Immunotherapy for Treatment of Resistant Cancer

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ABSTRACT

BACKGROUND: Cancer is the leading cause of mortality in developed countries and second cause of disease in developing countries. The aetiology of cancer resistance is prolonged exposure to carcinogenic agents and adaptation of cancer associated lifestyles, such as: smoking, diet, alcohol, sun exposure, and environmental to name just a few.

OBJECTIVE: This literature review aimed to highlight different immunotherapeutic approaches in the treatment and management of resistant cancer.

METHOD: A literature review of pertinent literature was conducted to ascertain the effectiveness of immunotherapy in resistant cancer. Google Scholar, PubMed and research gate were used to obtained related journal articles.

RESULTS: Immunotherapy has proven to be the best therapeutic approach to be applied in the treatment of resistant cancer as it induces the immune system to produce specific antibodies to cancer surface antigens.

CONCLUSION: Immunotherapy is the best therapeutic approach to be employed in treatment and management of resistant cancer.

KEYWORDS: immunotherapy, monoclonal antibodies, therapeutic strategies, resistant cancer, CRISPR application, biotherapy.

INTRODUCTION

Cancer remains the leading cause of mortality in countries that are developed and a second cause of mortality in low socioeconomic countries. Cancer burden has been identified as increasing in developing countries. This is due to constant exposure to carcinogenic agents, aging, adoption of westernised diets and cancer associated lifestyles such as smoking, high alcohol consumption and physical inactivity.1 The burden of cancer is projected to increase globally to up to 50% by the year 2030 and will be distributed mostly in low- and middle-income countries.2

The most common diagnosed types of cancer in the world that cause morbidity and mortality among men and women include cancer of the lungs with an incidence rate of 11.6% of the total cases that are globally reported. Lung cancer has a global mortality of 18.4%, which is then followed by breast cancer (11.6%), cancer of the colon (10.2%) and cancer of the prostate (7.1%).3 The major cause of treatment failure is the ability of cancerous cells to develop resistance to chemotherapy.4

Resistant cancer is a type of cancer that does not respond to therapy. Drug resistance has been the major problem in the treatment of cancer.4 Almost all types of approaches used in the management and treatment of cancer can lead to its resistance, excepting cancer that is treated through surgical procedures. Ultimately there are some patients that either do not respond positively to the applied treatment (known as intrinsic resistance), or become resistant during treatment which is known as acquired resistance. In some instances, patients become resistant to only one drug and remain sensitive to other drugs (single drug resistance), while other patients will become resistant to many drugs, known as multidrug resistance.5
The fight against cancer has remained a global battle; therefore, several aggressive therapeutic approaches will be developed with the aim of inhibiting cancer cells from proliferating and metastasising. Traditional approaches in the treatment and management of cancer such as chemotherapy and radiotherapy have shown less effectiveness. Chemotherapy and radiotherapy are often restricted in the treatment of cancer due to their increased side effects. Radiation therapy is an effective way of treating systemic cancer however, prolonged exposure to radiation contributes to tissue damage, resistance and pain. On the other hand, chemotherapeutic drugs have short-term side effects and they are often administered alongside surgical procedures. Although surgery has proven to be an effective approach in treating and managing tumours it is most effective in early stages and less effective in cancers that have undergone metastasis.

Cancer immunotherapy has proven to be the most effective approach to be considered in the treatment and management of resistant cancer since its first description in 1985. Immunotherapeutic drugs are biological substances that induce the immune system of a patient to produce antibodies that will target cancer cells and mitigate adverse effects that are related to the traditional therapeutic options. The immune system uses different techniques in the detection of cancerous cells such as the recognition of expressed tumour specific antigens, or by the recognition of molecules that appear different from the normal expressed antigens. Cancer immunotherapy has significantly contributed in the development of monoclonal antibodies and immune system checkpoints.

The therapeutic resistance of cancer occurs as cancerous cells become resistant to treatment such as radiotherapies, chemotherapies, and targeted therapy, via different treatment mechanisms. These include, genetic modifications in the genome of cancerous cells or the environmental conditions where cancer cells are residing. Therefore, different types of immunotherapy have been developed and these include monoclonal antibodies (mAbs), antigen presenting cells (APCs), biotherapy and targeted therapy.

BIOTHERAPY

Biotherapy of resistant cancer is a treatment technique that involves the use of immunotherapeutic drugs as they stimulate the immune system to respond to their stimulation and then produce cancer modifiers. Biotherapeutic drugs have the ability to stop or suppress tumour cell growth activities, help the immune cells in the identification of the tumour cells, and help in the repairing of cells that have been damaged by other therapeutic techniques. Treatment of cancer through the use of biotherapy involve the use of monoclonal antibodies (mAbs), cytokines, cancer vaccines, retinoids and gene therapy.

Monoclonal Antibodies in Resistant Cancer Treatment

mAbs are a type of antibody that are produced by B-lymphocytes in response to the introduction of foreign proteins (antigens). Unlike polyclonal antibodies that bind on different sites of the antigen, monoclonal antibodies bind to a single specific site of the antigen (e.g. cancerous cells) called an epitope, and stimulate the immune system to destroy them. Once they are introduced in the body, they bind on cancer cells to enable other cells of the immune system to easily identify the cells and destroy them. Monoclonal antibody efficacy in cancer immunotherapy is based on three different mechanisms which include:

1. Inhibition of factors that activate neoplasm proliferation;
2. Activation of antibody dependant cellular toxicity which is mostly bound to monoclonal antibodies used in targeted cancer therapy; and
3. Activation of complement dependant cellular toxicity.

Monoclonal antibodies are produced through immunisation of a mouse against a targeted cell. This process allows the immune system of the mouse to produce specific anti-human antibodies against that specific human antigen. The produced antibodies are then fused with the cancer cells that are cultured.

There are different types of monoclonal antibodies that are used in cancer therapy and these are either naked monoclonal antibodies or conjugated monoclonal antibodies. Naked monoclonal antibodies are modified proteins that are able to work without being bound by drugs or radioactive materials. Naked monoclonal antibodies are the most common type of monoclonal antibody used in the treatment of cancer. They bind on to the antigenic receptors of cancer cells and mediate their destruction. In conjugated monoclonal antibody cancer therapy, monoclonal antibodies are conjugated to a specific drug. Drug conjugated monoclonal antibody therapy are also known as antibody drug conjugates (ADCs). Antibody drug conjugates are targeted to a specific site that is affected by cancer as a way of overcoming side effects that have been observed in traditional resistant cancer chemotherapy.

Currently a few monoclonal antibodies have been approved for the treatment of cancer and these include rituximab, trastuzumab, bevacizumab, cetuximab and gemtuzumabozogamicin.

HYBRIDOMA CELL PRODUCTION

Monoclonal antibodies are a typical example of antibodies that are produced from hybridoma technology which involves injecting a mouse with a specific antigen. The injected antigens stimulate the immune system of the mouse to produce specific antibody producing cells which are then procured from the spleen of the mouse and subsequently fused with cancer immune cells called myelomas illustrated in Figure 1. The hybridoma cells obtained from fusion of isolated B-cells and cancer cells, are then cultured onto a selective media called hypoxanthine aminopterin thymidine (HAT). This medium allows the growth of only fused cells (Hybridoma), because unfused cancer cells are unable to synthesise hypoxanthine guanine phosphoribosyl transferase, an enzyme responsible for nucleic acid synthesis. However, hypoxanthine aminopterin thymidine medium is employed in production of monoclonal antibodies.
Monoclonal Antibodies Used in the Treatment of Resistant Cancer

Rituximab
Rituximab are monoclonal antibodies that target specific surface antigens of B-lymphocytes known as the cluster of differentiation 20 (CD20). It was the first type of monoclonal antibody to be used in the treatment of non-Hodgkin lymphomas and low-grade lymphomas. Currently, it has been approved in the treatment of CD20 B-lymphocyte cancers or any B-lymphocyte induced infections.\textsuperscript{12} It binds onto the CD20 surface antigens of the B-lymphocytes and activates the complement system to destroy the cancerous cell.\textsuperscript{13}

Trastuzumab
Trastuzumab are a set of monoclonal antibodies that interfere with the human epidermal growth factor receptor 2.\textsuperscript{14} It is the only approved monoclonal antibody for the treatment of human epidermal growth factor receptor 2 positive carcinomas such as breast cancer.\textsuperscript{15} Trastuzumab binds to the human epidermal growth factor receptor which are composed of distinct receptors. Through this process a tyrosine kinase is activated which triggers the regulation of various cellular activities such as cell division, cell death, and the ability to adhere to substances. Just like any other therapeutic methods, trastuzumab has adverse effects, which include anaemia, rash, dyspnoea, neutropenia and it may lead to cardiovascular related medical conditions. Trastuzumab accounts 2% to 7% of cardiac dysfunction cases.\textsuperscript{16}

Bevacizumab
Bevacizumab is a monoclonal antibody that is used to treat different types of cancer. It inhibits the formation of new blood vessels therefore leading to the blockage of endothelial cell differentiation. Its use is critically monitored because of its life-threatening cardiovascular side effects.\textsuperscript{17}

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Cetuximab
Cetuximab is a monoclonal antibody that targets a family of human epidermal receptors of tyrosine kinase. Cell proliferation is initiated as soon as the receptors are activated by ligand binding to the tumour cells. Cancers that result from the mutation of the epidermal growth factor receptors have been identified as the cause of human epithelial carcinomas. In order to treat the cancer effectively, strategies that target these receptors have been employed in previous decades. Despite it being effective in the treatment of cancer, it is well recognised as an important cause of both intrinsic and acquired resistance.\textsuperscript{18}

Cytokines in Resistant cancer Treatment
Cytokines are proteins that are released after the activation of the immune system.\textsuperscript{19} They help in communication by maintaining intracellular signals of the cells. Cytokines are immunomodulating agents that regulate cellular functions by binding to specific surface antigens of cancer cells, they enhance the activity of cytotoxic T lymphocytes (CTLs) and the cytotoxic effect natural killer cells thereby promoting tumour penetration and destruction.\textsuperscript{20} There are two forms of cytokines and these include interleukins and interferons.
Cytokines may either be paracrine and autocrine but never exocrine. Autocrine action involves release of chemicals that exert their effect on the cell that induced their production while paracrine action of cytokines involves secretion of chemicals by one cell that will exert their effect on cells other than those that induced their production. Their function is to block the cancer cell proliferation process through inhibition of protein synthesis. When cytokines become unregulated, they intend to form other medical complications such as Alzheimer's disease.10

Cancer Vaccines in Resistant Cancer Treatment

Vaccines are genetically modified agents resembling those that may cause a disease. They may be viruses, bacteria or surface proteins of antigens. Cancer vaccines are specifically developed from a patient’s cancer cells.11 The reason why most cancer vaccines are developed from a cancer patient they are then attenuated by the use of radiation or heat, thereby making the harmless.12,13 Attenuated cancer vaccines help the immune system to produce specific antibodies against the causative agent of that specific cancer. A demonstration illustrating the capabilities of attenuated herpes simplex virus (HPV) was made in the year 1999 by Ioda as it showed the effects of attenuated HPV with regards to it being able to induce the immune system to produce anti-herpes simplex virus antibodies.14 Currently researchers are working on the integration of vaccines with other forms of immunotherapies as a way of improving the effectiveness of immunotherapy.15

Retinoids in Resistant Cancer Treatment

Retinoids are a class of vitamin-derived chemical compounds. They may be synthesised or be naturally occurring vitamin A analogues.16 Once retinoids are introduced in the body, they will bind to retinoid binding receptors or proteins thereby activating specific genes of the deoxyribonucleic acid (DNA). The regulatory regions on the DNA are also known as retinoic-acid response elements which regulate cell division, differentiation and cell apoptotic activities. During cancer immunotherapy, retinoids inhibit tumour cell invasion, proliferation and migration.17 Retinoids play a very important role in the immunotherapy of resistant cancer. As demonstrated in Figure 2, retinoids do not only inhibit cell proliferation and cancer development, they also induce tumour cell apoptotic activities.18 Retinoids have the ability of modifying cellular properties through activation of certain genes or repressing specific carcinogenic genes.19

There are two forms of retinoids, naturally occurring and synthetic retinoids. These retinoids are able to penetrate the cell membrane through a protein mediated transport system. Figure 2 illustrates the retinoic acid pathway which begins with retinol (ROH) binding onto retinol binding protein 4 (RBP4) forming ROH-RBP4 complex which later penetrates the cell membrane and enters the cytoplasm of the cell. In the cytoplasm, ROH has three fates its ether is converted into retinyl ester which are then stored into the liver or it gets bound to cellular retinol-binding protein (CRBP) to form CRBP-ROH complex. When ROH is metabolised into retinaldehyde by alcohol dehydrogenase (ADH) this reaction can be reversed by the enzyme short-chain dehydrogenase/reductase (SDR).

Figure 2. Illustrates the mechanism of action of retinoids. Adapted from Priya and Reyes 19
The produced retinaldehyde further more acted upon by the enzyme aldehyde dehydrogenase (ALDH) to form all-trans-retinoic acid (atRA). The formed atRA then diffuses into the nucleus or metabolised to form 9-cis-retinoic acid (9cRA). In the nucleus, atRA activates only retinoic acid receptors (RAR) whereas 9cRA activates both the RAR and retinoid X receptors (RXR). The activation of the theses receptors heterodimerise and bind to the sequence of DNA known as retinoic acid response element (RARE) and retinoid X DNA response element (RXRE). Binding to RARE and RXRE activates transcription for target genes. This pathway can either induce differentiation and or cell apoptosis of tumour cells.23,27

**Gene Therapy in Resistant Cancer**

Gene therapy is one of the important approaches used in the treatment and management of cancer. The significance of using gene therapy in resistant cancer treatment is that it selectively destroys cancer cells from the normal body cells. The rationale behind gene therapy is that the modified genes induce production of cytokinins that will destroy cancer cells.22 The *Escherichia coli* cytokine deaminase is one of the enzymes that are genetically modified to aid suicide gene therapy in the treatment of resistant cancer. Due to the ability of cytokine deaminase to converting the prodrug 5-fluorocytosine (5-FC) into its active form 5-fluorouracil (5-FU), it is used in the treatment of cancers such as prostate cancer. When 5-FU penetrates the cell, it will induce cellular apoptosis.24

Gene therapy can help in prolonging the lives of people who have cancers which are incurable such as multiple myeloma.23 Although genetic engineering is currently a promising approach employed in treatment and management of resistance cancer, it is nevertheless associated with challenges of its efficacy. Nucleic acid instability is one of the factors associated with poor efficacy as they are subjected to enzymes such as nucleases that will degrade them before they reach their targeted site.27 New genetic engineering techniques such as clustered regularly interspaced short palindromic repeats (CRISPR) have shown unprecedented clinical advantages in the discovery of novel targets in the treatment of cancer. It is used not only in cell culture and microorganism genetic editing but it can also be used in human genome editing as it provides immunotherapeutic advantages. CRISPR used in the manipulation of human genetic material (DNA and RNA) replicating immune defence mechanisms form bacteria and viruses there by providing immunity against the invasion of foreign nucleic acids.30

**Risks Associated with Resistance Cancer Immunotherapy**

Cancer immunotherapy in resistant cancer has transformed the history of cancer and the lives of patients with resistant cancer, thus by making the cure possible for patients who develop resistance from other therapies such as chemotherapy. Just like any other therapeutic approaches used in the treatment of resistant cancer, immunotherapy has different forms of side effects associated with a specific immunotherapeutic approach adopted.37

The use of monoclonal antibodies in the treatment of cancer may lead to common side effects in immunotherapy. The signs and symptoms include; flu-like symptoms, fatigue, skin rash and muscle pains. Life threatening adverse effects of using immunotherapy include reduced hematopoietic activities, which may lead to white blood cell, red blood cell and platelet associated complications such as thrombotic thrombocytopenic purpura (TTP). Once the hematopoietic cell line is affected, complications of the cardiovascular system such as heart failure may develop. There are multiple mechanisms that are associated with the development of heart failure of which one of them is as a result from underproduction of red blood cells which in turn will lead to low red blood cell count. Low red blood cell count results in reduced oxygen to various body tissues for metabolic reactions. In anaemic patients, this will trigger a host of hemodynamic alterations that will lead to an increase in myocardial workload, causing adverse left ventricular remodelling. Apart from hematopoietic activities, some monoclonal antibodies bound to a drug in targeted immunotherapy are developed to halt the development of blood vessels by tumour cells thereby leading to bleeding.30,36

**Most clinics and hospitals use chemotherapy, hormonal therapy, radiotherapy and surgical therapy, the effectiveness of these techniques are less compared to that of immunotherapy**

**CONCLUSION**

Immunotherapy remains the best approach in the treatment of resistance cancer as compared to chemotherapy, which has a lot of therapeutic side effects. In the skeletal muscle of patients with cancer, chemotherapeutic agents induce oxidative stress onto the tissues by decreasing antioxidant levels leading to immune cell destruction and muscle weakness.23,33 Although most clinics and hospitals use chemotherapy, hormonal therapy, radiotherapy and surgical therapy, the effectiveness of these techniques are less compared to that of immunotherapy. The discovery of the epidermal growth factor receptors has opened the doors to understanding the signalling pathways used by cancer cells.22 Immunotherapy helps in boosting of the immune system in cancer patients with less adverse effects.39 Through the use of immunotherapy, many biomarkers have been identified which are now used in diagnosis of various tumours.40 The development of tumours is influenced by underlying genetic defects resulting in uncontrolled proliferation of tumour cells.35 Although CRISPR has been developed in the last decade, cancer related deaths are still being reported. However, CRISPR has shown effectiveness in dysregulation of cancers through targeting the epigenetic regulatory system and it is the future therapeutic approach to be adopted in the treatment cancer.50
ACRONYMS

9cRA - 9-cis-retinoic acid, ADCs - Antibody drug conjugates, ADH - Alcohol dehydrogenase, ALDH - Aldehyde dehydrogenase, APCs - Antigen presenting cells, aTRA - All-trans-retinoic acid, CD20 - Cluster of differentiation 20, CRABP - Cellular retinoic acid binding protein, CRBP - Cellular retinol-binding protein, CRISPR - Clustered regularly interspaced short palindromic repeats, CTLs - Cytotoxic T lymphocytes, CYP26A1 - Cytochrome P450 Family 26 Subfamily A Member 1, CYP26B1 - Cytochrome P450 Family 26 Subfamily B Member 1, CYP26C1 - Cytochrome P450 Family 26 Subfamily C Member 1, DNA - Deoxy ribonucleic acid, Fc - Fluorocytosine, FU - Fluorouracil, HAT - Hypoxanthine-aminopterin-thymidine, HPV - Herpes Simplex Virus, mAbs - Monoclonal antibodies,RAR - Retinoic acid receptor, RARE - Retinoid X receptor element, RNA - Ribonucleic acid, ROH - Retinol, RXR - Retinoid X receptor, RXRE - Retinoid X DNA response element, SDR - Short-chain dehydrogenase/reductase, TIP - Thrombocytic thrombocytopenic purpura.

REFERENCES


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