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Review article

Immunotherapeutic Approach to Anal Cancer

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Abstract

Anal cancer is a rare disease that accounts for 0.4% of all cancer diagnoses in the United States. Its incidence has increased by 2.2% annually. The risk factors associated with anal cancer are Human Papilloma Virus (HPV), Human Immunodeficiency Virus (HIV), sexual practices, smoking and immunosuppression. Immunotherapy is an alternative to chemotherapy and radiation that uses a patient's own immune system to fight the cancer. This can be done by giving the patients man-made immune system proteins to stimulate their immune system. In this paper, we will discuss the current trends in utilizing immunotherapy to fight anal cancer along with the different molecules involved in the proliferation of the disease.

Keywords: Anal Cancer; Immunotherapy; Immunosuppression; Kinase; Cytokine; Multiclonal Antibodies; Interferons

Introduction

Anal cancer is a rare disease that accounts for only 1-2% of all the world's cancers [1]. In 2014, SEER estimated 7,210 new cases and 950 deaths were due to anal cancer in the United States. The five-year survival rate of patients diagnosed with anal cancer is 65.5%. That statistic also represents 0.4% of overall cancers in the United States. According to SEER, the age-standardized rate of incidence is 1.8 per 1,00,000 persons. It includes different types of the histopathological and genetic characteristic. The male-to-female ratio for susceptibility to this cancer is 3:4. Higher incidences were observed between the age of 55 to 64 [2].

Squamous cell carcinoma is the most common type of anal cancer, followed by adenocarcinoma and malignant melanoma, which is quite rare [3]. Anal cancer may be asymptomatic at earlier stages, but later on, bleeding and discomfort may be observed. Other symptoms associated with anal cancer include: pain, itching, anal discharge, swelling of lymph nodes in the groin area, changes in bowel movements and fecal diameter [4].

Various risk factors associated with anal cancer include infection of Human Papilloma Virus (HPV), Human Immunodeficiency Virus (HIV) infection, sexual practices, smoking, history of other sexually transmitted diseases and immunosuppression [5]. Patients suffering from HIV are prone to HPV infection, viral infections, as well as anal intra-epithelial neoplasia (AIN), which is reported to be a precursor of anal cancer. It is postulated that the downregulation of immune response due to HIV makes the patients susceptible to HPV infections in the anal region, resulting in the abnormality of the epithelial layer. The two important factors associated with HPV-related anal cancer are: shielding effects from cell-mediated response and chromosomal instability due to loss of heterozygosity (LOH) [6]. The progression of anal cancer in HIV-negative patients is supported by the LOH due to mutations of tumor suppressor genes like p53, DCC and/or APC.

Tumor suppressor genes p53, p21, p27, p16, and retinoblastoma gene (Rb) have all been associated with anal cancer. Tumorigenic HPV infections can result in p53 gene mutation, removing the ability to suppress growth of a cancer cell [7]. Both p21 and p27 act as CDK inhibitors and are associated

with preventing the progression of G1 to S-phase of the cell cycle [8]. P16 is also a CDK inhibitor that inhibits cell proliferation [9]. The RB gene is a tumor suppressor gene, however, in a study done by Crook et al., no alterations in the locus of RB were observed in anal tumors [10]. Cyclins are a class of protein that acts as the cell cycle regulator at some specific transition phases along with CDKs.[11] It includes Cyclin A, D1 and E that usually regulate the transition from G1 to S phase. Along with this, Cyclin A additionally regulates the transition from G2 to M phase [12,13]. Minichrome Maintenance Protein 7 (MCM7) functions as a regulator in DNA replication and is expressed in all the phases of the cell cycle. The protein is degraded in the cells that are fully differentiated [14].

Immunotherapy

A. Monoclonal Antibodies (MABs):

There are no MABs that are currently approved by FDA for anal cancer. However, many MABs are under clinical trials in phases I-III as shown in Table 1 below:

1. Cetuximab [15]: It's a recombinant monoclonal antibody that possesses anti-neoplastic activity. It adheres to the EGFR at extracellular site and hence, prevents it from getting activated and dimerized. This subsequently results in inhibition of signal transmission and further inhibition of tumor cell proliferation. In a case series of 7 heavily pretreated anal cancer patients, cetuximab was given in combination with irinotecan. In contrast to previous studies, KRAS mutations were demonstrated in a number of patients in this case series. Response (defined as partial or minor) was seen in the KRAS wild type patients (n = 5) with no response demonstrated in the patients with KRAS mutations [16].

2. Panitumumab [17]: It is a monoclonal antibody that inhibits cell proliferation by adhering to the EGFR. It also inhibits the activation of tumor cells' EGF, hence, preventing EGFR expression and cell proliferation. The addition of Panitumumab to M/5-FU/RT has been shown to be a tolerable regimen with a good compliance and an acceptable safety profile in a study presented in ASCO 2014 [18].

Drug	Clinical trial identifier number	Phase	Study design	Target
Cetuximab	NCT00955240	Phase-II	Treatment	EGFR
Panitumumab	NCT01581840	Phase I/II	Safety/Efficacy Study, Open Label	EGFR
Trastuzumab + Interleukin-12	NCT00004074	Phase-I	Safety Study, Open Label	Human Epidermal growth factor receptor 2 (HER2)

Table 1. Monoclonal antibody drugs [15-19]

3. Trastuzumab [19]: It is a recombinant monoclonal antibody that targets HER2. In tumor cells that overexpress HER2, it generates cytotoxicity by adhering to HER2 with the help of

antibodies. Combination treatment with this monoclonal antibody and interleukin 12 are under further investigation in several types of solid tumors as well as in anal cancer.

B. Checkpoint Inhibitors:

An important part of the immune system is its ability to tell between normal cells in the body and those it sees as "foreign." This lets the immune system attack the foreign cells while leaving the normal cells alone. To do this, it uses "checkpoints" – molecules on certain immune cells that need to be activated (or inactivated) to start an immune response. Cancer cells sometimes find ways to use these checkpoints to avoid being attacked by the immune system.

1. Nivolumab: It is a monoclonal antibody with immunomodulating action. It prevents the binding of PD-L1 and PD-L2 ligands, resulting in inhibition of PD-1. This activates T-cells and associated immune responses, such as T cell associated cytotoxicity against tumors. Its efficacy and safety profile in metastatic refractory anal squamous cell carcinoma is under investigation in a phase II clinical trial as in table 2 below.

Drug	Clinical trial identifier number	Phase	Study design	Target
Nivolumab	NCT02314169	Phase-II	Efficacy Study, Open Label	Programmed death-1 receptor (PD-1)

Table 2. Checkpoint inhibitor drugs [20]

C. Cytokine Therapy:

There is no biological that is currently approved by FDA for anal cancer. However the biologicals that are under clinical trials in phase I-III are in Table 3 below:

1. Aldesleukin: It is an endogenous cytokine Interleukin-2 (IL-2), which helps in the regulation of the immune system and also has anti-neoplastic activities. Aldesleukin adheres and activates IL-2 receptor.

2. Recombinant Interleukin-12: It is recombinant cytokine interleukin-12 having anti-neoplastic activities. It activates interferon-gamma (IFN) production by adhering to its cell surface receptor, resulting in tumor apoptosis.

Drug	Clinical trial identifier number	Phase	Study design	Target
Aldesleukin + Tumor Infiltrating Lymphocytes (TIL)	NCT01585428	Phase-II	Safety/Efficacy Study, Open Label	White Blood cells
Recombinant Interleukin-12	NCT00003046	Phase-I	Efficacy Study, Open Label	IFN-gamma-inducible protein 10 (IP-10)

Table 3: Cytokine therapy [21]

D. Vaccine Therapy:

There is no FDA approved vaccine for anal cancer. Other biologicals that are under clinical trials in phase I-III are mentioned in Table 4 below:

1. Gardasil [22]: A highly purified form of VLPs, which are non-infectious in nature. It is obtained from HPV's capsid (L1) protein. It boosts the humoral response against HPV positive cells. It is a cancer preventing vaccine. The U.S. Food and Drug Administration has recently approved the vaccine Gardasil for the prevention of anal cancer and associated precancerous lesions due to human papillomavirus (HPV) types 6, 11, 16, and 18 in people ages 9 through 26 years.

Gardasil is already approved for the same age population for the prevention of cervical, vulvar, and vaginal cancer and the associated precancerous lesions caused by HPV types 6, 11, 16, and 18 in females. It is also approved for the prevention of genital warts caused by types 6 and 11 in both males and females.

2. Lovaxin C [23-24]: It comprises the strain of *Listeria monocytogenes* (Lm), a bacterium in live-attenuated form. It acts as an immunomodulator and has antineoplastic activity. It helps to boost the immune system by generating a response through cytotoxic T-lymphocytes (CTL), which act against the HPV 16 E7 expressing cancer cells resulting in tumor lysis. Preliminary data from a phase 1/2 trial of ADXS-HPV in HPV-associated anal cancer in combination with chemoradiation showing complete response and no recurrences to date in all treated patients.

3. HspE7: It is a chimeric protein obtained through recombinant technique. It consists of heat shock protein 65 (Hsp65) and HPV protein E7. Hsp65 boosts the immune response while E7 protein embodies a tumor antigen targeted by lymphocytes. Palefsky et al. developed another Bacilli-Chalmette-Guerin-derived heat-shock protein (Hsp65) fused to the E7 protein of HPV 16. Of 82 HIV-positive patients with high grade anal squamous intraepithelial lesion (HSIL) who were recruited and given the vaccine, half demonstrated moderate regression towards normal. The same vaccine showed a 71% regression to LSIL from HSIL within six months [25].

4. MKC1106-PP: It is a plasmid based regimen that improves the immune response. It consists of a recombinant technology based therapeutic plasmid (pPRA-PSM encoding fragments) along with two peptides (E-PRA and E-PSM). It targets both antigens: PRAME and PSMA. The clinical trial on anal cancer patients has been completed and the results would be available by the end of 2015.

5. HPV-16 E6: It is a synthetically prepared HPV type 16 oncoprotein E6 peptide sequence. It boosts the immune system

against E6 oncoproteins through the CTL response, which leads to rupture of tumor cell.

6. Vicoryx: It is a peptide fragment of human p16 kinase inhibitor. It activates a T-cell response specific to peptides. A phase I/IIa trial of the therapeutic vaccine Vicoryx was conducted. Vicoryx was used to treat 26, HPV positive, p16INK4a cancer patients in an open label, single center trial. Inclusion criteria for the trial were a history of advanced HPV-positive cervical, vulvar, vaginal, penile, anal and head and neck cancers with diffuse expression of p16INK4a in the tumor, after standard therapy. After an initial interim safety assessment (10 patients), the primary endpoint was the induction of a cellular and/or humoral immune response against the therapeutic vaccine. Secondary endpoints were safety of immunization with VicO-ryx and tumor response according to RECIST.

7. V503: A highly purified form of VLPs, which is non-infectious in nature. It is obtained from HPV's capsid (L1) protein. It boosts the immune system, resulting in CTL response against HPV positive cells. It has been suggested as a preventive vaccine for HPV associated cancers. Available studies comparing this 9-valent vaccine with approved Gardasil has been shown a better response and acceptable safety profile. Currently a Biologics License Application (BLA) for this investigational, nonavalent, HPV vaccine V503 has been submitted to the US Food and Drug Administration. Standard review was granted. The nonavalent HPV vaccine appears to be safe and effective in preventing persistent infection and precancerous lesions associated with HPV types 16/18/31/33/45/52/58, as well as genital warts related to HPV types 6 and 11.

Drug	Clinical trial identifier number	Phase	Study design	Target
Gardasil	NCT00090285	Phase-III	Efficacy Study, Double Blind	HPV Antigens
V503	NCT01651949	Phase-III	Safety/Efficacy Study, Open Label	Cytotoxic T-lymphocytes (CTL)
Lovaxin C (ADXS11-001)	NCT01671488	Phase-I/II	Safety/Efficacy Study, Open Label	HPV 16 E7 antigen
HspE7	NCT00052897	Phase I/II	Open Label	E7 antigen
Vicoryx	NCT01462838	Phase-I/II	Safety/Efficacy Study, Open Label	p16INK4a (cyclin dependent kinase inhibitor)
MKC1106-PP	NCT00423254	Phase-I	Safety/Efficacy Study, Open Label	PSMA/PRAME
HPV-16 E6 vaccine	NCT01653249	Phase-I	Safety Study, Open Label	E6oncoprotein

Table 4. Vaccine Therapy [22-30]

E. Suggested targeted therapies for anal cancer:

Kinase Inhibitors:

There is no tyrosine-kinase inhibitor that is currently approved by FDA for anal cancer. Some of the tyrosine kinase inhibitors that are under clinical trials in phase I-III are mentioned in Table 5 below.

1. Erlotinib: It is a quinazoline derivative that possesses anti-cancer properties. It competes with adenosine triphosphate and reversibly adheres to the EGFR tyrosine kinase. Thus, EGFR is unable to phosphorylate and hence, inhibits the cascade of signaling pathways, resulting in tumor growth inhibition.

2. Vandetanib: It is a tyrosine kinase inhibitor that inhibits VEGF associated proliferation of endothelial cells and also reduces the permeability of tumor vessels. It also inhibits the process of angiogenesis and cell proliferation by inhibiting EGFR.

3. Crizotinib: It is a tyrosine kinase inhibitor that regulates tumor cell growth. It competes with adenosine triphosphate and adheres to the ALK. It also halts the c-Met signaling pathway.

Drug	Clinical trial identifier number	Phase	Study design	Target
Crizotinib	NCT01828112	Phase-III	Open Label	Anaplastic Lymphoma Kinase (ALK)
Erlotinib	NCT00397384	Phase-I	Safety / Efficacy Study, Open Label	EGFR
Vandetanib	NCT00532909	Phase-I	Safety / Efficacy Study, Open Label	VEGFR

Table 5. Tyrosine Kinase Inhibitors [31-33]

Conclusion

Anal cancer can be described as an unwanted cell growth in the anus. Western countries show a higher rate of incidence of anal cancer. It might be due to a primary infection with HPV or HIV. Smoking, sexual practices and immunosuppression are considered the risk factors associated with anal cancer. Loss of heterozygosity of chromosomes may lead to the development of anal cancer. Although there is no FDA approved immunotherapy for anal cancer, Gardasil has been approved as a preventive vaccine therapy. Other clinical trials are going on for many other classes of immunotherapeutics like MABs, adoptive cell therapy, vaccine therapy, as well as targeted therapies such as tyrosine kinase inhibitors and interferons. Proper preclinical and clinical designs are the important pillars in understanding the future of immunotherapy in treating cancer patients.

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