

# A Comprehensive Review on the Application of Cornerstone Combination Therapy for the Cancer Treatment

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## Abstract

The current review focuses on cancer, the second most important disease across the globe. Cancer is a non-communicable disease leading to an alteration in the body at the genetic level. Nearly 130 types of cancers were reported across the world. The classification, diagnostic and treatment methods currently being reported were discussed. It was understood that surgery is the most commonly used therapy for benign tumors or the initial stage tumors. Of the conventional monotherapies, chemotherapy has gained importance to cure or suppress cancer in an economical manner. Besides cure, many side effects, tumor sensitivity, were observed which can range from mild to life-threatening in some patients. Hence, combination therapy combinations of conventional mono-therapy came into existence to overcome the side effects economically within less time. In combination therapy, the toxicity of the drugs being used can be minimized as they target the alternate pathway. Of various combinations, targeting the enzymes responsible for tumor growth and metastasis by small molecule inhibitors has gained importance because of high specificity (under clinical studies). Hence, combination therapy is a cornerstone therapy which needs to be evaluated thoroughly among large numbers of people across the world.

**Keywords:** Cancer, conventional monotherapy, combination therapy, enzymes, side effects, tumor sensitization, toxicity, cornerstone therapy.

## Introduction to Cancer

Cancer is a non-communicable, multistep, multifactorial, and microevolutionary transformation leading to the replicative immortality of altered cells within the body. Cancer is a directional multifactorial cellular disorder that causes changes at the gene level leading to its malignancy. Worldwide, cancer has gained as the second most important disease based on the number of deaths every year next to heart-related disorders 1. The first person to identify and name cancer is Hippocrates<sup>2</sup>. More than 100 kinds of malignancies have been reported by analysts, out of which 22% are because of tobacco, 10% were because of food habits and obesity, viral infections constitute 15% and hereditary constitutes 5-10%.

## Global Statistics on Cancer

Based on the WHO statistics, it was understood that the incidence rate of mortality is approximately 8 million in a year. Researchers in 2007 have reported the mortality rate as 45 percent which increases to 65 percent by 2030 3. Based on the GLOBOCAN cancer statistics 2018 the estimation of no of cancer cases is ap-

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proximately 18.1 million and the mortality rate was approximately 9.6 million 4. Based on the total number of deaths Lung cancer stands first with the incidence mortality of 18.4%. The incidence mortality rate of breast, prostate, colorectal, stomach, and liver cancers were 11.6 %, 7.1%, 6.1%, 9.2 %, 8.2%, and 8.2% respectively. The most commonly seen cancers among males are lung, followed by other cancers, (prostate and gastrointestinal cancers). Besides, in females the most diagnosed cancer is breast cancer followed by others. Lifestyle and geographical distribution are some of the most important factors associated with cancer 5. In 2012, approximately 14.1 million cases were reported across the world, out of which the incidence rate of mortality rate is 8.2 million 6. It was understood that the number of deaths reported from middle and low-income countries was relatively high in comparison with high-income countries. Of the new cancer cases reported across the world 57% constituted from low-income countries and the number of deaths was approximately 67% of the reported cases. Early detection and treatment results will improve the survival rates, reduce the healthcare burden and improves the overall economy.

Each year increased survival rate results in saving 2.4 -3.7 million lives each year7. The factors contributing for the cancer progression are genetic disorders, and most of the cancers are radiation, obesity, stress, smoking, geographical, race, and occupation 2, 8. It was also reported that diabetes was one of the main causes of cancer. Some of the reasons are the limited expression of tumor suppressor gene and alteration of both metabolic and signaling pathways leading to the production of various angiogenic factors as well as nutrients required for their progression 9. It was also reported that Radiation therapy is the first diagnostic method used to diagnose cancer. The other treatments currently available in the market are phototherapy 10, combination therapy 11, immunotherapy 12, chemotherapy 13, enzymatic therapy 14, radical therapy, adjuvant therapy, and many

others. Some of the commercially available drugs to treat cancer or reduce the cell division of cancer cells are Vincristine, Daunorubicin, Cyclophosphamide, L-Asparaginase 15, 16, L-Glutaminase 15, 17, 18, etc. with observed side effects.

### **Classification of Cancer**

Mainly the cancers are classified based on their moment, organ, and tissue origin. These are further sub-classified into different types.

#### **Tumor classification based on their moment**

Based on their moment inside the body, fluid tumors are classified into benign (static) and malignant (metastatic).

#### **Benign tumor**

The static noncancerous abnormal lumps inside the body are benign tumors. Because of their restricted moment, it is easy to remove them by surgery. The weight of the tumor in some cases will be pounds and large benign tumors sometimes press the surrounding vital organs. Most of the benign tumors do not reoccur after their removal and in rare cases, it was also reported that their reoccurrence can be seen at the same place. Ex: intestinal polyps, uterus fibroids. Benign tumors can be fatal if they grow inside the brain as it is difficult to remove them by surgery. It was also reported that in some cases the benign tumors become malignant. Ex: polyps in the intestine.

#### **Malignant tumor**

The cancerous tumors invading from one organ to other organs with the help of body fluids (blood and lymph) are called malignant tumors. This moment is known as metastasis.

#### **Cancer metastasis**

The moment of cancer cells is called metastasis. It was also reported that the 90 % mortality rate of cancer patients depends on the tumor metastasis 19 which can be decreased by the early detection of the tumor. Detach-

ment, migration, invasion, and adhesion are the four metastatic steps involved in the tumor progression from the primary site to the distal site through blood and lymph flow 19. Metastasis results change in both metabolic as well as signaling pathways resulting in the synthesis of nutrients and growth factors required by the tumor cells for their progression. It was also understood from the literature that the tumor at the distal site is more fatal in comparison with tumors at their primary or origin site. It is also important to understand the origin of the tumor during the diagnosis of the tumor. Many targeted therapies by small molecule inhibitors, radiation therapy, and chemotherapy were currently in use.

#### **Tumor classification based on origin**

Based on the primary site of tumor origin, the tumors were defined based on their organ origin. Ex: Breast cancer, lung cancer, renal cancer, brain cancer, and others.

#### **Tumor classification based on tissue type**

The international classification of diseases for oncology classified cancers was classified into 6 types based on tissue types. Ex: Sarcomas, Lymphomas, Central Nervous System (CNS) related cancers, and many. Some of the reported cancers are carcinomas (skin cancer), leukemia (blood cancer), sarcomas (connective tissue cancers), lymphomas (immune system) myeloma (progenitor cells), and CNS cancers (brain and spinal cord).

#### **Carcinomas**

Cancer of epithelial origin which covers both inner and outer surfaces of the body is called Carcinoma. DNA damage or alterations of genes leads to Carcinoma 20, 21. Skin cancer, oral cancers, breast cancer, ovarian cancer, gastrointestinal cancer, liver cancer are some of the reported carcinomas. The most common causative agents are alcohol, virus, tobacco, mutations, and many. Based on their origin again carcinomas were subclassified into

a). Adenocarcinoma: glands related cancer associated with mucosa, and b). Squamous cell carcinoma: originates from squamous epithelium. The most common adenocarcinoma across the world is gastrointestinal cancer (GI) and is associated with the gastrointestinal tract. It constitutes about ~ 95% of the reported carcinomas reported worldwide.

**Gastrointestinal cancer:** GI cancer is multi-step and multi-functional related to both the upper and digestive tract: stomach, esophagus, intestine, liver, pancreas is called gastrointestinal cancer. Many factors contribute to gastrointestinal cancer. It includes biological agents like *Helicobacter pylori*, inflammation, and other physiological factors. The most important factor causing gastrointestinal cancer is oxidative stress. Hence, supplementation with antioxidant foods regulates cancer. The symptoms observed in gastrointestinal cancer are mostly digestion problems, bleeding, weight loss, abdominal pain, vomiting, and others vary from one type of cancer to other. It was understood that Smoking, obesity, food, and drinking lifestyle habits mainly influence the progression of cancer and sometimes are involved in the initiation of the tumor also. In some cases, Geographical distribution also influences the incidence of GI cancer 22. **Diagnostics and Treatment of GI cancer:** The diagnostic methods used for the detection of gastrointestinal cancers are endoscopy and biopsy. The treatment of each cancer varies from person to person depending on the origin and location of the tumor. Chemotherapy is the most commonly used method to cure gastrointestinal cancers. Radiation and surgery were the other methods used for the diagnosis of GI cancer. A balanced diet enriched with phytochemicals was also reported to prevent cancer.

#### **Leukemia**

Cancer-related to blood is called Leukemia. The most commonly affected blood cells are White Blood Cells (WBC). Based on the onset of symptoms, the leukemia was classified

into acute (where the tumor cells multiply quickly) and chronic (the progression of the cancer is very slow). Leukemia was classified into two types as myelogenous leukemia and lymphocytic leukemia based on their progenitor origin 23-25. Based on both progenitor origin and onset of symptoms, the leukemia was classified into 4 types (Figure 01). The most commonly observed symptoms in leukemic patients are sweating, fa-

tigue, weight loss, fever, swollen lymph nodes, bleeding, etc. As leukemia is malignant, it can spread to other organs like the heart, kidneys, and lungs. Many diagnostic tests are developed to identify the type of leukemia and chemotherapy, transplantation, targeted therapy, radiation therapy, and immune therapy was used to treat leukemic patients.

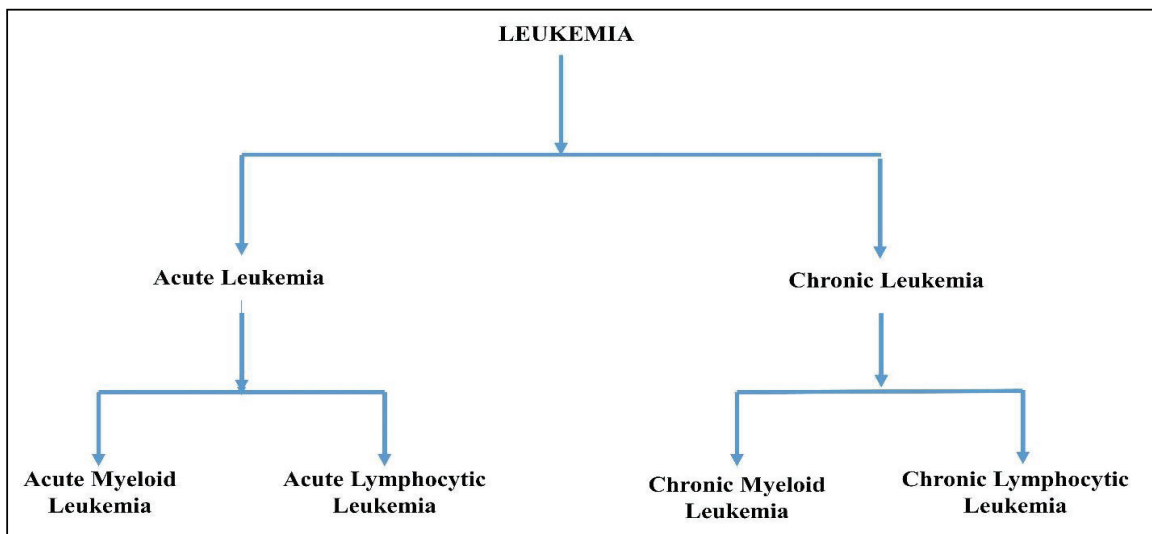


Figure 01. Classification of Leukemia

### Sarcoma

Sarcoma 26 is cancer associated with connective tissues like muscles, bones, tendons, and others. Osteosarcoma 27 is the most reported Sarcoma. Nearly 50 different types of sarcomas are reported. Based on the tissue type sarcoma are classified into soft tissue sarcoma and osteosarcoma. The symptoms observed among sarcoma patients are lumps, bone pain, breathing problems, swelling, etc. Sarcoma causative agents are genetic disorders, radiation, hereditary, and other bone disorders. Biopsy, CT scan, MRI scan, and ultrasound are the diagnostic methods used to treat sarcoma. Surgery, radiation therapy, chemotherapy, targeted therapy. Ex: Osteosarcoma (Bone cancer)28, angiosarcoma 29 (sarcoma associated with blood vessels), etc.

### Lymphoma

Cancer-associated with the lymphatic system is lymphoma where there is an abnormality in the cell division of lymphocytes leading to their accumulation in lymph nodes which results in the alteration of lymph nodes function. Stomach, breast, spleen, lymph nodes thymus are some organs associated with lymphoma. These are also referred to as solid cancers which were further sub-classified into Hodgkin lymphoma 30 and non-Hodgkin lymphoma 31. The causes of lymphoma were viral infection, weak immune system, radiation, etc. The symptoms observed in lymphoma patients are cough, fever, weight loss, fatigue, etc. Blood tests and imaging tests (CT scan, MRI, X-ray) can be used to diagnose lymphoma. Chemotherapy, radiation therapy, immunotherapy, and targeted

therapy were used to treat both Hodgkin lymphoma and non-Hodgkin lymphomas.

### **Myeloma**

The cancer associated with the abnormal division of blood plasma cells is Myeloma 32. It starts in the soft tissue of bone marrow. The causes of multiple myeloma are age, heredity, obesity, etc. pain, fatigue, fever, weight loss, anemia, etc. are observed in the advanced stages of myeloma. Smoldering myeloma is the initial stage of myeloma. It affects bones, kidneys, and other organs that are diagnosed by blood, imaging, and urine tests 33. Immunotherapy, chemotherapy, adjuvant therapy, radiation therapy are the most commonly used therapeutic methods to treat myeloma.

### **Central nervous system associated cancers**

The cancers associated with both the brain and spinal cord are CNS cancers 34. The most commonly associated cancer is a glioma. Glioma is associated with the connective tissue called glial cells 35. Both benign (non-cancerous) and malignant cancers were reported. astrocytoma, ependymoma 36, germ cell tumor, and other types of CNS cancers. The other types of cancers reported are mixed type (Ex: carcinosarcoma, mesodermal tumor, and others). Radiation, viruses, and head injury are some of the causes of CNS cancers. The symptoms observed are nausea, vomiting, headache, vision problems, etc. MRI, CT scan, biopsy are the diagnostic methods used to detect CNS cancers.

### **Stages of cancer**

Alterations in the genes lead to gain or loss of the gene leading to tumorigenesis. Mutagens (physical and chemical) are the environmental factors leading to the progression of cancer. Some of the cancers are caused by biological agents like human papillomavirus, Epstein–barr virus, etc. Stages are nothing but the extent of cancer within the body. The stages mainly describe the size, organs/tissues affected, and whether the cancer is metastasized. It mainly helps to diagnose cancer. Currently, im-

aging techniques, biopsy, tissue examinations were used to study cancer in detail. Generally, the appearance of cancer symptoms to its metastasis to other parts of the body is classified into 4 stages. A higher number indicated an advanced stage which sometimes leads to the death of the patient. It was also reported that some cancers start with Stage 0 (less harmful) and the cancers at this stage can be treated by surgery. The process taking place at different stages of cancer was described as follows: Stage 0 - in situ cancer (in place cancer), Stage I-Small tumor developed in a particular organ/tissue, Stage II-Tumor size increased and spread outside the particular organ/tissue, Stage III-Tumor spreads to the surrounding organs, and Stage IV-Metastasis and spreads the tumors across the body. Symptoms of Cancer: The symptoms observed among cancer patients are weight loss, lumps, bleeding, cough, pain fatigue, breathing-related problems, and others 1, 23.

### **Diagnosis and treatment of tumor**

**Tumor Diagnosis:** To treat cancer, it is important to know the type, size, and stage of cancer. Both imaging and lab tests are in use for the detection of tumor 37.

Complete blood count (Leukemia), blood chemistry profile, and cytogenetic analysis (base on the white blood cell count) are the blood tests used for the detection of cancers.

Urinalysis helps in the detection of kidney and uterus related cancers.

Biopsy- where surgically removed tissue is sent for examination and various types of biopsy are performed based on the tumor location and tumor type. Fine needle aspiration (FNA), core biopsy, Excisional biopsy, Incisional biopsy, Endoscopic biopsy, Laparoscopic biopsy, and skin biopsy are the different types of biopsy in use.

Cytology tests are used for the examination of body fluids like cerebrospinal fluid (brain cancer), pleural fluid (lung cancer), and

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others. The variants of cytology tests are scrape cytology and Pap smear tests.

X-rays, CT scan, ultrasound, MRI scan 38, Mammography 39, Endoscopy, and others are the imaging tests used for the detection of tumors.

Screening tests differ from one type of cancer detection to other types. Ex: Sigmoidoscopy (colorectal cancer) 40, HPV test (cervical cancer) 41, PSA test (prostate cancer) 42.

### **Treatments of cancer surgery**

Surgery is one of the most commonly used treatments to cure cancer with exceptional cancers associated with leukemia. Surgery is more effective to treat the cancer at initial stages and it can be used in combination with chemotherapy and other treatments for the reduction of size tumors (ex: brain tumors) 43-45. Surgery can be used to cure, debulk, and palate cancer. The side effects associated with surgery are bleeding, anesthesia complications, and infections. Many advanced surgeries like lumpectomy, radical mastectomy, video-assisted thoracoscopic surgery 46 (lung cancer treatment) are being used to remove the tumor within less time besides unaffacting/minimal effect on the surrounding tissues or organs. Robotic surgery 43, Laser surgery 47, electrosurgery 48, and cryosurgery 49 are the special variants of surgery being used to treat cancer.

### **Radiation therapy**

In Radiation therapy 50 the tumor cells were destroyed by the use of high energy X-rays. Radiation therapy can be given externally or internally. The internal radiation therapy is known as brachytherapy by the implantation of radioactive material. Some of the tumors which cannot be removed by the surgery are removed by Radiation therapy 51. Radiation therapy can be used in combination before the surgery for the reduction in tumor size as neoadjuvant therapy (ex. Lung cancer) and after surgery as adjuvant therapy (ex. Mastectomy). To relieve pain, palliative radiation therapy is used. Exter-

nal beam radiation therapy, Intensity-modulated radiation therapy (IMRT) 52, brachytherapy 53, proton therapy, systemic radiation therapy, and gamma knife 54 were the different ways of radiation therapy. The side effects observed in the patients treated with radiation therapy are redness, fatigue, pneumonia, etc.

### **Immunotherapy**

Immune system boosting with the help of inbuilt or synthetic compounds (synthesized in the laboratory) to defend against the cancer cells is known as Immunotherapy. Immunotherapy destroys the tumor or it is being used to suppress the growth of the tumor cell. Monoclonal antibodies (a kind of targeted therapy) 55, 56, T-cell therapy 57, oncolytic virus therapy 58, checkpoint inhibitors, etc. are some of the Immunotherapies 12, 59 in use. FDA in 2015 approved the Oncolytic virus - the genetically modified virus having an anti-tumor vaccine to treat advanced melanoma tumor cells. The virus used was the T-VEC (talimogene laherparepvec) 60. Usage of interleukins and interferons comes under non-specific immunotherapy which can be used in combination with chemotherapy or radiation therapy 61. Allergies and inflammation are the most commonly seen symptoms and the patients treated with Immunotherapy.

### **Hormonal therapy**

Hormonal therapy 62 is also known as endocrine therapy used to treat sensitive cancers, recurrence minimization, and others. Increased production of the hormones results in the development of cancers like breast cancer, ovarian cancer, prostate cancer, etc. 63. Endocrine therapy aids in tumor suppression by blocking the stimulating effect of the cancers or by the hormones produced. Tamoxifen is one such compound being used to treat breast cancer 64 20. The route of hormonal inhibitors was via oral or intravenous or surgery (removal of hormone-producing organs). In some cases, the removal of the organs by surgery prevents cancers. (Ex: oophorectomy) with some side effects like dryness etc. It can be given in combi-

nation with other therapies to reduce the size of the tumor (neoadjuvant therapy) or as adjuvant therapy or to kill the metastasis or recurred tumor. Some of the commercially available hormonal drugs are Abiraterone 65, Exemestane, Leuprolide etc. against prostate, breast, and uterine cancer 66.

### **Chemotherapy**

The application of chemically synthesized small molecule inhibitors against cancer cells is known as chemotherapy 13. In chemotherapy, the drugs inhibit the signaling pathways triggering the tumor cell multiplication besides induces apoptotic signal which leads to the death of cancer cells. Vincristine, Daunorubicin, Cyclophosphamide, etc. are the commercially available drugs to treat different cancers with some observed side effects like fever, hair loss, weight loss, inflammation, allergies, hypersensitivity reactions, inflammation, and others 67. In most of the cases, the side effects are short term with exceptional long-term effects associated with heart problems. The chemotherapeutic mainly targets the cell multiplication of the tumor cells. Chemotherapy is more effective against rapidly multiplying tumors besides indolent tumors. Neoadjuvant, adjuvant and palliative chemotherapy are the variants of chemotherapy. The route of administration is via intravenous, oral or directly into the fluids surrounding the organs. The diagnosis depends on the stage of cancer and the financial status of the patient. Various side effects 13 are observed in the chemotherapy treatment. Some are immediate effects (hair loss, skin, and gastric problems) and some effects are observed are late and these effects depend on the dose and toxicity of drugs administered during treatment. Based on their toxicity the side effects were classified into four groups as Mild symptoms- grade 1, moderate symptoms- grade 2, severe symptoms- grade 3 and life threatened symptoms as grade 4 (Neurotoxicity 68, 69, paralysis, comma, etc.) psychological problems, nausea, vomiting, gastrointestinal disorder, drug resistance, infertility, carcinogenicity, fatigue, sleep disorder, mucosi-

tis. The application of phytochemicals (phenols and others) can minimize drug resistance. Targeted therapy 13 has gained importance which uses small molecule inhibitors, monoclonal antibodies, enzymes 70, cytostatic drugs, and oncolytic virus 58 to treat cancer prognosis. The efficacy of cytostatic drugs depends on tumor response, survival time, and toxicity. Chemotherapy is effective against lymphomas and carcinomas 71.

### **Enzymatic therapy**

Application of enzymes as drug 72, 73 to treat diseases has gained importance because of its economic feasibility and high specificity towards its substrate. It will uphold various physiological conditions and eliminate it from the body in a reasonable time. Currently, enzymes are being used to treat gastrointestinal disorders, Gaucher's disease, liver failure, gout, coronary heart disease, cancer, etc. Many enzymes like L-Asparaginase and L-Glutaminase are being studied because of their ability to treat cancer with minimal side effects as these compounds convert the metabolites required by the tumor supplement for the production of energy. The enzymes supplied by the external route of administration will convert metabolites into unusable form leading to the death of tumor cells due to lack of energy supplements. Muller et.al worked on the application of Folylpolyglutamate synthetase validation by UPLC-MS/MS-based method (quantifies L-Glutamic acid) on the leukemic cell lines as well as clinical samples 74. In addition, the enzymes 16 were also used in suicide gene therapy (Enzyme prodrug therapy) in which converts the prodrug administered into tumor toxic form leading to the death of tumor cells. It was also reported that modified enzymes have high stability with observed fewer side effects against cancer patients. Hence, this therapy can be given individually or in combination with other chemotherapeutic drugs that may have lesser side effects.

### **Combination therapy**

The most common problem associated

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with many therapies is drug resistance 75, 76, and sensitization of the tumor cells which can be prevented by the application of combination therapy. The approval time for a drug to enter into the market takes approximately 15 years. Unlike conventional methods that are not specific towards tumor cells, combination therapy-specific towards its targets. The non-specific nature of conventional methods leads to many side effects. The treatments available in the market are not economically feasible and consume a lot of time having side effects. Hence, a combination of therapeutic approaches can be used to overcome the drawbacks of the mono-therapy 56. The cornerstone therapy which has gained importance in recent days is combination therapy. In combination therapy, various treatments used to treat cancer are combined to minimize or remove the cancer tumors in an effective way. Combination therapy is an effective measure against drug resistance which induces the apoptotic signal/or hinders the tumor growth 59, 77, 78. Even in combination therapy side effects are observed but with less toxicity as various pathways are targeted. The first proven concept of combination therapy against leukemic cancer was postulated by Emil Frei, James F. Holland, and Emil J. Freireich in 1965 79.

Combination therapy is a synergic approach that involves a combination of anti-cancer drugs to specifically target the cancer cells with minimal side effects. The drawbacks of monotherapy are drug resistance as the cancer cells chose alternate pathways for multiplication, sensitization 80 and other drawbacks in monotherapy is the drug targets both tumor and normal cells and these can be prevented by the application of combination therapy which uses cell cycle regulators and apoptotic inducers cost-effectively. The usage of antagonist drugs in combination therapy prevents the cytotoxicity of normal cells 81-83. The emerging variant of combination therapy is restrictive combination targeting the tumors based on the presence or absence of specific markers on both normal and tumor cells.

Drug reposting is the use of the commercially available drugs which are being used to treat various diseases to diagnose cancer. Hence the application of the repurposed drug in combination with cytotoxic drugs will be effective in diagnosing tumors. Carbonic anhydrase which is used to treat glaucoma and sickness has anti-cancer potential 84. Raplog 12 is another drug that is used in graft rejection as an immunosuppressant that has anti-cancer properties. Histone deacetylase inhibitors 85 in combination with Trichostatin A an antifungal antibiotic to treat cancer is under clinical trials 86. The first FDA approved repurposed drug is Vorinostat 87 used to treat cutaneous T-cell lymphoma. Romidepsin 57 and panobinostat 88 are the other drugs that have the efficacy to treat cutaneous T-cell lymphoma and multiple myeloma respectively 89. The combination of romidepsin and gemcitabine (Phase I) is being used to treat solid tumors of the pancreas, lung, breast, and ovaries 90 with observed side-effects like nausea, vomiting, and hematological abnormalities. The combination of itraconazole and penitrexol is under clinical trials to treat lung cancer 91, 92, and pancreatic cancer 93. This combination inhibited the endothelial cell proliferation of cell lines both in-vitro and in vivo. The anti-cancer effect against HT29 colon cancer cell lines by using Bevacizumab and curcumin combination 94 was demonstrated by Yue et.al. Kato et.al used gefitinib and erlotinib combination to treat lung cancer 95. Amado et.al in 2008 used panitumumab in combination with cetuximab 96 to treat colon cancer 55. It was reported by Quinn et.al that the application of combination therapy to prevent ductal adenocarcinoma. They demonstrated that the drugs target the selective apoptotic pathway leading to the death of tumor cells. The combination of pan-Bcl-2 inhibitor and minocycline 97 was used for the diagnosis of pancreatic cancer. The combined effects of the drugs were proved both in-vitro and in vivo.

Oncolytic therapy is the use of an oncolytic virus to target the tumor cells besides leaving normal cells. It was understood that



conventional therapies are less effective for metastatic cancer. The combination therapy of oncolytic virus 58 with conventional therapies is under clinical trials. Re-engineering of the virus at the gene level to specifically target the tumor cells is being carried out in adenovirus, herpesviruses, etc. dl1520, CV890, CV706 administration either by intratumoral, intraperitoneal, or intravenous in combination with 5-fluorouracil treat head and neck squamous cell carcinoma, gastric carcinoma is under clinical trials stage II. It was also reported that many wild type viruses have anti-tumor properties. The wild type viruses having cytotoxic properties are Vaccinia virus, Newcastle disease virus, parvovirus, etc. The most common side effects observed with oncolytic therapy are flu-like symptoms. The combination of oncolytic viruses and radiation therapy is at the preclinical stage. These experiments were reported on human glioma xenograft models with or without p53 mutant by dl1520 injections followed by radiation 58. Ivana Bozic in 2013 worked on the effect of combination therapy myeloma patients to overcome drug resistance. They developed a mathematical model to analyze the number of lesion w.r.t. treatment. They have given combination therapy to 20 myeloma patients administered with vemurafenib and observed that the results are effective for the long term unless any mutation is observed. In the case of mutation observance,

triple therapy is suggested 82. The developed mathematical model was also used to study the combined effect against pancreatic and colorectal metastatic cancers.

Feldmann et.al worked on the application of combined therapy by using the inhibitors that block the hedgehog signaling pathway for the diagnosis of pancreatic cancer. They reported that there is a significant decrease in the tumor size and metastasis phenomena in mice when a combination of Gemcitabine and Cyclopamine is administered 98. Hence, hedgehog signaling inhibitors may be a new emerging solution for pancreatic cancer treatment 99. Frew et.al worked on the combination therapy of histone deacetylase inhibitors and mouse-specific monoclonal antibodies MD5-1 against mouse breast cancer models. They reported that these both have been used as monotherapy to treat various tumors which are under clinical trials 86. Hence, the combination of Vorinostat with MD5-1 induces anti-tumor properties in mouse breast cancer models as the combination induces activation of both intrinsic and extrinsic apoptotic pathways 21, 75, 100, 101 both in-vitro and in vivo. Various combinations of drugs are under clinical trials showing promising results. Some of the combinations of drugs that are being studied were shown below Table 01.

Table 01. Various Combination of drugs under Clinical trial studies

COMBINATIONS	ANTI-TUMOR PROPERTIES	REFERENCE
VEGFR antibody DC101+Vinblastine	Tumor regression in neuroblastoma xenograft models	102
Bevacizumab+Paclitaxel	Metastatic breast cancer	103
Sorafenib+ Dacarbazine/Capercitabine	Breast cancer (phase 2 trials)	78
Cetuximab +Gemcitabine	Pancreatic cancer ( phase 2)	104
Erlotinib+Gemcitabine	Pancreatic cancer ( phase 3)	105
Bevcizumab + Everolimus	Melanoma and colorectal cancer	106
Imatinib + Peginterferon	Gastrointestinal stromal tumor patients	107
Vorinostat + MD5-1	Brest cancer mouse models	86
Gecitabine + Cyclopamine	Pancreatic cancer	98, 99

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Oncolytic virus dl1520+ 5-fluorouracil	Head and neck squamous cell carcinoma, gastric carcinoma (Phase 2)	58
pan-Bcl-2 inhibitor + Minocycline	Pancreatic cancer	97
Gefitinib + Erlotinib	Lung cancer	95
Panitumumab + Cetuximab	Colon cancer	96
Bevacizumab + Curcumin	HT29 colon cancer cell lines	94
Itraconazole + Penetrexol	Lung cancer	92
Histone deacetylase inhibitors +Trichostatin A	Various Cancer	86
Romidepsin+ Gemcitabine	Solid tumors	90

It was reported by many researchers that targeting the metabolic / signaling pathway enzymes which are involved in tumor progression can be effective in tumor suppression with minimal side effects. Elisa Bergaggio et.al work on the inhibitors that target the mutated isocitrate dehydrogenases which lead to cancers 14. These enzymes can be used as markers for the detection of cancers. Hence, potential targets need to be studied against these mutated enzymes 14. Another researcher William j. Israelsen et.al worked on pyruvate kinase regulators to treat cancer 108. The pyruvate kinases are involved in glycolysis and are understood to be 4 types having unique properties. Of which pyruvate kinase isoform 2 is involved in both cell cycle progression 108. Hence, pyruvate kinase inhibitors may be an emerging idea for tumor treatment. Khushwant et.al reviewed the application of small molecule kinase inhibitors which are involved in tumor suppression 109. They have reported that nearly 37 kinase inhibitors were approved by the FDA to treat breast and lung cancer patients, while many others are in clinical phase studies 109.

Biagio Ricciuti worked on enzymatic inhibitors that control angiogenesis 9, 110 as promising emerging targets. Small molecules targeting nitric oxidase and metalloproteinase are not effective in clinical studies. Besides, heparanase, NADH oxidase, lysyl oxidase small molecule inhibitors showed promising results studying these enzyme inhibitors can be effective. Iqra Sarfraz worked on the malic en-

zyme 2 111 inhibitors as this enzyme is over expressed and is seen in various cancers- hepatomas, lung cancer, melanomas, osteosarcomas, etc. when et.al 111 reported NPD389 inhibitors against malic enzymes which need to be further evaluated. Embonic acid is a competitive small molecule inhibitor of malic enzyme 112 which induces senescence on H1299 cell lines and the effect of embonic acid 113 on other cell lines needs to be evaluated further. Similarly targeting phosphoglycerate mutase-1 114 by small molecule inhibitors can be further evaluated with combination therapy.

### Summary of the review

It was understood from the literature that based on the statistics, cancer is the second most important disease which needs attention for the cure. A diverse range of cancers was identified across the world which is influenced by geographical distribution, food habits, pollution, and other environments and mutagens (both physical and chemical). Various treatments are currently in use with many side effects, some of them are life-threatening. In addition to the present treatment, and many clinical trials are going on the application of combination therapy to treat cancer as well as minimize the side-effects within less time. Various combinations are currently in clinical studies of which some of them are effective and some are not and it depends on the tumor properties and combinations of therapy that need to be changed for different types of cancer. Hence,

combination therapy will be a promising emerging therapy that needs to be thoroughly evaluated further by targeting the enzymes responsible for tumor prognosis. Hence, if this therapy succeeds shortly, it will be economical with minimal side-effects as the combination of therapies used the toxicity caused by the monotherapy.

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