoma patients were treated with humanized monoclonal antibody against PDL-1. The data obtained showed that a 6-17% regression in tumor was induced in patients in 24 weeks (Brahmer et al., 2012). In an in vivo

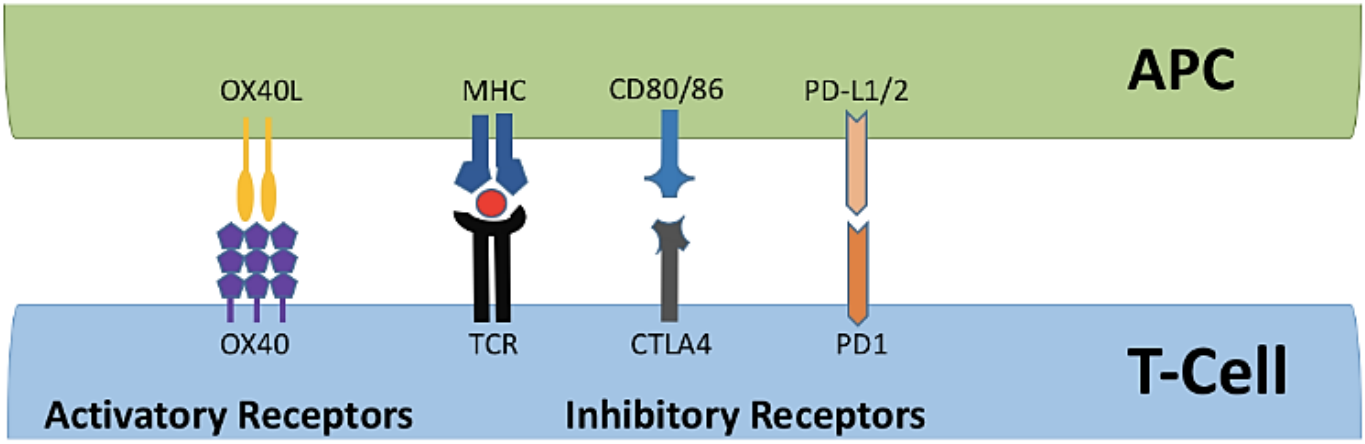

Figure 3. Activatory and Inhibitory receptors on T-cell-APC synapse.

study carried out by Vandeveer et al. (2015), anti-PDL-1 antibody (Avelumab) was tried on murine bladder tumor model expressing high levels of PD-L1. Administration of PDL-1 blocker, resulted in significant tumor reduction and improvement in survival time in mice (Vanderveer et al., 2015). PD-L1 and CTLA-4 targeting monoclonal antibodies are not the only targeted molecules in cancer immunotherapy. There are many targets in cancer signalling pathway that needs to be blocked (Fig. 3).

Antagonistic and agonistic monoclonal antibodies: Monoclonal antibodies targeting B7 checkpoint mediators (CTLA-4, PD1) are good contributors in cancer immunotherapy. However, alternative approaches are also needed as some patients are resistant against these co-inhibitory receptor blockade (Croft et al., 2010). Tumor necrosis factor receptor checkpoints gained a lot of importance at this point as they could be a good target in monoclonal antibody based cancer immunotherapies (Aspeslagh et al., 2016). CD134 (OX-40) is one of those targets that is also a co-stimulatory molecule. It is usually expressed by activated immune cells. So a monoclonal antibody targeting this co-stimulatory molecule would be a good candidate triggering immune system against cancer cells. One of the first anti-OX40 (agonistic) mAb was tried in the early 2000s. Preliminary data suggests that, using anti-OX40 not only significantly improves T-cell function, but also prevents immunosuppression caused by tumor infiltrating Tregs (Aspeslagh et al., 2016; Marabelle et al., 2013).

CD137 is another signalling receptors being targeted agonistically. It is a monoclonal antibody target that also belongs to tumor necrosis factor receptor family that is important in both innate (DC and NK cells) and adaptive (T-cells) immune responses against tumor cells. Its main function in tumor immunology is to increase autoimmune responses and induce anti-tumor immune responses via modulating tumor microenvironment (Makkouk et al., 2016). CD137 activates downstream signal transduction by activating NF□B transcription factor that is important in immune cell regulation. CD137 activates this signalling cascade via interacting with TRAF 1 and TRAF2. Research on CD137 has risen the idea to produce an agonistic mAb that induces CD137 activity against tumor cells. There are two agonistic mAbs produced against CD137 which are in clinical studies at the moment. One of them is humanized IgG4 mAb (Urelumab) and the other one is human IgG2 mAb (PF-05082566). The very first clinical trial of urelumab was carried out in 2005 and the data obtained were promising, suggesting the activation of both CD4 and CD8 T-cells upon using agonistic mAb (Sznol et al., 2008). In 2012, it was shown that, it has significant effect on low doses with small toxicity levels. Urelumab is currently being tried in clinic with other monoclonal Abs as combinational treatments (Makkouk et al., 2016). IgG2 mAb (PF-05082566) is also in clinical trials stage especially showing a response in disease stabilization (Segal et al., 2014). This mAb is also being tried in combinational therapies along with other monoclonal antibodies (Makkouk et al., 2016).

Recent advances in tumor biology and immunology risen different molecules important for tumor microenvironment. One of these molecules are ecto-5'-nucleotidase also known as CD73. It is a key molecule important in AMP degradation into adenosine that results with the promotion of angiogenesis and immune-suppression in tumor niche promoting cancer

Table 2. Showing the immune checkpoint inhibitors, agonistic mAbs, tyrosine kinase inhibitors and growth factor inhibitors mentioned in the review including their main roles in cancer immunotherapy.

	Antibody target	Effect
Checkpoint Inhibitors	PD-1, PD-L1 (Nivolumab, Pembrolizumab)	*Immune checkpoint inhibitors, *Boost Immune response upon blockade
	CTLA-4 (Ipilimumab, Trelimumab)	*Immune checkpoint inhibitors, *Boost Immune response upon blockade
Agonistic and Antagonistic Monoclonal Antibodies	CD73 (Antagonistic mAb)	*Immune infiltrate increases, *CD73 adenosine production decreases, *Tumor growth and metastases decreases
	CD134 (OX-40) (Agonistic mAb)	*TNF necrosis factor checkpoint agonist. *Provides co-stimulation on APC-T-cell interaction *Prevents Treg suppression
	CD137 (urelumab) (Agonistic mAb)	*Belongs to TNF receptor family *Agonistic mAbs increase autoimmune responses and induce anti-tumor immune responses via modulating tumor microenvironment
Growth Factor Blockers	EGFR (Trastuzumab, Cetuximab)	*Trastuzumab and cetuximab have similar activities on tumor cells as they both target the EGFR
	VEGF-A (Bevacizumab)	*Bevacizumab block angiogenesis via blocking VEGF-A
Tyrosine Kinase Inhibitors	BRAF-V600 (Vemurfenib)	*Found to prolong survival rate of patients via blocking tyrosine kinase activation.

development. CD73 targeting monoclonal antibodies gave a great response in cancer treatment studies that are given along with either anti-PDL-1 or anti-CTLA-4 (Antonioli et al., 2016). Adenosine levels can be increased while cells are in hypoxia via A2A adenosine receptor (A2AR)-dependent mechanism. So a mechanism blocking this receptor also leads to blockade of immunosuppression and expansion of CTL response (Hathfield et al., 2014; Hathfield et al., 2015). Young et al. (2016) carried out a pre-clinical study on mice aiming to block A2AR and CD73 receptors and investigate their effect on tumor cells. They have blocked A2AR and CD73 (via anti-CD73) alone and together respectively. When they blocked A2AR they investigated an increase in CD73 activity. Data obtained from the study indicates that using co-blockade CD73 and A2AR might be a good alternative way for tumor immunotherapy as they reduce metastasis, tumor growth and induce immune response against them. This study would provide a preliminary data for future studies in this field (Young et al., 2016).

Bi-specific T-cell engaging monoclonal antibodies are another type of antibodies currently being tried in cancer treatment studies. These antibodies are formed from bispecific antibodies and aim to activate T-cells against tumor cells via engaging both of them. Upon engagement, T-cells are being stimulated and specifically bind to the tumor cells. These antibodies are constructed via attachment of their variable fragments together. One of the antibodies used is specific for a molecule on tumor cell, whereas other antibody is specific for T-cell activating

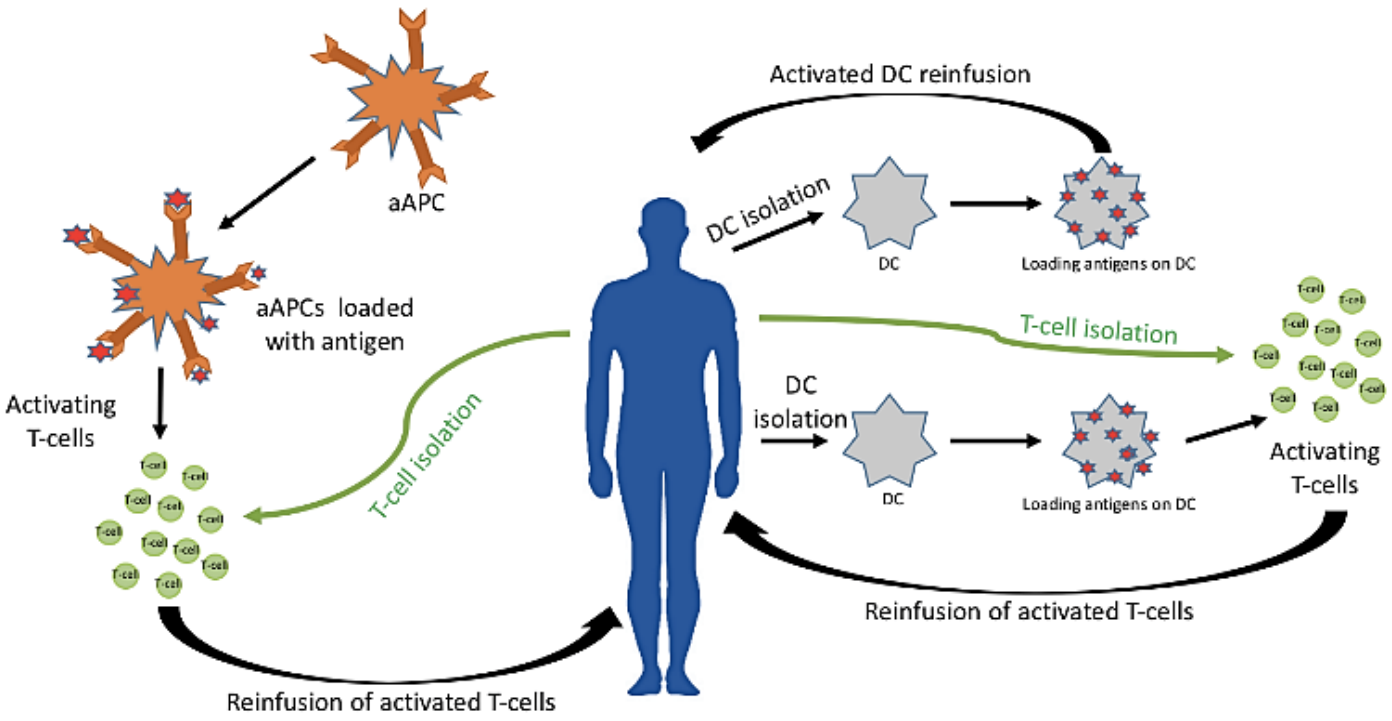

Figure 4. Various strategies being tried on cancer immunotherapy.

receptors. Blinatumomab is an example of bi-specific antibodies that is also approved by FDA for the treatment of B-cell acute lymphoma (Prezepiorka et al., 2015) (Table 2).

Tyrosine kinase inhibitors: Monoclonal antibody based therapies are not only limited for blocking immune checkpoints and ligand receptor interactions. They can also be used for the manipulation of intracellular signalling pathways such as tyrosine kinase inhibitors. Vemurafenib is one of the monoclonal antibody based therapies that targets BRAF-V600 which is a mutated protein kinase. This drug was tested in melanoma patients and has been found to prolong the survival rate of the patients (Sosman et al., 2012).

Active immunotherapy: Cancer immunotherapy can be instigated in two groups which are passive and active immunotherapy. Passive immunotherapy is based on monoclonal antibody therapies that do not activate or boost immune response against target cells. Active immunotherapy is mainly based on the vaccination of patient with tumor antigen hence boosting host immune system against the targeted tumor cells. This is the main reason why this type of treatment strategy is described as active immunotherapy (Vachelli et al., 2012). Active immunotherapy mainly depends on adaptive immune system which is based on antigen presenting cells (APCs) responsible for presenting tumor associated antigens to host immune cells. This leads to the activation of CD4 and CD8 T-cells (Vachelli et al., 2012). Cancer vaccination studies have been carried out via various different strategies for instance; by using peptides, proteins, tumor lysates, nucleic acids, DNA's, whole tumor cells, recombinant viruses etc. (Kirkwood et al., 2012; Vachelli et al., 2012).

Autologous cell transfer: Autologous cell transfer is one of the active immunotherapeutic approaches that is being tried. Autologous cell transfer is taking host immune cells (T-cells), activating them in vitro and then reinfusing them back into the patient (Fig. 4). Autologous cell transfer is usually applied after a chemotherapy in order to decrease Treg response and the competition with other T-cells (Eggermont et al., 2014).

Chimeric antigen receptor T-cells (CARTs) are another example of adoptive cell transfer based immunotherapies. In this type of treatment, T-cells of the patient are being isolated and they are stimulated by using CARTs. CARTs consist of an extracellular domain that recognizes the receptor on tumor cell and an intracellular domain that is responsible for activating T-cells. CD19 (B-cell) specific CARTs induced T-cell therapies showed

a considerable remission in B-cell lymphoma patients (Kochenderfer et al., 2012).

DC based vaccines: In 2016, a study on acute myeloid leukemia patients was carried out by Willemen et al. (2016) by using monocyte derived dendritic cells pulsed with tumor-associated antigen. Monocytes were isolated and matured into dendritic cells in vitro. Hyaluronic acid-mediated motility receptor (RHAMM) was chosen as tumor-associated antigen and induced to monocyte derived DCs via mRNA electroporation. Patients were vaccinated with the formulation and data obtained was found to be very promising due to the RHAMM specific T-cells formation at the site of vaccination (Willemen et al., 2016).

In a clinical study, Brossart et al. (2015) investigated the effect of various peptides pulsed with autologous DCs isolated from advanced ovary and breast cancer patients. They demonstrated that, the most effective CTL response inducing peptide was HER2/NEU derived E75 which was effective for more than 6 months following injection. This study has clinical significance as DC pulsed single peptide vaccination could induce long lasting immune response against tumor cells in ovary and breast cancer patients providing preliminary data for future approaches (Brossart et al., 2015).

Many vaccines that are formulated against various cancers have failed to induce a strong T-cell response in vivo. The main problem behind it is that Lymph Nodes (LNs) are the primary organs responsible for antigen uptake (Melero et al., 2014; Palucka et al., 2013). In order to overcome this problem and induce a strong antigen presentation to Dendritic Cells for developing a strong CD8 T-cell response, recent biotechnological inventions are currently tried to be used. Qian et al. (2016) tried to use nanoparticles in order to deliver tumor antigenic peptides to LNs efficiently. The idea behind the use of nanoparticles was that conventional delivery techniques have limited cargo capability and toxicity to the host cells (Jeanbart et al., 2014). Qian et al. (2016) developed an AP-carried SR-B1-targeted fluorescent nanoparticle (a-Ap-FNP) that targets scavenger receptor (SR-B1). They demonstrated the successful delivery of a-Ap-FNP to LN when injected into mice footpad or tail. This new strategy for drug delivery could be a powerful tool to elicit a strong T-cell response against tumor cells (Qian et al., 2016).

Sipuleucel-T is one of the examples of a targeted active immune therapy that is discovered against prostate cancer (Fig. 5). It is one of the first cell-based immunotherapy that is approved by FDA in 2010. PBMC of the patient is being isolated and activated by Granulocyte macrophage colony stimulating factor (GM-CSF) and a recombinant protein consisting of prostate antigen prostatic acid phosphatase (PAP). These cells are being fused and activated ex-vivo and then re-infused into the patient to activate the tumor specific T-cells (Bilusic et al., 2012). In a study, Sipuleucel-T was found to prolong survival times of the patients with prostate cancer (Kantoff et al., 2010).

Targeting cancer stem cells (CSC) via DC based immunotherapy: Cancer stem cells are the major danger for patients following the conventional treatment methods. The main reason behind it is their resistance to the conventional cancer treatment techniques. Dashti et al. (2016) tried a dendritic cell based vaccine that is pulsed with cancer stem cell lysates on mouse malignant melanoma model. CSC pulsed dendritic cell based vaccine induced a significant prophylactic effect and depression in tumor dimension in tumor bearing mice. They also demonstrated that IFN-γ and IL-4 levels of mice injected with DC based vaccine showed a significant increase suggesting a potential strategy to develop a better effective vaccine against cancer treatment (Dashti et al., 2016).

Problems in DC based vaccination and studies to overcome problems: DC based vaccination against tumor cells provides a lot of advantage including actively boosting the immune cells, including antibody producing B-cells and killer T-cells against target tumor cells. However, DC isolation and antigen presentation to them is consuming huge amounts of time apart from the difference in the clinical outcomes between different individuals due to the differences in the quality of DC generated ex-vivo (Steenblock et al., 2009; Palucka et al., 2013). In order to overcome this problem, DCs are tried to be produced artificially which is known as artificial antigen presenting cells (aAPCs). These aAPCs are rigid in structure, can be produced in different sizes, have distinct shape and they provide necessary signals needed for T-cell activation (Turtle et al., 2010). In a recent study carried out by Varela et al., nanoparticle-based aAPCs were used in vitro and tried to enrich the specificity of T-cells isolated from human donor. T-cell mixtures isolated from human donors were incubated with nanoparticle-based aAPCs presenting melanoma antigen MART1. After the enrichment and expansion protocol, they have

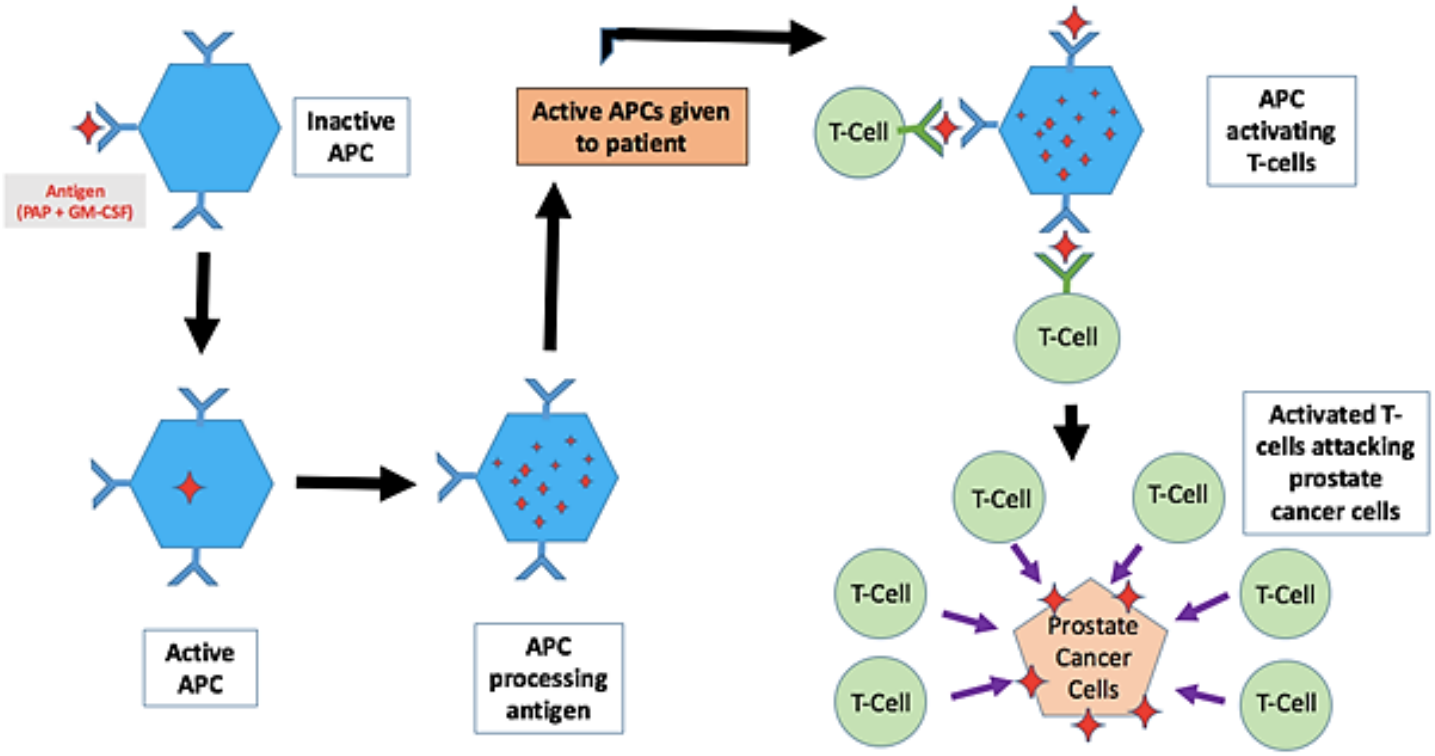

Figure 5. Sipuleucel-T activity mechanism.

tested T-cells on day 0, 7 and 14. They have demonstrated the specificity of T-cells that are incubated with aAPCs were 70% specific for target antigen (MART1). This strategy could be regarded as a huge candidate for the next generation adoptive cell transfer (ACT) technology as artificial APCs would provide advantage for saving time and increasing effectiveness of antigen presentation to T-cells (Varela et al., 2016).

Another major problem in DC based vaccination is the difficulty in obtaining a sufficient amount of functional DCs from bone marrow (BM) of patients. Kitadani et al. (2016) tried to overcome this by using pluripotent stem cell derived DCs in mice in their previous study. They found that iPSDCs were equivalent to bone marrow derived stem cells. They have also tried this technique in humans by isolating pluripotent stem cells and converting them to human iPSDCs in vitro. IPSDCs were tried on CEA-positive cancer stem cells and obtained promising results with cancer specific T-cell stimulation (Kitadan et al., 2016).

DC vaccines are applied either sub-cutaneously or intradermally to the patients which might cause DCs to die while the process is being applied and only small percentage of DCs might be able to reach the draining lymph nodes. So, intranodal injections are being tried in DC immunization to overcome this problem. This process leads to direct travel of DCs and antigen to the lymph node (Banchereau and Palucka, 2005; Harao et al., 2015; Morse et al., 1999).

Peptide based vaccines: Peptide based vaccines are mainly targeting peptides expressed on tumor cells (TAAs) and inducing a CD8 and/or CD4 specific T-cell responses. The main advantage of this type of approach is the increased specificity and less toxicity of the response when compared to other types of therapies (Fig. 6) (de Paula Peres et al., 2015).

Ghaffari-Nazari et al. (2015) tried to improve immune response against in mice HER2 positive breast cancer model by using a synthetic multi-epitope peptide (P5+435 HER2/neu), universal Pan DR epitope along with CpG-oligonucleotide adjuvant (Toll-like receptor agonist adjuvant). Data obtained demonstrated that IFN-γ, CD8 and CD4 positive cells expanded and tumor size diminished by 40% in vaccinated mice when compared to control group showing a significant tumor specific immune response formation in vaccinated mice (Ghaffari-Nazari et al., 2015).

HER2/NEU is a great candidate for cancer

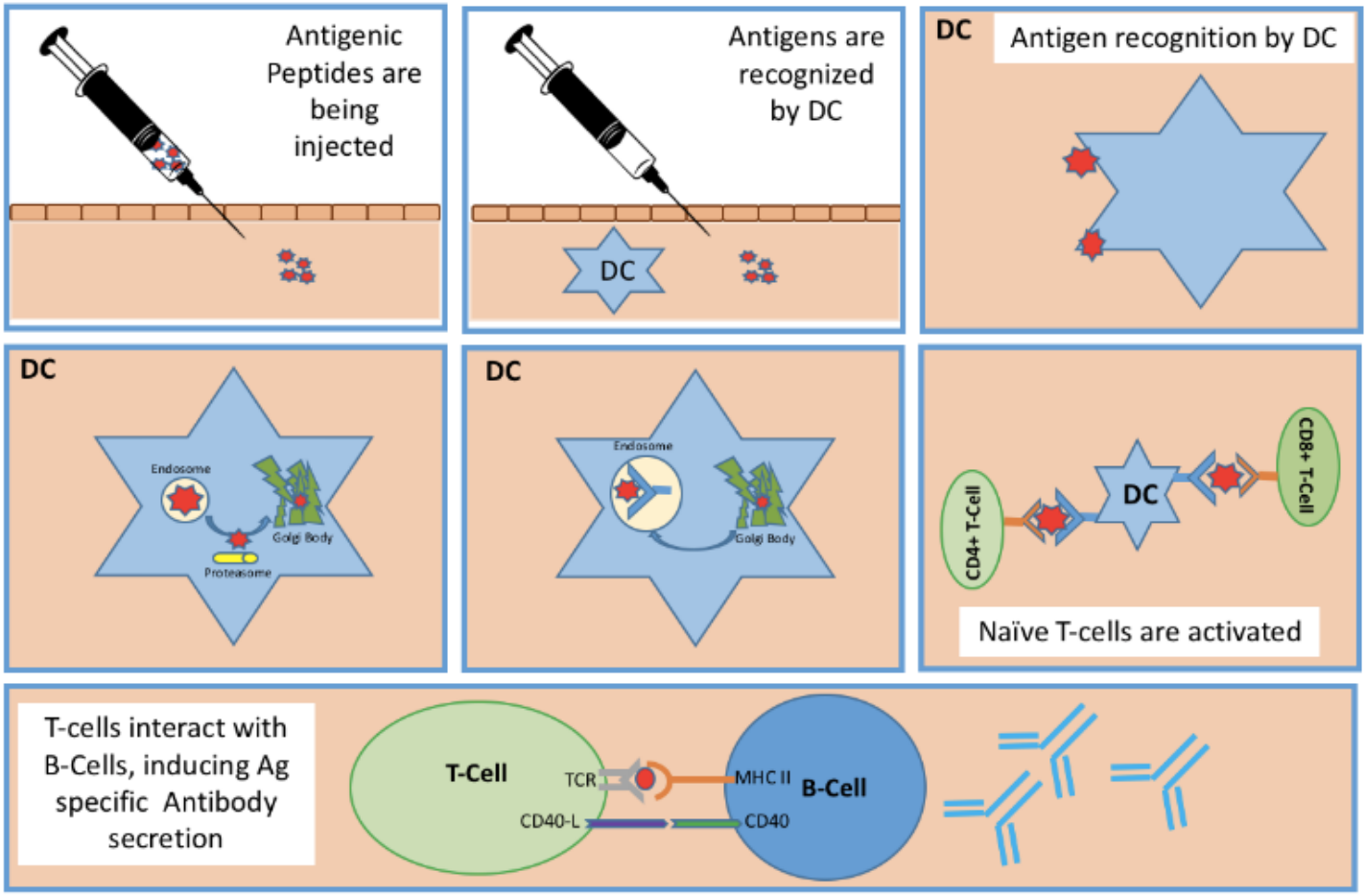

Figure 6. Peptide vaccines activation mechanism. Tumor associated peptides were injected to patient and it is being taken into endosomes and being processed in the host. After peptide processing, they are being presented to naïve T-cells via APCs and inducing a tumor specific T-cell response.

immunotherapy especially for the patients suffering from HER2 positive breast cancer. E75 is the immunological peptide of the HER2 protein that is given to patients in clinical trials along with GM-CSF. In the clinical study, it was observed that, a E75 positive CTL production increases towards the end of 4 weeks' period. Disease free survival in vaccinated groups significantly increased when compared to control group, demonstrating the positive effect of this peptide based vaccine (Peoples et al., 2005). Same group performed another study of the same formulation (NeuVax) and obtained very promising results providing a 5-year disease free survival and lower toxicity when compared to previous studies showing the effectivity and safety of the formulation (Mittendorf et al., 2012).

MUC1 is a tumor associated antigen that is a membrane-associated glycoprotein and expressed in malignant cells in a highly glycosylated form (Singh and Hollingsworth, 2006). MUC1 is regarded as a good candidate for peptide based vaccine due to its overexpression in more than 70% of the cancers (Kohlgraf, 2004). A vaccine called Stimuvax, L-BLP25, a liposomal vaccine containing 25 immunologic amino acids from MUC1 is currently in phase III trials in clinic. This vaccine is a peptide based vaccine targeting MUC1 peptide, and causing a Th1 type response via antigen presentation with APCs (Mehta et al., 2012; Paula Peres et al., 2015).

DPX-0907 is a universal Th-peptide (obtained from tetanus toxin) used vaccine along with HLA-A2 specific peptides derived from tumor associated antigens (TAAs). Phase I clinical study was carried out in breast, ovarian and prostate cancer patients. The results obtained by group was very promising with 70% increased TAA specific CTL response along with production of memory cells specific for target antigens (Bernstein et al., 2012).

In cancer vaccines, peptides used are generally the molecules expressed on tumor cells that are usually

synthesized. However, there are some different approaches like OncoVAX that uses patients own cancer cells together with a fresh frozen Bacillus Calmette-Guerin (BCG) adjuvant. Patient-specific, autologous, metabolically active, irradiated and non-tumorigenic cells are used in OncoVAX. Clinical studies showed that this vaccine has significant effect on stage II colon cancer patients and induced a lower recurrence rate when compared to the control group (Sun et al., 2016; Uyl-de Groot et al., 2005).

Therapies via using microorganisms: In a study carried out by Vendrell et al. (2011), *Salmonella typhi* based multiple treatment was tested in a mouse model for breast cancer. They have investigated a multiple treatment strategy by injecting *S. typhi* based CVD915 (attenuated *S. typhi* strain) into the tumor, peritoneal tissue and draining lymph node. They proved that using this live attenuated anticancer vaccine induced a strong Th1 type response and a significant reduction in tumor size proving its potential to be used for anticancer therapy (Vendrell et al., 2011).

Apart from bacteria, viruses are also important live organisms in cancer immunotherapy. Oncolytic viruses are natural/genetically modified that can infect and replicate inside tumor cells leading to tumor death. Up on tumor lysis, tumor antigens and some intrinsic danger signals stimulate antigen presenting cells leading to the activation of immune cells (Bartlett et al., 2013; Sathyanaryanan and Neelapu, 2015). T-Vec is an oncolytic immunotherapy that is currently being investigated in clinical treatment stage. This herpes simplex virus Type-1 isolated vaccine is engineered to replicate inside tumor cells and produce GM-CSF as an adjuvant for anti-tumor immune response. In a clinical trial, it is investigated that using T-Vec has improved the overall survival rate of patients (Johnson et al., 2015).

Foreignizing tumor cells for targeted immunotherapy: Another problem in cancer immunotherapy is the fact that tumors might contain self-antigens that are not detected and rejected by host immune system. Lee et al. (2015) investigated a different strategy and aimed to foreignize tumor cells by injecting non-self-antigenic polymers into them. They have conjugated a tumor targeting hyaluronic acid with ovalbumin (OVA) (foreign antigen). Mice bearing TC-1 tumor was treated with the conjugate and eventually they accumulated inside the tumor tissue successfully. According to the results, a decrease in tumor growth was achieved as a result of the ova specific CTL cells produced upon vaccination. Data is suggesting an alternative strategy for immune rejection in cancer immunotherapy (Lee et al., 2015).

Nucleic acids in immunotherapy: Recent advances in different types of immunotherapeutic approaches have failed over years and a strong immune response against target tumor cells have not been achieved. Unsuccessful results over years caused scientists to try alternative ways of fighting against cancer. At this point, using directly nucleic acids against tumor cells was born as an alternative strategy. This strategy is based on using double-stranded RNA to induce specific immune proteins such as cytokines (Kabilova et al., 2014). Nucleic acids can be induced in vivo by both DNA and RNA. RNA is more preferred due to the fact that it cannot be incorporated into host genome hence it does not cause any risk of oncogenesis (Hoerr et al., 2000). mRNA based DC induction is a very powerful tool for antigen presentation to DCs as it can code for all kind of transcribed proteins as well as being economic and more safe (Kreiter et al., 2011).

Van Nuffel et al. (2012) demonstrated in a study that, DCs loaded with tumor encoding mRNA induce antitumor immunity as well as increasing DC activity (Van Nuffel et al., 2012). They have participated the study on melanoma patients, and used mRNA sequence encoding full length of tumor antigen together with DCs. The study demonstrated that, using this vaccine strategy induced naïve T-cell repertoire to mature into tumor specific CD4 and CD8 T-cells (Van Nuffel et al., 2012). Stimulation of DCs with mRNA also provided the recognition of multiple epitopes from the same antigen. Data also showed that, melanoma tumors escape risk has also been reduced due to multiple epitope presentation including HLA type I and II. Using of broad spectrum of antigenic epitopes encoding mRNA also made it possible for extending this DC based therapy to all patients regardless of their HLA types (Van Nuffel et al., 2012).

Cytokines and growth factors in cancer immunotherapy: Successful immunotherapy for cancer needs a strong immune activation against targeted tumor cells. In order to perform a strong immune activation, a couple of mediators are needed to be activated such as cytokines and growth factors for effective antigen presentation to T-cells (Palmer et al., 2002).

Rok Lee et al. (2017) used a DNA technology to form

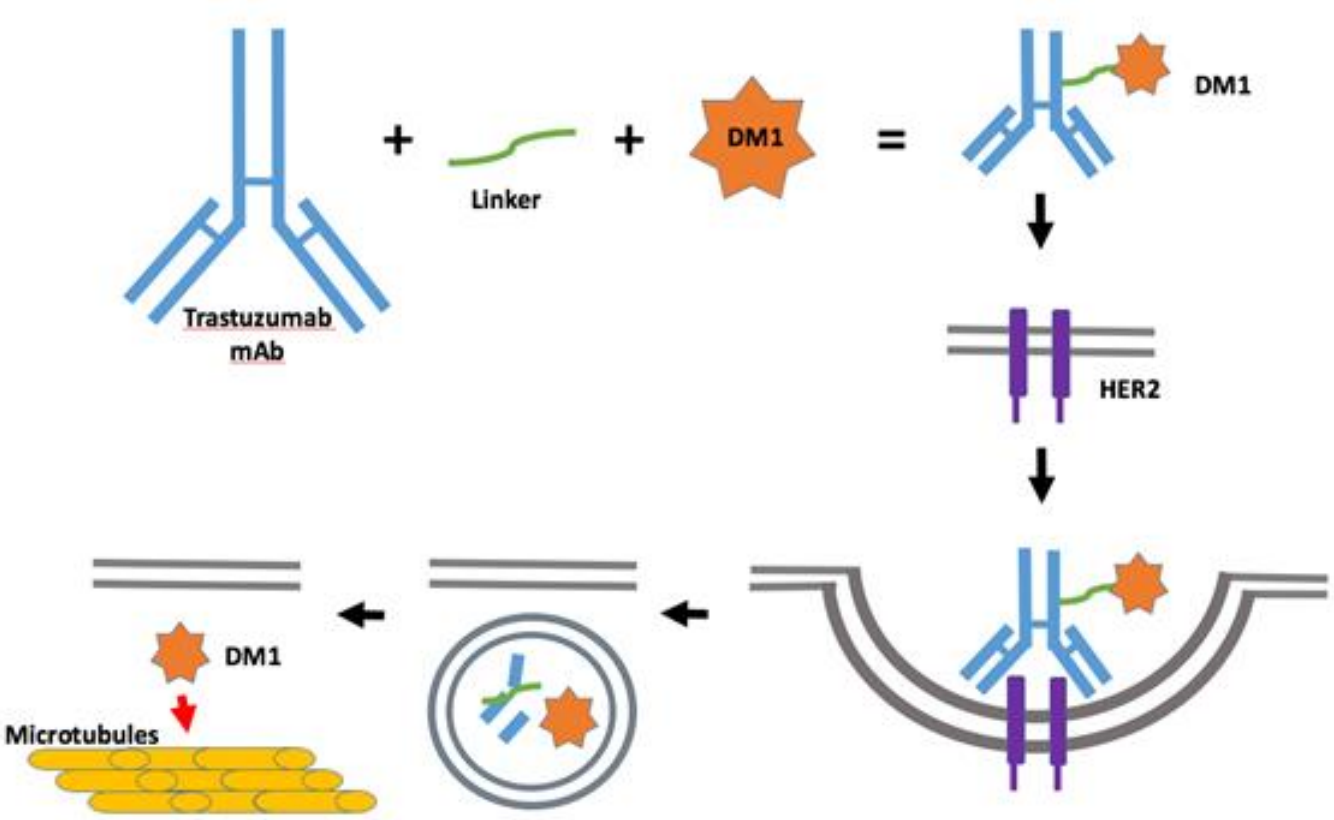

Figure 7. Structure, components and mechanism of action of T-DM1. Drug attaches to target tumor cells via trastuzumab, and it penetrates into the target cells. Inside the cell, DM1 inhibits polarization of microtubules causing the cell cycle arrest of tumor cell.

a cDNA fusion protein inducing both GM-CSF and IL-18. GMIL-18 vaccine was tested in vivo on mouse colon cancer. The results of the study demonstrated that GMIL-18 vaccine induced differentiation of DCs apart from the increase in CD4, CD8 cell infiltration into tumor cells and the induction of IFN-γ in mouse solenocytes. This study suggested GMIL-18 fusion protein could be a good candidate for immunotherapy for cancer (Rok Lee et al., 2017).

Combinational therapeutic approaches: Despite of the advances in both immunotherapy and cancer biology, current therapeutic approaches do not show expected outcomes when applied. In order to overcome this obstacle, combinational approaches such as drug-antibody conjugates are currently being tried in literature (Martinez et al., 2016).

Antibody-drug conjugates (ADCs) are formed from a monoclonal antibody that is conjugated to a cytotoxic drug via a covalent bond. ADCs are mainly designed to protect healthy cells from the cytotoxic drug via the specific antibody targeting tumor in the formulation. T-DM1 is one of the examples of combinational approaches against advanced HER2 positive breast cancer. Drug is formed from trastuzumab (mAb) conjugated with a cytotoxic drug DM1 important in cell death induction and cell division inhibition (Fig. 7) (Lewis et al., 2008). This drug-conjugate is recently being approved by both European Medicines Agency (EMA) and FDA (Amiri et al., 2014). T-DM1 mechanism activates when antibody (Trastuzumab) binds to the tumor cells. This ligation allows internalization of the DM1 (cytotoxic drug) into the targeted tumor cells. Trastuzumab not only allows specific targeting of the DM1 to tumor cells, but also labels tumor cells for Neutral Killer (NK) recognition, promotes antibody dependent cell mediated cytotoxicity (ADCC) and reduction in tumor angiogenesis (Scaltriti et al., 2007). DM1 used in the formulation is a derivative of anti-mitotic drug that inhibits polymerization of tubulin (Fig. 7) (Juntilla et al., 2011).

Conclusion

The discovery of new techniques and strategies in

immunology and cancer treatment are the new era of cancer immunotherapy as the current techniques are not sufficient and powerful in eliminating cancer cells. Better understanding of the mechanistic pathways of cancer and immunology lightens new treatment strategies. This area of research is currently very popular as it is found to be the most powerful way to eliminate cancers. This review aimed to summarize recent strategies carried out in cancer immunotherapy field.

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