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Prospective Therapeutic Strategies for Cervical Cancer

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Abstract – Cervical cancer is one of the leading causal cancer-related fatalities in the world. Cervical cancer patients can be treated by conventional treatment such as surgery, radiotherapy, chemotherapy, medications and combination treatments. Currently, more targeted treatments are being developed to cure cervical cancer. The treatments include immunotherapy, virotherapy and gene therapy which will be discussed in this paper. In immunotherapy, the synergy of CTLA-4 suppression and PD-1/PDL-1 immune checkpoint inhibition targeting their corresponding pathways enhanced the human immune system resulting a promising treatment effects. Oncolytic viruses such as Newcastle disease virus selectively infect and kill cancerous cells/tissues without harming normal cells/tissues. This character has made them a potential modality in combating cancer which popularly known as oncolytic virotherapy. Gene therapy delivers modified genetic materials to the target cancer cells via viral and non-viral vectors. It is used to target the abnormal gene, to increase cells' susceptibility towards drugs or conventional therapy, to induce tumour cells apoptosis, to enhance tumour cell immunogenicity recognition and to inhibit the oncogene expression. The objective of this minireview is to add to the general knowledge on aforementioned therapeutic strategies against cervical cancer.

Keywords: cancer, cervical, immunotherapy, oncolytic, gene therapy

Introduction

Cervical cancer is a cancer that developed in woman's cervix and one of the leading causal cancer-related fatalities in the world. Cervical cancer is asymptomatic during early stage, otherwise it can be treated via surgery or radiation upon detection. Infection by human papillomavirus (HPV) strains HPV-16 and HPV-18, also known as the high-risk HPVs, is the most common cause of cervical cancer. Any activities which lead to the exposure of HPV infection such as having multiple sexual partners and engaging in sexual contact at early age in life may also cause the disease (National Health Science, 2018). Other possible reasons of cervical cancer include aberrant activation of hepatocyte growth factor/c-mesenchyal-epithelial transition (HGF/c-Met) signalling pathway (Boromand *et al.*, 2017) and overexpression of microRNA-9 (miR-9) (Zhang *et al.*, 2018). Most cervical cancer cases are preventable by routine screening and vaccination, nonetheless, metastatic cervical cancer often results in poor prognosis (Yung *et al.*, 2013). Screening of the cancer for early detection can be done via PAP smear test where the general practitioner would swab a small sample of cells from the cervix area and observed for any abnormalities under microscopes (National Health Service, 2018). Cervical cancer may be treated via surgery, radiotherapy, chemotherapy, medications and other targeted treatments including combination therapy (American Cancer Society, 2018; National Health Service, 2018). This

minireview will be focusing on three therapeutic strategies, namely immunotherapy, oncolytic virotherapy and gene therapy. The exploration of these strategies may add to the general knowledge on aforementioned therapeutic strategies against cervical cancer even to those who are not in the field of medicine.

Immunotherapy

Cancer immunotherapy is defined as the utilization of naturally derived or synthetically generated components to stimulate or enhance body immune response to fight against cancer. Immunotherapy is able to restore the damped anti-cancer immune response (Drake *et al.*, 2014). The general concept of immunotherapy is to achieve a response against tumour by stimulating immune defences, which are mostly impaired among cancer patients (Disis, 2014; Mandal and Chan, 2016).

The roles of immune checkpoints in regulating immunity

The key requirement of immune system is crucial for self-tolerance, to prevent the immune cells from attacking cells indiscriminately. To prevent autoimmunity, activation of immune checkpoints pathway is vital to regulate activation of T cells at multilevel steps during an immune response (Fife and Bluestone, 2008; Goldrath and Bevan, 1999). Immune checkpoints are referred as the plethora of inhibitory pathways of the immune system for the maintenance of self-tolerance and immune homeostasis (Pardoll, 2012).

Under normal conditions, a balance between T cell activation and the inhibitory pathways are used to prevent autoimmunity or immune deficiency. Pardoll (2012) described that the expression of immune checkpoint proteins could be dysregulated by tumours as an important immunity resistance mechanism. In context of cancer condition, overexpression of inhibitory T cell receptors on their own cell surface permits inhibition of anti-tumour immune response. In most cancer immunotherapies, cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death receptor-1 (PD-1) are the main inhibitory receptors that being expressed on T cells (Chen and Flies, 2013; Pardoll, 2012), also referred as immune checkpoints that belong to immunoglobulin superfamily (Brunet *et al.*, 1987; Ishida *et al.*, 1992).

CTLA-4 immune checkpoint expressed on the surface of T cells interacts with ligand cluster of differentiation 80 (CD80) and for PD-1, it interacts with its programmed death ligand-1 (PDL-1) on antigen presenting cells (APCs). According to Fife *et al.* (2009), CTLA-4 drives the immune checkpoint inhibitors, as it halts potentially autoreactive T cells at the initial stage of naïve T cell activation. In contrast, PD-1 pathway which primarily occurs in peripheral tissues, will regulate the previously activated T cells at the later stages of immune response. Expression of PD-1 also being presented on B cells, dendritic cells (DCs), monocytes and natural killer (NK) T-cells upon activation (Brunet *et al.*, 1987; Riley, 2007).

Functions of PD-1 and its ligand, PDL-1, in cervical cancer

High expression of PDL-1 is commonly observed on cell surface of solid tumours. This expression has a large proportion on tumour infiltrating lymphocytes (TILs) (Ahmadzadeh *et al.*, 2009). It has been reported that expressions of PDL-1 are 95% of cervical intraepithelial neoplasia and 80% of squamous cell carcinomas (Mezache *et al.*, 2015). Within tumour microenvironment, PDL-1 is expressed for oncogenic signalling or induced to inflammatory cytokines (Jenkins *et al.*, 2018). The complexity of PDL-1 neither guarantees nor precludes response to PD-1/PDL-1 blockade. However, murine studies had confirmed the contribution of PDL-1 on both tumour cells and immune cells are vital to determine response to PD-1 blockade (Figure 1(A)) (Juneja *et al.*, 2017, Lau *et al.*, 2017).

Discovery of PD-1 and PDL-1 pathways emerges as a result of the necessity to control the degree of inflammation at the site of antigen expression. The cytokines produced by T cells will modulate PDL-1 expression in tissues hence activate the PD-1 proteins (Mahoney *et al.*, 2015). This condition will lead to immune tolerance, where the immune system loses the control to mount an inflammatory response.

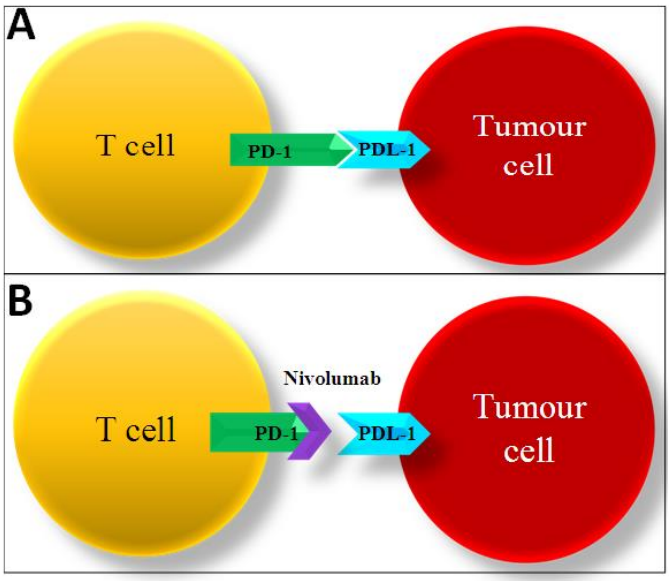

Figure 1: (A) Immune checkpoint, programmed death receptor-1 (PD-1) expressed on T cell surface binds to its programmed death ligand-1 (PDL-1) on tumour cell. (B) Nivolumab inhibits the interaction of PD-1 to its ligand PDL-1 (e.g. Lau *et al.*, 2017; Topalian *et al.*, 2014).

PD-1/PDL-1 immune checkpoint inhibition

Pharmacologically, the inhibitors of PD-1/PDL-1 prevent the interaction between PD-1 and its ligand, PDL-1, thus facilitating a positive immune response to kill the tumour. Several studies have indicated that antibodies that inhibit PD-1 and PDL-1 have prognostic capacities on many advanced malignancies and an efficient way to maintain the function of effector T cells. Inhibition of PD-1/PDL-1 interactions by specific antibodies may serve as an effective anti-tumour therapy. PD-1 pathway blockades will restore the activity of anti-tumour T cells that had become quiescent (Buchbinder and Desai, 2016).

Monoclonal antibodies (mAbs) have been used as immune checkpoint inhibitors that inhibit the interaction of PD-1/PDL-1 pathway and overcome the conventional therapy for cancer treatment. mAbsare able to reduce solid tumours, suppress advanced tumours and metastasis, and diminish the toxicity within tolerable limits, contributing to the survival of cancer patients (Naidoo *et al.*, 2015; Topalian *et al.*, 2014). The checkpoint inhibitors are designed to either block PD-1 or PDL-1, hence turn on T-cell mediated immunity (Figure 1(B)).

PD-1/PDL-1 immune checkpoint inhibitor against cervical cancer

On December 2014, the Food and Drug Administration (FDA) had approved Nivolumab (Opdivo, Bristol-Myers Squibb), for treatment of patients suffers from metastatic or unrespectable melanoma (Alsaab *et al.*, 2017). Nivolumab is PD-1 specific monoclonal antibody and it prevents the interaction of PDL-1 towards PD-1. In a clinical trial conducted by Hollebecque *et al.* (2017), treatment using Nivolumab demonstrated encouraging clinical outcomes among women with recurrent or metastatic cervical, vaginal and vulvar cancers. The study showed that progression-free survival rate (73.9%) was observed after three months, and overall survival (87.1%) was observed after six months. The overall response rate (ORR) across those three tumour types was 20.8% and the disease control rate (DCR) was 70.8%. For future prospect, combination of immune checkpoint inhibitors is ongoing in clinical trials which involve co-targeting CTLA-4 and PD-1, in combination or sequential, in advanced-stage of melanoma patients. This synergism may be resulting in the amplification of T cells in lymphoid organs and tumour tissue by CTLA-4, while inhibition of PD-1 overcomes the immune suppression in tumour tissues (Ribas, 2012). This combination treatment is now being investigated in ovarian and cervical cancers (Ribas, 2012).

Oncolytic Virotherapy

Oncolytic viruses selectively infect and kill cancerous cells/tissues without harming normal cells/tissues (Ferguson *et al.*, 2012; Fukuhara *et al.*, 2016; Russell *et al.*, 2012). This character of oncolytic viruses has made them a potential modality in combating cancer which popularly known as oncolytic virotherapy nowadays. Oncolytic virotherapy is generally divided into two approaches using either naturally-occurring oncotropic viruses such as Newcastle disease virus, parvovirus, vesicular stomatitis virus and reovirus or using genetically modified viruses, which are engineered to attain selective oncolysis ability, such as adenovirus, herpes simplex virus and vaccinia virus (Motalleb, 2013).

Viruses have attracted interest as potential anti-cancer therapeutic agents since early 19th century when tumour regressions have been documented following virus infection or vaccination, mostly seen in immunosuppressed patients (Liu *et al.*, 2007). This is the foundation for clinical trials where body fluids containing animal or human viruses were used to transmit infections to cancer patients (Russell *et al.*, 2012). Among the earliest reports was the regression of cervical carcinoma after administration of rabies vaccine in 1912 (DePace, 1912). In 1956, a clinical trial using live adenoidal pharyngeal conjunctival against cervical cancer showed selective oncolytic effect of the virus limited to cancerous tissues (Smith *et al.*, 1956). The practice was eventually abandoned due to uncontrolled toxicity (Fukuhara *et al.*, 2016). However, with the invention of recombinant DNA technology, modification of viruses to improve their safety and anti-tumoural efficacy became possible (Kirn *et al.*, 2001).

Advantages and disadvantages of oncolytic virotherapy

Oncolytic viruses destroy tumours by various mechanisms. A direct cell lysis can be achieved via the production of proteins that have direct cytotoxic effects on the tumour cells, through transgenes expression (Mullen and Tanabe, 2002). Elicitation of specific and non-specific immune response may enhance sensitivity of tumour cells to chemotherapy and radiotherapy (Goldufsky *et al.*, 2013).

Oncolytic virotherapy is generally safe (Ferguson *et al.*, 2012; Goldufsky *et al.*, 2013; Liu *et al.*, 2007) as it lacks cross resistance with other therapeutic agents (Kirn *et al.*, 2001; Motalleb, 2013). Oncolytic viruses allow for the insertion and expression of transgenes in tumour cells to achieve specific effect (Goldufsky *et al.*, 2013), while offering synergistic activity with other therapeutic approaches (Prestwich *et al.*, 2008). It is also possible to monitor virus spread in tumours through transgene expression monitoring (Russell *et al.*, 2012). Moreover, the amplification of input dose is possible as virus replicates and release new virions (Sze *et al.*, 2013).

Nonetheless, oncolytic virotherapy does carry some drawbacks. The disadvantages include the presence of pre-existing immunity to the virus as a result of primary infection and/or previous immunization or oncolytic virotherapy, which limits the virus spread (Ferguson *et al.*, 2012). In addition, virus neutralization by antibodies, inactivation by complements, non-specific uptake by other tissues such as the liver and spleen, and poor virus discharge from the vascular compartment following intravenous administration have been reported (reviewed by Wong *et al.*, 2010).

Enhancing viral delivery

Various approaches to enhance viral delivery to tumour cells have been suggested. Viruses can be delivered intra-tumourally to avoid arrest by immune cells, although systemic delivery would be required for metastatic cancer therapy (Ferguson *et al.*, 2012; Russell *et al.*, 2012). The usage of non-human animal viruses to prevent their rapid eradication by pre-existing antibodies has also been suggested (Kelly and Russel, 2007).

Other suggestions are ultrasound delivery of viruses using microbubbles (Liang et al., 2010), utilization of carrier cells to hide and deliver viruses to tumour beds (Russell *et al.*, 2012) and polymer coating of viruses which can enhance their intravenous delivery to tumours (Fisher and Seymour, 2010). Immune suppression could also be used to increase intratumoural virus spread but this approach could diminish cross-priming of anti-cancer immunity (Russell *et al.*, 2012).

Despite the clinical achievement of oncolytic virotherapy, efficacy has not been observed in all patients and cancer types (Plitt and Zamarin, 2015). Future researches should focus on optimal choice of viruses, tumour types and stages of disease, viral dosage, routes of delivery, and recognizing possible combinations that may boost their pharmacological mechanisms of action (Goldufsky *et al.,* 2013).

Oncolytic virotherapy against cervical cancer

Development of an increasingly effective oncolytic virotherapy has also increases the possibility of toxicity to normal cells. Therefore, current researches are trying to control the virus replication in normal cells upon delivery and expression to occur strictly at the targeted cancer cells. The ability to selectively stimulates replication at tumour cells only and diminish the replication if toxicity is evidenced could provide better safety and efficacy of oncolytic virotherapy.

In one study, Kanerva *et al.* (2008) uses adenoviruses containing the cyclooxygenase-2 (Cox-2) or vascular endothelial growth factor (VEGF) promoter to restrict viral replication to target tissues expressing the promoters, which are the tumour tissues. Expressions of Cox-2 and VEGF have been linked with tumour invasiveness and angiogenesis and undetected in the normal epithelial lining of the cervix (Cao and Prescott, 2002). Overexpression of Cox-2 and VEGF leads to chemotherapy resistance and poor survival rate of cervical cancer patients. Kanerva *et al.* (2008) also concluded that prior pre-treatment with anti-inflammatory reagent dexamethasone, on cervical cancer cells *in vitro,* able to reduce the replication of oncolytic adenovirus carrying Cox-2 and VEG-F promoters in cancer cells. The usage of this steroid offers a safety switch for oncolytic virotherapy in case the tumour-specific promoters mediate any side effect in clinical trial.

Other than adenoviruses, a novel oncolytic Sindbis virus has been shown to successfully induce the cytopathic effects and apoptosis of two cervical cancer cells HeLaS3 and C33A. Its *in vivo* study demonstrated a site-specific and significant cervical tumour regression in nude mice upon intraperitoneal and intravenous virus inoculations (Unno *et al.,* 2005).

It is suggested that combination of oncolytic virotherapy with other therapeutic agents may increase anti-cancer effects (Motalleb, 2013). Application of recombinant herpes simplex virus type I increases anti-tumour activity against cervical cancer when combined with radiation therapy (Blank *et al.,* 2004). Valproic acid also improves oncolytic effect of rat parvovirus H-1PV synergistically against cervical cancer (Li *et al.,* 2013). Combination of neoadjuvant chemotherapy with a recombinant human oncolytic adenovirus-p53 also offers better efficacy, safety and synergism in treating locally advanced cervical cancer patients of stage IB2 to IIIA (Xiao *et al.,* 2017).

Gene Therapy

Gene therapy can be used to treat genetically inherited disease or cancer by transferring genetic materials into patients' target cells to enhance or inhibit a specific protein expression (Podolska *et al.*, 2012; Scholz and Wagner, 2012) without affecting the normal cells (Carrington, 2015). Gene therapy, also known as targeted therapy, may cause changes in the genetic materials of the patients (Kumar, 2016) and can be classified into two main groups, the germ line gene therapy and the somatic gene therapy (Ibraheem *et al.*, 2014).

According to Ibraheem *et al.* (2014), germ line gene therapy involved alteration of the gene therapy in the germ cell of reproductive system, while somatic gene therapy occurs when genetic modification took place in the non-reproductive system cells. The germ line gene therapy is transmissible throughout several generations but the somatic gene therapy is restricted to only the patient who is treated with it (Ibraheem *et al.,* 2014). Germ line gene therapy is transmissible due to the integration of the gene into the chromosome of the targeted genome location, whilst somatic gene therapy targeted the non-heritably genetic material, hence limits its transmission (Stribley et al., 2002).

In cancer treatment, gene therapy is used to target the abnormal gene, to increase the susceptibility of the cell towards drugs or conventional therapy, to induce cell apoptosis, to block oncogene expression

and to enhance tumour cell immunogenicity recognition (Das *et al.*, 2014). Gene therapy can be applied to a patient by either a direct transfer or by using living cell-based approach (Figure 2).

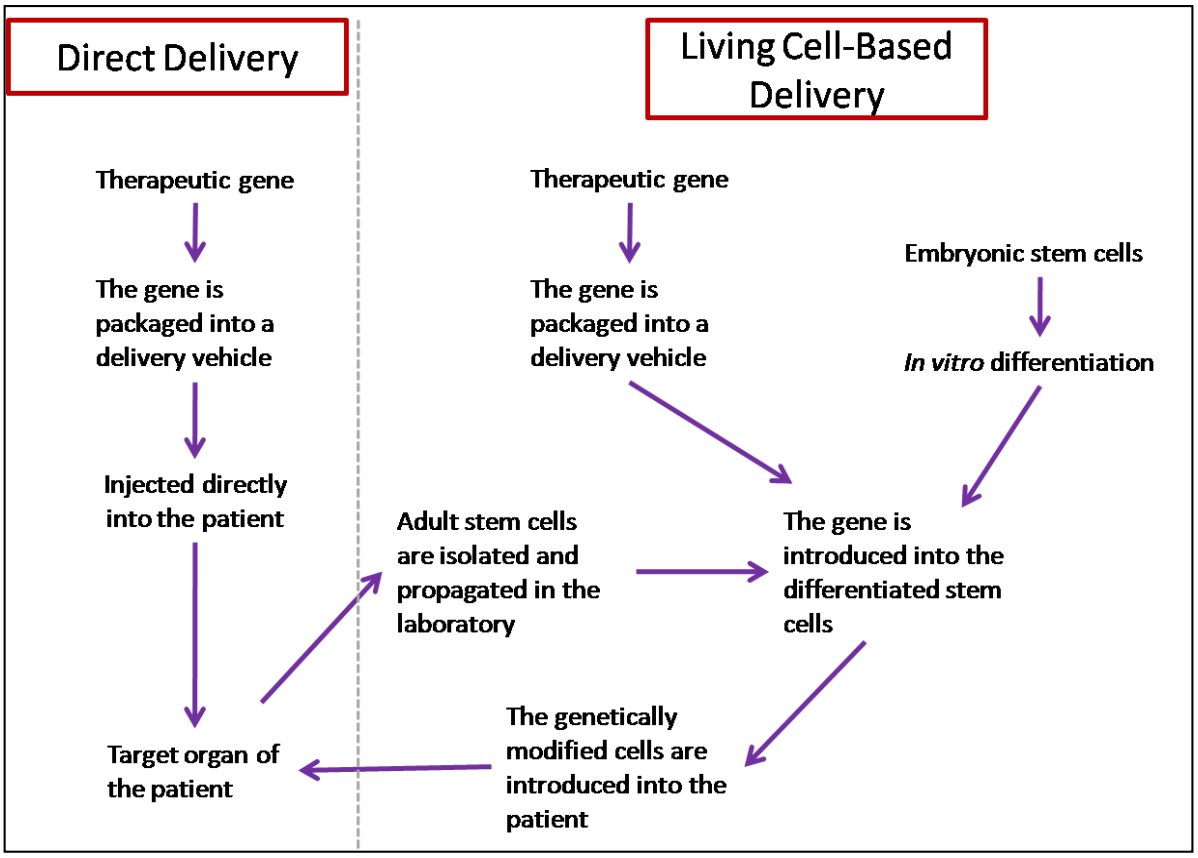

Figure 2: Representative of process flow for the two types of gene delivery approach for gene therapy; direct and living cell-based delivery (reproduced from NIH Stem Cell Information (2016)).

In direct transfer, the gene of interest will be packed in liposomes or any other biological microparticles. These microparticles are then injected directly to the patients which resulted in gene or protein expression at the targeted organ. Gene therapy using living cells involves isolation and propagation of patient's cells, introduction of therapeutic gene into the cell and re-introduction of the transformed cells to the patient (NIH Stem Cell Information, 2016).

For a successful delivery of the genetic modification component, "vehicles" are designed to create a secure and efficient genetic material carrier towards the target (Ibraheem *et al.*, 2014). These carriers are important to carry genetic materials in a stable manner, and at the same time must cross the cell membrane and deliver the gene of interest to the targeted organ. There are many considerations for the development of a carrier, such as delivery method to the target, uptake mechanism by the targeted cell, arrival and recognition at the target (El-Aneed, 2004; Ibraheem *et al.*, 2014; Narayan and Murty, 2010). There are now a wide variety of carriers that are used for gene therapy.

Types of carrier used in gene therapy

Traditionally, methods for gene therapy against cancer includes viral vectors, non-viral vectors (naked DNA), plasmids, bacteria vectors, liposomes, polymers, and molecular conjugates (Liu *et al.*, 2014; Teo *et al.*, 2016). More recently, carriers are developed based on proteins, such as the polyethylene glycol-polylactic acid (PEG-PLA) block copolymer (Liu *et al.*, 2014).

Viral vectors are constructed by manipulating the viral genome through removing and/or replacing the virulence gene before adding with the gene of interest. Viral vectors are efficient as they can infect and replicate inside a host cell by releasing their genome into the host's intracellular environment (Shen and

Post, 2007). Meanwhile, for non-viral vectors which consist of combination of naked DNA with nanoparticles or chemicals and delivered into cells via physical or chemical aid offers few advantages. These advantages include ease of preparation and scale up and ability to accommodate various size of therapeutic DNA. In addition, the non-viral vectors do not exert any type of immune responses in targeted cells thus can be inoculated into patient repeatedly (Schmidt-Wolf and Schmidt-Wolf, 2003). The development of non-viral vectors and protein carriers is intended to reduce the toxicity effects on host cells upon administration (He *et al.*, 2010).

Gene therapy against cervical cancer

Recently, a group of researchers was able to inactivate two oncogenes of high-risk HPV-18, using Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 (CRISPR/Cas) technology. Kennedy *et al.*, (2014) designed a single guide RNAs (sgRNAs) specific for E6 or E7 gene deletion and insertion mutations. The Cas9/sgRNA was delivered to the cells via transfection or lentiviral transduction. The resulting cleavage of HPV genome induces host cell tumour suppressor p53 and retinoblastoma (Rb) proteins to perform their functions, leading to cell cycle arrest, senescence, and apoptosis of the infected cells.

In another study, a high gene transfer efficiency was observed using an adeno-associated virus (AAV) vector encoding short hairpin RNA (shRNA) against the E6 and E7 of HPV-16 in three different cervical cancer cell lines (BOKU, SiHa and SKG-IIIa cells) (Sato *et al.*, 2018). shRNA is known to constantly inhibiting target gene expression for longer periods of time. Sato *et al.* (2018) demonstrated that the AAV-shRNA was able to reduce the mean volume of 8-mm major axis cervical tumours in mice. Furthermore, the expression levels of E6 and E7 was decreased, whereas the expression levels of tumour suppressors p53, p21 and pRb proteins were increased upon treatment, compared to the control, without exhibiting any adverse effects to the host.

Conclusion

With the advancement of science and technologies, higher chances of cancer recovery are possible. Although some of the treatments are still in the phase of clinical trials, promising results are evidenced, thus, approval of such treatments are imminent. Exploring the causes of the diseases and ways to modify our genome or tweaks our immunity enable researchers to develop more targeted treatments to cure all types of cancer, hence minimise cancer-related deaths in future.

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References

Ahmadzadeh, M., Johnson, L. A., Heemskerk, B., Wunderlich, J. R., Dudley, M. E., White, D. E., & Rosenberg, S. A. (2009). Tumor antigen specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood*, 114, 1537-1544.

Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., & Iyer, A. K. (2017). PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. *Frontiers in Pharmacology*, 8(AUG), 1–15.

American Cancer Society: Treating Cervical Cancer. Retrieved on December 3, 2018 from https://www.cancer.org/cancer/cervical-cancer/treating.html

Blank, S. V, Rubin, S. C., Coukos, G., Amin, K. M., Albelda, S. M., & Molnar-Kimber, K. L. (2004). Replication-selective herpes simplex virus type 1 mutant therapy of cervical cancer is enhanced by low-dose radiation. *Human Gene Therapy*, 13(5), 627–639.

Boromand, N., Hasanzadeh, M., ShahidSales, S., Farazestanian, M., Gharib, M., Fiuji, H., Behboodi, N., Ghobadi, N., Hassanian, S. M., Ferns, G. A., & Avan, A. (2017). Clinical and prognostic value

of the C-Met/HGF signaling pathway in cervical cancer. *Journal of Cellular Physiology*, 233(6), 4490-4496.

Brunet, J. F., Denizot, F., Luciani, M. F., Roux-Dosetto, M., Suzan, M., Mattei, M. G., & Golstein, P. (1987). A new member of the immunoglobulin superfamily-CTLA-4. *Nature*, 328(6127), 267–270.

Buchbinder, E. I., & Desai, A. (2016). CTLA-4 and PD-1 pathways similarities, differences, and implications of their inhibition. *American Journal of Clinical Oncology: Cancer Clinical Trials*, 39(1), 98–106.

Cao, Y., & Prescott, S. M. (2002). Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. *Journal of Cellular Physiology*, 190, 279–286.

Carrington, C. (2015). Oral targeted therapy for cancer. *Australian Prescriber*, 38(5), 171-176.

Chen, L., & Flies, D. B. (2013). Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nature Reviews Immunology*, 13(4), 227–242.

Das, S., Menezes, M., Bhatia, S., Wang, X., Emdad, L., Sarkar, D., & Fisher, P. (2014). Gene therapies for cancer: strategies, challenges and successes. *Journal of Cellular Physiology*, 230(2), 259-271.

DePace, N. G. (1912). *Sulla scomparsa di un enorme cancro vegetante del collo dell'utero senza cura chirurgica* [Disappearance of a huge cervical cancer without surgical treatment]. *Ginecolgia*, 9, 82-89.

Disis, M. L. (2014). Mechanism of action of immunotherapy. *Seminars in Oncology*, 41 (Suppl. 5), S3–13.

Drake, C. G., Lipson, E. J., & Brahmer, J. R. (2014). Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. *Nature Reviews Clinical Oncology*, 11, 24-37.

El-Aneed, A. (2004). An overview of current delivery systems in cancer gene therapy. *Journal of Controlled Release*, 94(1), 1-14.

Ferguson, M. S., Lemoine, N. R., & Wang, Y. (2012). Systemic delivery of oncolytic viruses: Hopes and hurdles. *Advances in Virology*.

Fife, B. T., & Bluestone, J. A. (2008). Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. *Immunological Reviews*, 224, 166-182.

Fife, B. T., Pauken, K. E., Eager, T. N., Obu, T., Wu, J., Tang, Q., Azuma, M., Krummel, M. F., & Bluestone, J. A. (2009). Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. *Nature Immunology*, 10(11), 1185-1193.

Fisher, K. D., & Seymour, L. W. (2010). HPMA copolymers for masking and retargeting of therapeutic viruses. *Advanced Drug Delivery Reviews*, 62(2), 240-245.

Fukuhara, H., Ino, Y., & Todo, T. (2016). Oncolytic virus therapy: a new era of cancer treatment at dawn. *Cancer Science*, 107(10), 1373-1379.

Goldrath, A. W., & Bevan, M. J. (1999). Selecting and maintaining a diverse T-cell repertoire. *Nature*, 402, 255-262.

Goldufsky, J., Sivendran, S., Harcharik, S., Pan, M., Bernardo, S., Stern, R. H., Friedlander, P., Ruby, C. E., Saenger, Y., & Kaufman, H. L. (2013). Oncolytic virus therapy for cancer. *Oncolytic Virotherapy*, 2, 31-46.

He, C., Tabata, Y., & Gao, J. (2010). Non-viral gene delivery carrier and its three-dimensional transfection system. *International Journal of Pharmaceutics*, 386(1-2), 232-242.

Hollebecque, A., Meyer, T., Moore, K. N., Machiels, J-P. H., De Greve, J., & López-Picazo, J. M. (2017). An open-label, multicohort, phase I/II study of nivolumab in patients with virus-associated tumors: Efficacy and safety in recurrent or metastatic (R/M) cervical, vaginal, and vulvar cancers. *Journal of Clinical Oncology*, 35(15), 5504-5504.

Ibraheem, D., Elaissari, A., & Fessi, H. (2014). Gene therapy and DNA delivery systems. *International Journal of Pharmaceutics*, 459, 70-83.

Ishida, Y., Agata, Y., Shibahara, K., & Honjo, T. (1992). Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO Journal*, 11(11), 3887-3895.

Juneja, V. R., McGuire, K. A., Manguso, R. T., LaFleur, M. W., Collins, N., Haining, W. N., Freeman, G. J., & Sharpe, A. H. (2017). PD-L1 on tumour cells is sufficient for immune evasion in immunogenic tumours and inhibits CD8 T cell cytotoxicity. *Journal of Experimental Medicine*, 214(4), 895-904.

Jenkins, R. W., Barbie, D. A., & Flaherty, K. T. (2018). Mechanisms of resistance to immune checkpoint inhibitors. *British Journal of Cancer*, 118(1), 9-16.

Kanerva, A., Lavilla-Alonso, S., Raki, M., Kangasniemi, L., Bauerschmitz, G. J., Takayama, K., Ristimäki, A., Desmond, R. A., & Hemminki, A. (2008). Systemic therapy for cervical cancer with potentially regulatable oncolytic adenoviruses. *PLoS ONE*, 3(8), e2917.

Kelly, E. & Russel, S. J. (2007). History of oncolytic viruses: genesis to genetic engineering. *Molecular Therapy*, 15(4), 651-659.

Kennedy, E., Kornepati, A., Goldstein, M., Bogerd, H., Poling, B., & Whisnant, A., Kastan, M. B., & Cullen, B. R. (2014). Inactivation of the human papillomavirus E6 or E7 gene in cervical carcinoma cells by using a bacterial CRISPR/Cas RNA-guided endonuclease. *Journal of Virology*, 88(20), 11965-11972.

Kirn, D., Martuza, R. L., & Zwiebel, J. (2001). Replication-selective virotherapy for cancer: Biological principles, risk management and future directions. *Nature Medicine*, 7(7), 781-787.

Kumar, N. (2016). Cervical cancer, a nightmare for womanhood: review of recent advances. *Women's Health and Gynecology*, 2(2), 017-025.

Lau, J., Cheung, J., Navarro, A., Lianoglou, S., Haley, B., Totpal, K., Sanders, L., Koeppen, H., Caplazi, P., McBride, J., Chiu, H., Hong, R., Grogan, J., Javinal, V., Yauch, R., Irving, B., Belvin, M., Mellman, I., Kim, J. M., & Schmidt, M. (2017). Tumour and host cell PD-L1 is required to mediate suppression of antitumour immunity in mice. *Nature Communications*, 8, 14572.

Li, J., Bonifati, S., Hristov, G., Marttila, T., Valmary-Degano, S., Stanzel, S., Schnölzer, M., Mougin, C., Aprahamian, M., Grekova, S. P., Raykov Z, Rommelaere J, & Marchini, A. (2013). Synergistic combination of valproic acid and oncolytic parvovirus H-1PV as a potential therapy against cervical and pancreatic carcinomas. *EMBO Molecular Medicine*, 5(10), 1537-1555.

Liang, H. D., Tang, J., & Halliwell, M. (2010). Sonoporation, drug delivery, and gene therapy. Proceedings of the Institution of Mechanical Engineers, Part H: *Journal of Engineering in Medicine*, 224(2), 343-361.

Liu, T. C., Galanis, E., & Kirn, D. (2007). Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. *Nature Clinical Practice Oncology*, 4(2), 101-117.

Liu, B., Han, S., Tang, X., Han, L., & Li, C. (2014). Cervical cancer gene therapy by gene loaded PEG-PLA nanomedicine. *Asian Pacific Journal of Cancer Prevention*, 15(12), 4915-4918.

Mahoney, K. M., Rennert, P. D., & Freeman, G. J. (2015). Combination cancer immunotherapy and new immunomodulatory targets. *Nature Reviews Drug Discovery*, 14, 561-584.

Mandal, R., & Chan, T. A. (2016). Personalized oncology meets immunology: The path toward precision immunotherapy. *Cancer Discovery*, 6(7), 703-713.

Mezache, L., Paniccia, B., Nyinawabera, A., & Nuovo, G. J. (2015). Enhanced expression of PDL1 in cervical intraepithelial neoplasia and cervical cancers. *Modern Pathology*, 28, 1594-1602.

Motalleb, G. (2013). Virotherapy in cancer. *Iranian Journal of Cancer Prevention*, 6(2), 101-107.

Mullen, J. T., & Tanabe, K. K. (2002). Viral Oncolysis. *The Oncologist*, 7, 106-119.

Naidoo, J., Page, D. B., Li, B. T., Connell, L. C., Schindler, K., Lacouture, M. E., Postow, M. A., & Wolchok, J. D. (2015). Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. *Annals of Oncology*, 26(12), 2375-2391.

Narayan, G. & Murty, V. V. (2010). Integrative genomic approaches in cervical cancer: implications for molecular pathogenesis. *Future Oncology*, 6, 1643-1652.

National Health Science: Cervical cancer. United Kingdom: Department of Health and Social Care. Retrieved on December 3, 2018 from https://www.nhs.uk/conditions/cervical-cancer/

NIH Stem Cell Information Home Page. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services. (2016). Retrieved on April 24, 2018 from http://stemcells.nih.gov/info/Regenerative_Medicine/2006Chapter4.htm

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer*, 12(4), 252-264.

Plitt, T., & Zamarin, D. (2015). Cancer therapy with Newcastle disease virus: rationale for new immunotherapeutic combinations. *Clinical Investigation*, 5(1), 75-87.

Podolska, K., Stachurska, A., Hajdukiewicz, K., & Małecki, M. (2012). Gene therapy prospects-intranasal delivery of therapeutic genes. *Advances in Clinical and Experimental Medicine*, 21, 525-534.

Prestwich, R. J., Errington, F., Harrington, K. J., Pandha, H. S., Selby, P., & Melcher, A. (2008). Oncolytic viruses: do they have a role in anti-cancer therapy? *Clinical Medicine: Oncology*, 2, 83-96.

Ribas, A. (2012). Tumor immunotherapy directed at PD-1. *New England Journal of Medicine*, 366, 2517-2519.

Riley, J. L. (2009). PD-1 signalling in primary T cells. *Immunological Reviews*, 229 (1), 114-125.

Russell, S. J., Peng, K-W., & Bell, J. C. (2012). Oncolytic virotherapy. *Nature Biotechnology*, 30(7), 658-670.

Sato, N., Saga, Y., Uchibori, R., Tsukahara, T., Urabe, M., Kume, A., Fujiwara, H., Suzuki, M., Ozawa, K., & Mizukami, H. (2018). Eradication of cervical cancer in vivo by an AAV vector that encodes shRNA targeting human papillomavirus type 16-E6/E7. *International Journal of Oncology*, 52(3), 687-696.

Schmidt-Wolf, G., & Schmidt-Wolf, I. (2003). Non-viral and hybrid vectors in human gene therapy: an update. *TRENDS in Molecular Medicine*, 9(3), 67-72.

Scholz, C., & Wagner, E. (2012). Therapeutic plasmid DNA versus siRNA delivery: common and different tasks for synthetic carriers. *Journal of Controlled Release*, 161(2), 554-565.

Shen, Y., & Post, L. (2007). Viral vectors and their applications. In B. N. Fields, D. M. Knipe & P. M. Howley (Eds), *Fields Virology* (pp. 539-564). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Smith, R., Huebner, J., Rowe, P., & Thomas, B. (1956). Studies on the use of viruses in the treatment of carcinoma of the cervix. *Cancer*, 9(6), 1211-1128.

Stribley, J. M., Rehman, K. S., Niu, H., & Christman, G. M. (2002). Gene therapy and reproductive medicine. *Fertility and Sterility*, 77, 645-657.

Sze, D. Y., Reid, T. R., & Rose, S. C. (2013). Oncolytic virotherapy. *Journal of Vascular and Interventional Radiology*, 24(8), 1115-1122.

Teo, P., Cheng, W., Hedrick, J., & Yang, Y. (2016). Co-delivery of drugs and plasmid DNA for cancer therapy. *Advanced Drug Delivery Reviews*, 98, 41-63.

Topalian, S. L., Sznol, M., McDermott, D. F., Kluger, H. M., Carvajal, R., Sharfman, W. H., Brahmer, J. R., Lawrence, D. P., Atkins, M. B., Powderly, J. D., Leming, P. D., Lipson, E. J., Puzanov, I., Smith, D. C., Taube, J. M., Wigginton, J. M., Kollia, G. D., Gupta, A., Pardoll, D. M., Sosman, J. A., & Hodi, F. S. (2014). Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *Journal of Clinical Oncology*, 32(10), 1020-1030.

Unno, Y., Shino, Y., Kondo, F., Igarashi, N., Wang, G., Shimura, R., Yamaguchi, T., Asano, T., Saisho, H., Sekiya, S., & Shirasawa, H. (2005). Oncolytic viral therapy for cervical and ovarian cancer cells by Sindbis virus AR339 strain. *Clinical Cancer Research*, 11(12), 4553-4560.

Wong, H. H., Lemoine, N. R., & Wang, Y. (2010). Oncolytic viruses for cancer therapy: overcoming the obstacles. *Viruses*, 2(1), 78-106.

Xiao, J., Zhou, J., Fu, M., Liang, L., Deng, Q., Liu, X., & Liu, F. (2017). Efficacy of recombinant human adenovirus-p53 combined with chemotherapy for locally advanced cervical cancer: a clinical trial. *Oncology Letters*, 13(5), 3676-3680.

Yung, M. M. H., Chan, D. W., Liu, V. W. S., Yao, K. M., & Ngan, Y. S. (2013). Activation of AMPK inhibits cervical cancer cell growth through AKT/FOXO3a/FOXM1 signalling cascade, *BMC Cancer*, 13(1), 1-8.

Zhang, H., Zhang, Z., Wang, S., Zhang, S., & Bi, J. (2018). The mechanisms involved in miR-9 regulated apoptosis in cervical cancer by targeting FOXO3. *Biomedicine and Pharmacotherapy*, 102, 626-632.