NPTEL Phase – II (Syllabus Template)

Course Title: Bio-Organic Chemistry of Natural Enediyne Anticancer Antibiotics

Module 4:
Applications of Enediyne Antitumor Antibiotics: Defining Cancer and Its Various Type; Cancer-Treatment of Choice; Cancer-Combination Therapies; Therapeutic Applications of Enediyne Antitumor Antibiotics; The Approved Enediynes for Use as Anticancer Drugs; Enediynes Under Clinical Investigation; Immunoconjugates; Antibody–Drug Conjugates; Targeted Chemotherapy; Antibody-Enediyne Conjugate under Clinical Investigation Future Prospect and recent advances in Enediyne Research.

4.1. Defining Cancer and Its Various Type

4.1.1. Introduction (Taken from Wikepedia)

- **Cancer**, known medically as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumors are cancerous. Benign tumors do not grow uncontrollably, do not invade neighboring tissues, and do not spread throughout the body. There are over 200 different known cancers that afflict humans.

- Determining what causes cancer is complex. Many things are known to increase the risk of cancer, including tobacco use, certain infections, radiation, lack of physical activity, obesity, and environmental pollutants. These can directly damage genes or combine with existing genetic faults within cells to cause the disease. Approximately five to ten percent of cancers are entirely hereditary.

- Cancer can be detected in a number of ways, including the presence of certain signs and symptoms, screening tests, or medical imaging. Once a possible cancer is detected it is diagnosed by microscopic examination of a tissue sample. Cancer is usually treated with chemotherapy, radiation therapy and surgery. The chances of surviving the disease vary greatly by the type and location of the cancer and the extent of disease at the start of treatment. While cancer can affect people of all ages, and a few types of cancer are more common in children, the risk of developing cancer generally increases with age. In 2007, cancer caused about 13% of all human deaths worldwide (7.9 million). Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world.
4.1.2. Various Types of Cancer
4.1.2.1. Leukemia

Leukemia or leukaemia is a type of cancer of the blood or bone marrow characterized by an abnormal increase of immature white blood cells called "blasts." Leukemia is a broad term covering a spectrum of diseases. In turn, it is part of the even broader group of diseases affecting the blood, bone marrow, and lymphoid system, which are all known as hematological neoplasms.

4.1.2.2. Classification of Leukemia

<table>
<thead>
<tr>
<th>Four major kinds of leukemia</th>
<th>Acute</th>
<th>Chronic</th>
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<tbody>
<tr>
<td><strong>Lymphocytic leukemia</strong> (or &quot;lymphoblastic&quot;)</td>
<td>Acute lymphoblastic leukemia (ALL)</td>
<td>Chronic lymphocytic leukemia (CLL)</td>
</tr>
<tr>
<td><strong>Myelogenous leukemia</strong> (also &quot;myeloid&quot; or &quot;nonlymphocytic&quot;)</td>
<td>Acute myelogenous leukemia (AML) (or myeloblastic)</td>
<td>Chronic myelogenous leukemia (CML)</td>
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</table>
Clinically and pathologically, leukemia is subdivided into a variety of large groups. The first division is between its acute and chronic forms:

- **Acute leukemia** is characterized by a rapid increase in the number of immature blood cells. Crowding due to such cells makes the bone marrow unable to produce healthy blood cells. Immediate treatment is required in acute leukemia due to the rapid progression and accumulation of the malignant cells, which then spill over into the bloodstream and spread to other organs of the body. Acute forms of leukemia are the most common forms of leukemia in children.

- **Chronic leukemia** is characterized by the excessive build up of relatively mature, but still abnormal, white blood cells. Typically taking months or years to progress, the cells are produced at a much higher rate than normal, resulting in many abnormal white blood cells. Whereas acute leukemia must be treated immediately, chronic forms are sometimes monitored for some time before treatment to ensure maximum effectiveness of therapy. Chronic leukemia mostly occurs in older people, but can theoretically occur in any age group.

Additionally, the diseases are subdivided according to which kind of blood cell is affected. This split divides leukemias into lymphoblastic or lymphocytic leukemias and myeloid or myelogenous leukemias:

- In lymphoblastic or lymphocytic leukemias, the cancerous change takes place in a type of marrow cell that normally goes on to form lymphocytes, which are infection-fighting immune system cells. Most lymphocytic leukemias involve a specific subtype of lymphocyte, the B cell.

- In myeloid or myelogenous leukemias, the cancerous change takes place in a type of marrow cell that normally goes on to form red blood cells, some other types of white cells, and platelets.

Combining these two classifications provides a total of four main categories. Within each of these four main categories, there are typically several subcategories. Finally, some rarer types are usually considered to be outside of this classification scheme.

- **Acute lymphoblastic leukemia (ALL)** is the most common type of leukemia in young children. This disease also affects adults, especially those age 65 and older. Standard treatments involve chemotherapy and radiotherapy. The survival rates vary by age: 85% in children and 50% in adults. Subtypes include precursor B acute lymphoblastic leukemia, precursor T acute lymphoblastic leukemia, Burkitt's leukemia, and acute biphenotypic leukemia.

- **Chronic lymphocytic leukemia (CLL)** most often affects adults over the age of 55. It sometimes occurs in younger adults, but it almost never affects children. Two-thirds of affected people are men. The five-year survival rate is 75%. It is incurable, but there are many effective treatments. One subtype is B-cell prolymphocytic leukemia, a more aggressive disease.
• **Acute myelogenous leukemia** (AML) occurs more commonly in adults than in children, and more commonly in men than women. AML is treated with chemotherapy. The five-year survival rate is 40%, except for APL, which is over 90%. Subtypes of AML include acute promyelocytic leukemia, acute myeloblastic leukemia, and acute megakaryoblastic leukemia.

• **Chronic myelogenous leukemia** (CML) occurs mainly in adults; a very small number of children also develop this disease. Treatment is with imatinib (Gleevec in US, Glivec in Europe) or other drugs. The five-year survival rate is 90%. One subtype is chronic monocyctic leukemia.

• **Hairy cell leukemia** (HCL) is sometimes considered a subset of chronic lymphocytic leukemia, but does not fit neatly into this pattern. About 80% of affected people are adult men. No cases in children have been reported. HCL is incurable, but easily treatable. Survival is 96% to 100% at ten years.

• **T-cell prolymphocytic leukemia** (T-PLL) is a very rare and aggressive leukemia affecting adults; somewhat more men than women are diagnosed with this disease. Despite its overall rarity, it is also the most common type of mature T cell leukemia; nearly all other leukemias involve B cells. It is difficult to treat, and the median survival is measured in months.

• **Large granular lymphocytic leukemia** may involve either T-cells or NK cells; like hairy cell leukemia, which involves solely B cells, it is a rare and indolent (not aggressive) leukemia.

• **Adult T-cell leukemia** is caused by human T-lymphotropic virus (HTLV), a virus similar to HIV. Like HIV, HTLV infects CD4+ T-cells and replicates within them; however, unlike HIV, it does not destroy them. Instead, HTLV "immortalizes" the infected T-cells, giving them the ability to proliferate abnormally. Human T cell lymphotropic virus types I and II (HTLV-I/II) are endemic in certain areas of the world.
4.1.2.3. Signs and Symptoms of Leukemia

![Common symptoms of Leukemia](image)

- **Systemic**
  - Weight loss
  - Fever
  - Frequent infections

- **Lungs**
  - Easy shortness of breath

- **Muscular**
  - Weakness

- **Bones or joints**
  - Pain or tenderness

- **Psychological**
  - Fatigue
  - Loss of appetite

- **Lymph nodes**
  - Swelling

- **Spleen and/or liver**
  - Enlargement

- **Skin**
  - Night sweats
  - Easy bleeding and bruising
  - Purplish patches or spots

**Figure 1. Common symptoms of chronic or acute leukemia**
Damage to the bone marrow, by way of displacing the normal bone marrow cells with higher numbers of immature white blood cells, results in a lack of blood platelets, which are important in the blood clotting process. This means people with leukemia may easily become bruised, bleed excessively, or develop pinprick bleeds (petechiae).

White blood cells, which are involved in fighting pathogens, may be suppressed or dysfunctional. This could cause the patient's immune system to be unable to fight off a simple infection or to start attacking other body cells. Because leukemia prevents the immune system from working normally, some patients experience frequent infection, ranging from infected tonsils, sores in the mouth, or diarrhea to life-threatening pneumonia or opportunistic infections.

Finally, the red blood cell deficiency leads to anemia, which may cause dyspnea and pallor.

Some patients experience other symptoms, such as feeling sick, having fevers, chills, night sweats, feeling fatigued and other flu-like symptoms. Some patients experience nausea or a feeling of fullness due to an enlarged liver and spleen; this can result in unintentional weight loss. Blasts affected by the disease may come together and become swollen in the liver or in the lymph nodes causing pain and leading to nausea (Figure 1).

If the leukemic cells invade the central nervous system, then neurological symptoms (notably headaches) can occur. All symptoms associated with leukemia can be attributed to other diseases. Consequently, leukemia is always diagnosed through medical tests.

The word *leukemia*, which means 'white blood', is derived from the disease's namesake high white blood cell counts that most leukemia patients have before treatment. The high number of white blood cells are apparent when a blood sample is viewed under a microscope. Frequently, these extra white blood cells are immature or dysfunctional. The excessive number of cells can also interfere with the level of other cells, causing a harmful imbalance in the blood count.

Some leukemia patients do not have high white blood cell counts visible during a regular blood count. This less-common condition is called *aleukemia*. The bone marrow still contains cancerous white blood cells which disrupt the normal production of blood cells, but they remain in the marrow instead of entering the bloodstream, where they would be visible in a blood test. For an aleukemic patient, the white blood cell counts in the bloodstream can be normal or low. Aleukemia can occur in any of the four major types of leukemia, and is particularly common in hairy cell leukemia.
4.1.2.2. Carcinoma

Carcinoma is the medical term for the most common type of cancer occurring in humans. Put simply, a carcinoma is a cancer that begins in a tissue that lines the inner or outer surfaces of the body, and that generally arises from cells originating in the endodermal or ectodermal germ layer during embryogenesis. More specifically, a carcinoma is tumor tissue derived from putative epithelial cells whose genome has become altered or damaged to such an extent that the cells become transformed, and begin to exhibit abnormal malignant properties.

4.1.2.2.1. Pathogenesis of Cancer

Cancer occurs when a single progenitor cell accumulates mutations and other changes in the DNA, histones, and other biochemical compounds that make up the cell's genome. The cell genome controls the structure of the cell's biochemical components, the biochemical reactions that occur within the cell, and the biological interactions of that cell with other cells. Certain combinations of mutations in the given progenitor cell ultimately result in that cell (also called a cancer stem cell) displaying a number of abnormal, malignant cellular properties that, when taken together, are considered characteristic of cancer, including:

- the ability to continue to divide perpetually, producing an exponentially (or near-exponentially) increasing number of new malignant cancerous "daughter cells" (uncontrolled mitosis);
- the ability to penetrate normal body surfaces and barriers, and to bore into or through nearby body structures and tissues (local invasiveness);
- the ability to spread to other sites within the body (metastasize) by penetrating or entering into the lymphatic vessels (regional metastasis) and/or the blood vessels (distant metastasis).

If this process of continuous growth, local invasion, and regional and distant metastasis is not halted via a combination of stimulation of immunological defenses and medical treatment interventions, the end result is that the host suffers a continuously increasing burden of tumor cells throughout the body. Eventually, the tumor burden increasingly interferes with normal biochemical functions carried out by the host's organs, and death ultimately ensues.

Malignant neoplasms are exceptionally heterogeneous entities, reflecting the wide variety, intensity, and potency of various carcinogenic promoters. To date, no simple and comprehensive method for classifying them has yet been devised and accepted within the scientific community. Traditionally, however, malignancies have generally been classified into various taxa using a combination of criteria, including:
One commonly used classification scheme classifies these major cancer types on the basis of cell genesis, specifically:

1. Their (putative) cell (or cells) of origin
   1. **Epithelial cells** => carcinoma
   2. **Non-hematopoietic mesenchymal cells** => sarcoma
   3. Hematopoietic cells

1. **bone marrow**-derived cells that normally mature in the bloodstream => **Leukemia**
2. **bone marrow**-derived cells that normally mature in the lymphatic system => **Lymphoma**
3. **Germ cells** => **Germinoma**

Other criteria that play a role in a cancer diagnosis include:

- The degree to which the malignant cells resemble their normal, untransformed counterparts
- the appearance of the local tissue and stromal architecture
- the anatomical location from which tumors arise
- genetic, epigenetic, and molecular features

4.1.2. 2.2. Histological Types and Variants of Carcinoma

**Adenocarcinoma**: *(adeno = gland)* Refers to a carcinoma featuring microscopic glandular-related tissue cytology, tissue architecture, and/or gland-related molecular products, e.g., mucin.

**Squamous cell carcinoma**: Refers to a carcinoma with observable features and characteristics indicative of squamous differentiation (intercellular bridges, keratinization, squamous pearls).

**Adenosquamous carcinoma**: Refers to a mixed tumor containing both adenocarcinoma and squamous cell carcinoma, wherein each of these cell types comprise at least 10% of the tumor volume.

**Anaplastic carcinoma**: Refers to a heterogeneous group of high-grade carcinomas that feature cells lacking distinct histological or cytological evidence of any of the more specifically differentiated neoplasms. These tumors are referred to as Anaplastic or Undifferentiated carcinomas.

**Large cell carcinoma**: Composed of large, monotonous rounded or overtly polygonal-shaped cells with abundant cytoplasm.

**Small cell carcinoma**: Cells are usually round and are less than approximately 3 times the diameter of a resting lymphocyte and little evident cytoplasm. Occasionally, small cell malignancies may themselves have significant components of slightly polygonal and/or spindle-shaped cells.
There are a large number of rare subtypes of anaplastic, undifferentiated carcinoma. Some of the more well known include the lesions containing pseudo-sarcomatous components: spindle cell carcinoma (containing elongated cells resembling connective tissue cancers), giant cell carcinoma (containing huge, bizarre, multinucleated cells), and sarcomatoid carcinoma (mixtures of spindle and giant cell carcinoma). Pleomorphic carcinoma contains spindle cell and/or giant cell components, plus at least a 10% component of cells characteristic of more highly differentiated types (i.e. adenocarcinoma and/or squamous cell carcinoma). Very rarely, tumors may contain individuals components resembling both carcinoma and true sarcoma, including carcinosarcoma and pulmonary blastoma.

4.1.2. 2.3. Frequent Organ Sites of Carcinoma

- **Lung:** Carcinoma comprises > 98% of all lung cancers.
- **Breast:** Nearly all breast cancers are ductal carcinoma.
- **Prostate:** The most common form of carcinoma of the prostate is adenocarcinoma.
- **Colon and rectum:** Nearly all malignancies of the colon and rectum are either adenocarcinoma or squamous cell carcinoma.
- **Pancreas:** Pancreatic carcinoma is almost always of the adenocarcinoma type and is highly lethal.

Some carcinomas are named for their or the putative cell of origin, (e.g. [hepatocellular carcinoma](#), renal cell carcinoma).

4.1.2. 2.4. Types of Carcinoma

1. **Epithelial neoplasms**

2. **Squamous cell neoplasms**
   - (a) **Squamous cell carcinoma:** Squamous cell carcinoma (SCC or SqCC) is a cancer of a kind of epithelial cell, the squamous cell. These cells are the main part of the epidermis of the skin, and this cancer is one of the major forms of skin cancer. However, squamous cells also occur in the lining of the digestive tract, lungs, and other areas of the body, and SCC occurs as a form of cancer in diverse tissues, including the lips, mouth, esophagus, urinary bladder, prostate, lung, vagina, and cervix, among others. Despite sharing the name *squamous cell carcinoma*, the SCCs of different body sites can show tremendous differences in their presenting symptoms, natural history, prognosis, and response to treatment.

3. **Basal cell neoplasms**
   - (a) **Basal cell carcinoma:** Basal-cell carcinoma (BCC), a skin cancer, is the most common cancer. It rarely metastasizes or kills. However, because it can cause significant destruction and disfigurement by invading surrounding tissues, it is still considered malignant.

4. **Transitional cell carcinomas**
5. **Adenocarcinomas:**

   (a) **Adenocarcinoma:** *Adenocarcinoma* is a cancer of an epithelium that originates in glandular tissue. Epithelial tissue includes, but is not limited to, the surface layer of skin, glands and a variety of other tissue that lines the cavities and organs of the body. Epithelium can be derived embryologically from ectoderm, endoderm or mesoderm. To be classified as Adenocarcinoma, the cells do not necessarily need to be part of a gland, as long as they have secretory properties. Well differentiated adenocarcinomas tend to resemble the glandular tissue that they are derived from, while poorly differentiated adenocarcinomas may not.

   (b) **Linitis plastica:** *Linitis plastica*, also known as *Brinton's disease* or *leather bottle stomach*, is a morphological variant of diffuse (or infiltrating) stomach cancer. Causes of linitis plastica could be lye ingestion or metastatic infiltration of the stomach, particularly breast and lung carcinoma.

   (c) **Vipoma:** A *VIPoma* (also known as *Verner Morrison syndrome*, after the physicians who first described it) is a rare (1 per 10,000,000 per year) endocrine tumor, usually (about 90%) originating from non-β islet cell of the pancreas, that produce vasoactive intestinal peptide (VIP). It may be associated with multiple endocrine neoplasia type 1.

   (d) **Cholangiocarcinoma:** *Cholangiocarcinoma* is a medical term denoting a form of cancer that is composed of mutated epithelial cells (or cells showing characteristics of epithelial differentiation) that originate in the bile ducts which drain bile from the liver into the small intestine. Other biliary tract cancers include pancreatic cancer, gallbladder cancer, and cancer of the ampulla of Vater. Cholangiocarcinoma is a relatively rare neoplasm that is classified as an adenocarcinoma (a cancer that forms glands or secretes significant amounts of mucins). It has an annual incidence rate of 1–2 cases per 100,000 in the Western world, but rates of cholangiocarcinoma have been rising worldwide over the past several decades.

   (e) **Hepatocellular carcinoma:** *Hepatocellular carcinoma* (HCC, also called *malignant hepatoma*) is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of hepatic cirrhosis).

   (f) **Adenoid cystic carcinoma:** *Adenoid cystic carcinoma* (AdCC) is a rare type of cancer that can exist in many different body sites. It most often occurs in the areas of the head and neck, in particular the salivary glands; but has also been reported in the breast, lacrimal gland of the eye, lung, brain, Bartholin gland, trachea, and the paranasal sinuses. It is sometimes referred to as adenocyst, malignant cylindroma, adenocystic, adenoidcystic, ACC, AdCC.

   (g) **Renal cell carcinoma:** *Renal cell carcinoma* (RCC, also known as *hypernephroma*) is a kidney cancer that originates in the lining of the proximal convoluted tubule, the very small tubes in the kidney that transport GF (glomerular filtrate) from the glomerulus to the descending limb of the nephron. RCC is the most common type of kidney cancer in adults, responsible for approximately 80% of cases. It is also known to be the most lethal of all the genitourinary tumors. Initial treatment is most commonly a radical or partial nephrectomy and remains the mainstay of curative treatment. Where the tumor is confined to the renal parenchyma, the 5-year survival rate is 60-70%, but this is lowered considerably where metastases have
spread. It is relatively resistant to radiation therapy and chemotherapy, although some cases respond to immunotherapy. Targeted cancer therapies such as sunitinib, temsirolimus, bevacizumab, interferon-alpha, and sorafenib have improved the outlook for RCC (progression-free survival), although they have not yet demonstrated improved survival.

(h) Grawitz tumor

6. Adnexal and Skin appendage Neoplasms
7. Mucoepidermoid Neoplasms
8. Cystic, Mucinous and Serous Neoplasms
9. Ductal, Lobular and Medullary Neoplasms
10. Acinar cell neoplasms
11. Complex epithelial neoplasms

4.2. Cancer-Treatment of Choice

<table>
<thead>
<tr>
<th>Cancer, Treatment of Choice</th>
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<tbody>
<tr>
<td>• Localized Cancer: Surgery</td>
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<tr>
<td>• Locally Advanced:</td>
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<tr>
<td>If Surgery not possible,</td>
</tr>
<tr>
<td>Radio therapy</td>
</tr>
<tr>
<td>Chemotherapy</td>
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<tr>
<td>Radio + Chemo (Radio-sensitizer)</td>
</tr>
<tr>
<td>Chemo → Radio</td>
</tr>
<tr>
<td>Radio, Chemo → Surgery (Neo. Adjuvant Chemotherapy)</td>
</tr>
</tbody>
</table>

| If Surgery possible,         |
| Post-operative Radio or Chemo (Adjuvant Chemotherapy) |
| • Regional: Radiotherapy     |
|   En-bloc Surgery, if possible |
| • Metastatic Cancer: Chemotherapy (Palliative or Curative) |

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<thead>
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<th>Chemo (Adjuvant) therapy</th>
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4.2.1. Introduction to Cancer Chemotherapy

- **What is Chemotherapy?** Chemotherapy is a kind of treatment that uses drugs to attack cancer cells. It is called a "systemic treatment" since the drug, entering through the bloodstream, travels throughout the body and kills cancer cells at their sites. Chemotherapy, often shortened to just "chemo". The drugs may rarely be intended to have a local effect, but in most cases, the intention is to destroy cancer cells wherever they may exist in the body.

- Chemotherapeutic drugs are chemically designed to target cells that are dividing and growing rapidly. Once they reach the cancer cells, they act to retard their growth, eventually resulting in their destruction.

- Chemotherapy may be given at home, in a clinic or in a hospital. The frequency of chemotherapy can be daily, weekly, monthly or an on-off schedule depending on the type of drug, the body's response and the type of cancer. The chemotherapy is decided on the basis of the type of cancer. The dosage is calculated on the basis of the patient's body weight and the drug's toxicity.

- The chemotherapy is concerned with the whole body.

- Chemotherapy is used to treat:
  
  - early-stage invasive breast cancer to get rid of any cancer cells that may be left behind after surgery and to reduce the risk of the cancer coming back
  - advanced-stage breast cancer to destroy or damage the cancer cells as much as possible

- In some cases, chemotherapy is given before surgery to shrink the cancer.

- At present more than 50 anticancer drugs have been discovered. They are used in several ways:
  
  - Monotherapy or only one drug
  - Combination chemotherapy or a group of drugs which work together
  - Combined modality or chemotherapy along with other treatment such as surgery and radiotherapy

- The drugs are delivered to the affected cells in the following forms:
  
  - Oral (tablet form, by mouth)
  - Intravenous or Intramuscular (injected by needle into a vein or muscle)
  - Intrathecal chemotherapy (injected through a needle in the back)
4.2.2. History of Cancer Chemotherapy

**The National Chemo-therapy Program begins at the National Cancer Institute (NCI), a sys-
tematic programme for drug screening com-
mercises.**


**Lewis Goodman and Alfred Gilman use nitrogen mustard to treat a patient with non-Hodg-
kin’s lymphoma and demonstrate for the first time that chemotherapy can induce tumour re-
gression.**

**1942**

**Sydney Farber uses antifolates to successfully induce remissions in children with acute lymphoblastic leukemia (ALL).**

**George Hitchings and Gertrude Elion synthe-
size the purine analogue 6-mercaptopurine.**

**Roy Herz and Min Chiu Li demonstrate that meth-
trexate as a single agent can cure choriocarcinoma, the first solid tumour to be cured by chemotherapy.**

**Combination chemotherapy (POMP regimen) is able to induce long term remissions in children with ALL.**

**Vincent DeVita and col-
leagues cure lymphomas with combination chemo-
therapy.**

**The Food and Drug Admin-
istration (FDA) approves the alkylating agent cyclo-
phosphamide.**

**The NCI introduces ‘dis-
ease oriented’ screening using 60 cell lines derived from different types of hu-
man tumour.**

**A combination of cyclo-
phosphamide, methotrex-
ate and fluorouracil (CMF) was shown to be effective as adjuvant treatment for node-positive breast can-
cer.**

**Emil Frei and colleagues demonstrate that chemother-
apy given after surgi-
cal removal of esophageco-
ma can improve cure rates (adjuvant chemotherapy).**

**The FDA approves pacli-
taxel (Taxol), which be-
comes the first ‘blockbust-
er’ oncology drug.**

**The FDA approves bevacizumab (Avastin), the first clinically proven antiangiogenic agent, for the treat-
ment of colon cancer.**

**Studies by Dain Drucker lead to FDA approval of imatinib mesylate (Glivec) for chronic myelogenous leukaemia, a new paradigm for targeted therapy in oncology.**

**Goodman and Gilman: first use nitrogen mustard (alkylating agent) to treat cancer.**

**The researches at Harvard University define mutations in the epidermal growth factor receptor that confer selective responsiveness to the target-
ed agent gefitinib, indicating that molecular testing might be able to prospectively identify subsets of patients that will respond to targeted agents.**
4.2.3. How Chemotherapy Works?

- Chemotherapy medicines prevent cancer cells from growing and spreading by destroying the cells or stopping them from dividing.
- Cancer cells tend to grow and divide very quickly with no order or control. Because they're growing so fast, sometimes cancer cells break away from the original tumor and travel to other places in the body. Chemotherapy weakens and destroys cancer cells at the original tumor site AND throughout the body.
- Most normal cells grow and divide in a precise, orderly way. Still, some normal cells do divide quickly, including cells in hair follicles, nails, the mouth, digestive tract, and bone marrow (bone marrow makes blood cells). Chemotherapy also can unintentionally harm these other types of rapidly dividing cells, possibly causing chemotherapy side effects.
- When treating early-stage breast cancer, it's fairly common for chemotherapy to be given after surgery, as soon as you recover. Doctors call this "adjuvant" chemotherapy because it's given in addition to surgery, which is considered the primary treatment.
- In some cases, chemotherapy is given before surgery to shrink the cancer so that less tissue has to be removed. When chemotherapy is given before surgery, it's called "neoadjuvant" chemotherapy.
- In many cases, chemotherapy medicines are given in combination, which means you get two or three different medicines at the same time. These combinations are known as chemotherapy regimens. In early-stage breast cancer, standard chemotherapy regimens lower the risk of the cancer coming back. In advanced breast cancer, chemotherapy regimens make the cancer shrink or disappear in about 30-60% of people treated. Keep in mind that every cancer responds differently to chemotherapy.

4.2.4. Side Effects of Chemotherapy

Since chemotherapy also affects normal actively dividing cells such as those in the bone marrow, the gastrointestinal tract, the reproductive system and in the hair follicles, most patients experience some degree of side effects, which may include any or all of the following:

- **Nausea and vomiting:** This is a common side effect of chemotherapy. It can be controlled with anti-sickness drugs (anti-emetics).
- **Fatigue:** Chemotherapy affects different people in different ways. Some find they can lead fairly normal lives during treatment, but many find they become tired and have to take things more slowly. Just do as much as you can and be careful not to over-strain. Taking short naps may help.
- **Hair loss:** This is the least harmful side effect, yet can be the hardest to bear. The use of a cold compress around the scalp when taking chemotherapy helps stop hair loss to some extent. Hair will grow back surprisingly quickly once treatment is over.
- **Susceptibility to infections:** When the drugs act on cancer cells, they also destroy normal cells including white blood cells, which fight infections. When white blood cells are in short supply, the body's immune system is weakened making you susceptible to infections. Any fever should be reported to the doctor.
- **Decrease in blood cell count:** During chemotherapy, you may become anemic. Regular blood tests are done to ensure this does not happen. If necessary, blood transfusions are given.
- **Mouth sores and ulcers:** Some chemotherapy drugs cause sores and ulcers in the mouth. Regular use of a mouthwash is very important.

### 4.2.5. Drugs Used in Cancer Chemotherapy

- **Cytotoxic Agents**
  - Alkylating Agents
  - Antimetabolites
  - Cytotoxic antibiotics
  - Plant derivatives
- **Hormones**
  - Suppress nat’l hormone secr’n or antagonize hormone action
- **Misc (mostly target oncogene products)**
4.2.6. Role of Chemotherapy in Cancer Treatment

Role of Chemotherapy in Cancer Treatment

- (1) **Metastatic Cancer**: Palliative or
  - Curative Chemotherapy
- (2) **Adjuvant Chemotherapy**:
  - to eradicate or control micro-metastasis
- (3) **Neo-adjuvant Chemotherapy**
  - (Induction Chemotherapy) — to make Surgery of RT possible
  - to alleviate surgical damage
  - to eradicate micro-metastasis
- (4) **Hematological Malignancies**:
  - Primary Treatment
4.2.7. Cytotoxic Agents (Drugs) Used in Cancer Chemotherapy
4.2.7.1. Mechanism of Cancer Chemotherapy

**Mechanism of Cancer Chemotherapy (Cytostatic Drugs)**

**Anticancer Drugs are Antiproliferative**

- Affect cell division
  - Active on rapidly dividing cells
- Most effective during S phase of cell cycle
  - Many cause DNA damage
- Damage DNA $\rightarrow$ initiation of apoptosis (Programmed Cell Death)

**Anti-proliferation**

1. Blockage DNA Synthesis
   - Blockage purined pyrimidine synthesis
   - Inhibit DNA polymerase
2. Direct Damage to DNA
   - Breakage of DNA chain
   - Cross-linkage, inhibit depolarization
3. Inhibit Transduction (DNA $\rightarrow$ RNA)
4. Spindle toxin $\rightarrow$ Mitosis damage
5. Inhibit topoisomerase
4.2.7.2. Various Facets of Cancer Chemotherapy

Various Facets of Cancer Chemotherapy

- **Chemotherapy**
  - Chemotherapy is the use of drugs to inhibit or kill proliferating cancer cells, while leaving host cells unharmed, or at least recoverable.

- **Growth fraction**
  - Tumor cells can be classified as proliferating cells and non-proliferating cells.
  - The ratio of proliferating cells in the whole tumor tissue is called growth fraction (GF).
  - The faster the tumor cells proliferate, the bigger the GF is and the higher the sensitivity of tumor to a drug is.
  - Generally, in the early stage, the GF of a tumor is bigger and the effect of a drug on the tumor is better.

- **Proliferating cells**: Based on the DNA changes in cells, proliferating cycle of tumor cells can be divided into 4 phases:

  - **Pre-synthetic phase (Gap 1 phase or G1 phase)**: cells chiefly make preparations for the synthesis of DNA.
  - **Synthetic phase (S phase)**: cells are synthesizing their DNA.
  - **Post-synthetic phase (Gap 2 phase or G2 phase)**: DNA duplication has been finished and they are equally divided to the two of future sub-cells.
  - **Mitosis phase (M Phase)**: each cell is divided into two subcells. Some of these new cells enter the new proliferating cycle, the others become non-proliferating cells.

- **Non-proliferating cells**
  - Non-proliferating cells include **G0 phase cells (resting-phase cells)**.
  - G0 phase cells have proliferation ability but do not divide temporally.
  - When proliferating cells are suffered heavy casualties, G0 phase cells will get into proliferating cycle and become the reasons of tumor recurrence.
  - G0 phase cells are usually not sensitive to antineoplastic drugs, which is the important obstacle to tumor chemotherapy.
4.2.7.3. Cancer Chemotherapy: Mechanisms of Antineoplastic Drugs

**Cancer Chemotherapy: Mechanisms of Antineoplastic Drugs**

Most antineoplastic drugs act on the proliferating cycle of cell

1. **Destruction of DNA or inhibition of DNA duplication**
   - e.g. alkylating agents, mitomycin C.

2. **Inhibition of nucleic acid (DNA and RNA) synthesis**
   - e.g. 5-fluorouracil, 6-mercaptopurine, methotrexate, cytarabine.

3. **Interfering with the transcription to inhibit RNA synthesis**
   - e.g. dactinomycin, dauvorin, and doxorubicin.

4. **Inhibition of protein synthesis**
   - e.g. vinca alkaloids, epipodophylotoxins, and paclitaxel.

5. **Interfering with hormone balance**
   - e.g. adrenal corticosteroids, estrogens, tamoxifen etc.
4.2.7.4. Toxicity of Antineoplastic Drugs and Their Classification

**Toxicity of Antineoplastic Drugs**

1. **Short-term Toxicity**
   - Common side reactions usually appear earlier and many of them occur in rapid proliferating tissues such as marrow, gastrointestinal tract, and hair follicle.
   - myelosuppression, gastrointestinal tract symptom
   - Alopecia

2. **Long-term toxicity**
   - The long-term toxicity mainly occurs in patients who received chemotherapy many years ago.
   - **Examples:** carcinogenesis, teratogenesis and sterility.

**Classification of Antineoplastic Drugs**

On the basis of antineoplastic action on the phase of proliferation cycle, drugs are classified as:

1. **cell cycle non-specific agents** (phase nonspecific agents, CCNSA) (e.g. alkylating agents)
   - Act in all proliferating phases, even the G0
   - Effects are stronger.

2. **cell cycle specific agents** (phase specific agents, CCSA). (e.g. Antimetabolites, vinca alkaloids)
   - Just act on specific phases of the cell cycle
   - Effects are comparatively weaker.

On the basis of source and action mechanisms, the drugs are also classified as:

1. **alkylating agents**
2. **antimetabolites**
3. **natural products**
4. **hormones and antagonists**
5. **miscellaneous agents**
4.2.7.4.1. Alkylating Agents as Antineoplastic Drugs

**Alkylating Agents**

- Alkylating agents act via a reactive alkyl (RCH2-CH2-) group that reacts to form covalent bonds with nucleic acids.
- There follows either cross-linking of the two strands of DNA, preventing replication, or DNA breakage.
  - All alkylating agents are phase-nonspecific.
  - Kill rapidly proliferating cells, also kill nonproliferating cells.
- **Examples of Alkylating Agents**
  - **Mechlorethamine**
    - The first drug used in the treatment of cancer
    - At present, it is mainly used for Hodgkin's disease and non-Hodgkin's lymphomas.
  - **Cyclophosphamide**
    - Most widely used in clinical therapy for treatment of cancer at present.
    - It has no antineoplastic action outside the body and must be activated in the liver.

**Nitrogen Mustard Alkylating Agents**

![Mechlorethamine](image1)

**Mechlorethamine**

![Cyclophosphamide](image2)

**Cyclophosphamide**
4.2.6.4.2. Antimetabolites as Antineoplastic Drugs

**Antimetabolites**

- Antimetabolites are analogues of normal metabolites and act by competition, replacing the natural metabolite and then subverting cellular processes.

- **Examples of antimetabolites include**
  
  - **Folic acid antagonists** (e.g. Methotrexate)
    - Mimics folic acid, which is needed for synthesis of DNA, RNA and some amino acids
    - It acts mainly on the S phase cells.
    - It has a serious myelosuppression.
  
  - **Antipyrimidines** (e.g. 5-Fluorouracil, Cytarabine).
    - **5-Fluorouracil** is a fluorine-substituted analogue of uracil
    - Must be metabolically activated to a nucleotide, in this case FdUMP.
    - Then its metabolite inhibits the synthetase of deoxythymidine monophosphate, blocking DNA synthesis. Besides, as the fraudulent substance, its metabolite can also interfere with the synthesis of RNA.
      - A phase-specific drug.
  
  - **Antipurines** (e.g. 6-Mercaptopurine)
    - A structural analogue of hypoxanthin
    - It must be converted intracellularly to the nucleotide 6-mercaptopurine ribose phosphate and 6-methylmercaptopurine ribonucleotide, and then inhibit purine biosynthesis, causing inhibition of biosynthesis of nucleic acid.

![Methotrexate](image)

![5-Fluorouracil](image)

![6-Mercaptopurine](image)
4.2.7.4.3. Natural Products as Antineoplastic Drugs

### Natural Products

- This group is determined by the source of the drug
- The major classes of natural products include
  - antibiotics
  - vinca alkaloids
  - biologic response modifiers
  - enzymes
  - Epipodophyllotoxins
  - Taxanes

- **Antibiotic antineoplastic agents:** Damage DNA in cycling and noncycling cells.
  - **Example: Dactinomycin (actinomycin D)**
    - This drug binds noncovalently to double-stranded
    - DNA and inhibits DNA-directed RNA synthesis.
    - Dactinomycin is a **phase-nonspecific agent**, but it is more active against G1 phase cells.

- **Vinca (plant) alkaloids**
  - Vincristine and vinblastine are alkaloids derived from the periwinkle plant.
  - Binding to tubulin, interfere with the assembly of spindle proteins during mitosis.
  - Act in M phase to inhibit mitosis, blocking proliferating cells as they enter metaphase.
  - Both can cause bone marrow suppression and neurotoxicity
4.2.7.4.4. Hormones and Antagonists and Miscellaneous Agents as Antineoplastic Drugs

**Hormones and Antagonists**

- The growth of some cancers is hormone dependent. Growth of such cancers can be inhibited by surgical removal of hormone glands, increasingly, however, administration of hormones or antihormones is preferred.

- **Examples:**
  - Adrenocortical steroids to inhibit the growth of cancers of lymphoid tissue and blood.
  - Oestrogen antagonists (tamoxifen) is indicated for breast cancer.
  - Oestrogen is used for prostatic cancers.

**Miscellaneous Agents**

- **Examples: Hydroxyurea**
- Hydroxyurea inhibits ribonucleotide reductase. Inhibition of DNA synthesis.
- It is specific for the cells of S phase
- The major adverse effect of this drug is bone marrow depression.
4.3. Cancer-Combination Therapies

4.3.1. Principles of Combination Therapies

1. In order to enhance curative effect, to decrease the toxicity and to reduce the drug resistance, combination therapies are often used in the treatment.

2. Advantages of drug combinations:
   - They provide maximal cell kill within the range of tolerated toxicity.
   - They are effective against a broader range of cell cycle phases.
   - They may slow or prevent the development of resistance.

4.3.2. Aspects of Combination Therapies

1. Select drugs according to their phase specific characteristics:
   - The aim of this rule is to urge more G0 phase cells to enter the proliferating cycle so as to increase the amount of tumor cells killed by drugs.
   - For high GF tumor such as acute leukemia, phase specific drugs are firstly used to kill S or M phase cells, and then phase non-specific drugs are used to kill tumor cells in other phases, and finally the above two steps are repeated once again to kill new cell from G0 phase.
   - For low GF tumor such as solid tumors, phase non-specific drugs are firstly used to kill cells of all phases, and then phase specific drugs are used, and finally the above steps are repeated to kill the new cell from G0 phases.

2. Combinations of antineoplastic drugs with different action mechanism
   - can destroy tumor cells from various biochemical links at same time.

3. Combinations of antineoplastic drugs with other therapies
   - Examples: chemotherapy plus operation,
   - chemotherapy plus radiotherapy.

4. Combination of low-toxic drugs with high toxic ones
   - does not obviously increase the toxicity of antineoplastic drugs while the remarkable synergism of anticancer action is produced.
   - Example: bleomycin (light myelosuppression) + mitomycin (serious myelosuppression), which is often used to treat carcinoma of cervix.

5. Select drugs according to antineoplastic range (spectrum)

6. Use right dose
4.4. Therapeutic Applications of Enediyne Antitumor Antibiotics

4.4.1. Introduction

The enediynes are a class of natural products with fascinating architectures produced by certain microorganisms. They possess potent antitumor and antimicrobial activities with spectacular biological profiles and proven clinical efficacy. Nearly 20 discrete enediynes have been discovered such as neocarzinostatin (NCS), calicheamicins (CAL), esperamicins (ESP), dynemicins (DYN), lidamycin (LDM), kedarcidin and so on.

Most of the enediyne antibiotics show rapid and strong activities against cancer cells. They have much higher antitumor activity relative to widely used chemotherapeutic drugs such as adriamycin. Many enediynes are in clinical trials. In vitro experiments show that the IC\textsubscript{50} of the enediyne antibiotics is ranging 1~100 pg/ml. In animal models, they also markedly inhibit the growth of several tumor cells.

The enediyne antibiotics share a common mechanistic path for DNA strand scission. Enediynes are biologically inactive but undergo cycloaromatization reactions which give rise to cytotoxic and highly reactive diyl radicals. These highly reactive radicals are capable of abstracting hydrogen atoms from the DNA backbond to trigger DNA damage at low concentration. In the case of calicheamicin, this involves a cascade of reactions resulting in the formation of a post activated diyl core. That diyl core abstracts hydrogen atoms from the DNA backbone. On interception by molecular oxygen, an intermediate peroxide is formed which ultimately leads to strand scission by generation of a 5'-aldehyde.
4.4.2. Mechanisms of Action of Naturally Occurring Enediynes

The ene-diynyl antibiotics are known to arrest formation of malignant tumors by binding the two strands of DNA together. By tying the two DNA strands together, ene-diynes prevent them from unraveling and thus arresting the replication process. One of the ene-diynes, Dynemicin A (Figure 2), has been particularly effective. As shown in Scheme 1, the ene-diynyl portion of this molecule closes to form a cyclic structure. This cyclic structure has two radical centers. These radical centers interact with DNA, forming labile centers, which then form a covalent bond across the two strands, rendering it unable to unravel or replicate. Mutated or cancerous cells replicate faster than normal cells, so ene-diynes will have more of an effect on the mutated DNA, helping to prevent the spread of tumorous tissue. The major problem, however, is that Dynemicin A, and other ene-diynes, also react with healthy DNA, stopping all replication processes. As a result, these potential antibiotics are presently too toxic for widespread use in cancer therapy.

![Dynemicin A](image)

The enediyne antitumor antibiotics contain either DNA intercalating groups (such as DYN) or DNA minor groove binding function (such as CAL and NCS). The biological actions of these molecules are a result of three important functional domains. Each molecule contains an assemblage that consists of (a) an enediyne moiety; (b) a delivery system that communicates the enediyne moiety to its DNA target; and (c) a triggering device that, when activated, initiates the cascade of reactions that leads to generation of the reactive chemical species.

Thus, the Common modes of action of enediyne class of natural antibiotics:

- **intercalation into minor groove**
- **reaction (activation) with either a thiol of NADPH - generates radical**
- **radical cleavage of DNA**

The enediyne antibiotics share a common mechanism for producing radical cleavage of DNA: First, the enediynes undergo cycloaromatization reactions resulting in formation of highly reactive diradical intermediates. Second, these highly reactive radicals are capable of abstracting hydrogen atoms from the DNA backbond to trigger DNA damage. The key transformation of 3-ene-1,5-diyynes is a thermal rearrangement that was disclosed in the early 1970 by Masamune and Bergman that is commonly known as Bergman cyclization. The classical Bergman reaction is
believed to precede through a reactive diradical benzenoid species (a p-benzyne) which cleaves the DNA by abstracting H-atom from sugar-phosphate backbone of DNA.

Scheme 1. Mechanism of action of enediyne anticancer antibiotics: DNA cleavage initiated by C4'- or C5'-hydrogen atom abstraction.
Scheme 2. Mechanism of action of enediyne anticancer antibiotics: DNA cleavage initiated by (a) C4' or (b) C5' hydrogen atom abstraction.
Less than 20% of the strand breaks result from hydrogen atom abstraction at C4′- (Scheme 2) and C1′− (Scheme 3). NCS chromophore effects primarily single-stranded DNA cuts by the C(6) radical at C5′− (Scheme 2) of deoxyribose, whereas those double stranded lesions which are observed involve additional hydrogen abstraction by the C2- radical from C1′- or C4′- of the deoxyribose on the complementary strand.

Scheme 3. Mechanism of action of enediyne anticancer antibiotics: DNA cleavage initiated by C1′- hydrogen atom abstraction.
Below is an example of DNA cleavage shown by natural enediyne Calicheamicin (Scheme 4).

4.4.3. Site Specific Binding of Enediynes to DNA

It is known that enediynes cleave double strand DNA, causing both single and double strand cuts. The enediynes bind to the minor groove of double helical DNA at specific sites. The predominantly sequences of sites are:

(a) for Calichiamicin (CAL) at 5'-TCCT-3', 5'-TTGT-3' and 5'-ATCT-3';
(b) for Neocarzinostatin chromophore (NCS) at 5'-GGAGCGC-3'
(c) for Dynamicin (DYN) at 5'-CTACTACTGG-3', 5'-AG-3', 5'-AT-3', and 5'-GC-3'
(d) for kedarcidin at 5'-TCCT-3'
(e) for N1999A2 at 5'-GGT-3'
(f) for Esperamicin (ESP) at 5'-CTC-3', 5'-TTC-3', and 5'-TTT-3'
(g) for Lidamycin (LDM) at 5'-CTTTT-3', 5'-ATAAT-3', 5'-CTTTA-3', 5'-CTCTT-3', and especially 5'-GTTAT-3'.

![Mechanism of DNA Cleavage by Calicheamicins](image)
The studies suggest 4'-H atom abstraction along with 5'-H abstraction from the targeted DNA deoxyribose sugars (Scheme 2).

1. In the case of CAL, the diradical abstracts hydrogen atoms from duplex DNA at the C-5' position of the cytidine and the C-4' position of the three nucleotide base pairs removed on the 3'-side of the complementary strand, leading to cleavage of both strands of DNA.
2. NCS is converted into a diradical that attacks the C-5' position of the deoxyribose of mainly thymidylate residues in DNA.
3. In the ESP-mediated DNA degradation, thymidylate and deoxycytidylate residues at the C-4' and/or C-5' position are preferred cleavage sites.
4. CAL, ESP and DYN have potent DNA cleavage activity in the presence of thiol compounds and the characteristic of DNA cleavage is induction by NADPH.
5. NCS requires sulphydryl activation for the activity which results in lower selectivity and cytotoxic activity.
6. The LDM diradical also abstracts hydrogen atoms at the C4' and C5' position of the adenine residue on the opposite strand, inducing double-strand break.
7. LDM has higher DNA cleaving ability compared with other enediyne compounds such as NCS, ESP, CAL and kedarcidin. Even in the absence of thiols or reductants, LDM still induces high DNA breakage. LDM induces novel DNA interstrand cross-links and drug monoadducts under anaerobic conditions, which is similar to that in the center regions of large tumors.

Table 2: *In vitro* test results for anticancer activities of enediyne anticancer antibiotics

<table>
<thead>
<tr>
<th>Agent</th>
<th>Producing Strain</th>
<th>In vitro IC$_{50}$ (nM)</th>
<th>In Vivo ID$_{50}$ (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neocarzinostatin (NCS)</td>
<td><em>Streptomyces carzinostaticus</em></td>
<td>225-900</td>
<td>380</td>
</tr>
<tr>
<td>Lidamycin (LDM)</td>
<td><em>Streptomyces globisporus C-1027</em></td>
<td>0.01-0.5</td>
<td>0.25-0.5</td>
</tr>
<tr>
<td>Kedarcidin</td>
<td><em>Actinomycete strain L585-6</em></td>
<td>1</td>
<td>2-3.3</td>
</tr>
<tr>
<td>Calicheamicins (CAL)</td>
<td><em>Micromonospora echinospora ssp</em></td>
<td>6-9</td>
<td>0.5-1.5</td>
</tr>
<tr>
<td>Esperamicins (ESP)</td>
<td><em>Actinomadura verrucospora</em></td>
<td>0.3-8.3</td>
<td>0.1-0.2</td>
</tr>
<tr>
<td>Dynemicins (DYN)</td>
<td><em>Micromonospora chersina M956-1</em></td>
<td>0.9-10</td>
<td>30-60</td>
</tr>
</tbody>
</table>

IC$_{50}$ = half-inhibiting concentration; ID$_{50}$, half-inhibiting dosage.
4.5. Enediyne-Induced Cell Cycle Arrest

The DNA damage caused by enediyne leads to the initiation of cellular recovery mechanisms that involve activation of DNA damage response pathways, cell cycle arrest and apoptosis.

Calichlamicin (CAL) induced double-strand DNA cleavages are repaired slowly but completely. This process results in high levels of H2AX phosphorylation and efficient cell cycle arrest. Investigation of the mode of action of CAL in living cells, revealed that upon longer CAL exposure, genes involved in chromatin arrangement, DNA repair and/or oxidative damage, DNA synthesis and cell cycle checkpoint control as well as other nuclear proteins expression.

LDM-induced cell cycle arrests are associated with the status of the tumor suppressor gene p53. It is known that LDM at low concentrations induce G1 arrest in p53 wild-type MCF-7 cells. However at high concentration LDM caused both G1 and G2/M arrests with an increase of p53 and p21, and a decrease of phosphorylated retinoblastoma protein, Cdk1 and cyclin B1 protein levels. LDM also induced G2 arrest in p53-mutant human colon carcinoma HT-29 cells. LDM induced G2 arrest Lead to cytoplasmic localization of cyclin B1.

Treatment with Neocarzinostatin (NCS) was found to inhibit cellular proliferation through G2 cell cycle arrest. NCS also causes apoptosis induction in cervical cancer HeLa, INBL, CaSki and C33A cell lines. The mechanism of NCS-induced G2 block is closely related to X-ray-induced G2 block. Caffeine act as a stimulator of the recovery of HeLa-S3 cells from the arrested G2 cell cycle by NCS. NCS-induced inhibition of DNA synthesis and mitosis was found to be markedly reduced when caffeine was coadministered with NCS.

4.6. Enediyne-Induced Cell Death

After DNA damage, cells can be arrested at some phase of the cell cycle to facilitate DNA repair or induced apoptosis (Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms). DNA damage is a critical event preceding cellular apoptosis and appears to signal cell death through the mitochondria. The ability of the enediyynes to induce apoptosis is mostly related to their ability to make double-strand cuts in DNA. The simple enediyne models are less effective that natural enediynes because of the lack of DNA binding appendages like oligosaccharides/peripheral decoration with aromatic units in natural enediynes.

It was reported that CAL-induced apoptosis is independent of the death-receptor/ FADD-mediated signals. CAL triggered apoptosis in a p53-independent manner. The cell death occurs via activation of mitochondria, release of cytochrome c and activation of caspase-9 and -3.

Human promyelocytic leukemia HL-60 cells to undergo apoptosis with morphological changes, condensation of nuclear chromatin, and a typical ladder pattern of DNA fragments upon exposure with LDM. LDM-induced apoptosis is more effective in human colorectal cancer cells with wild type p53 than those with mutant or deleted p53. LDM in low dose causes blocking of apoptosis through the inhibition of the mitochondrial pathway. However, in high dose LDM causes rapid apoptosis through direct DNA damaging mechanism and the process is independent
of activation of p53 and caspase. In this case the apoptosis is not be blocked by a caspase inhibitor.

Neocarzinostatin (NCS) induces apoptosis in MCF-7 cells. This is characterized by decreased Bcl-2 and increased Bax levels that induce the release of cytochrome c from the mitochondria, and then activates caspase 9. Activation of caspase 9 induces sequential activation of caspase 7 and caspase 6 leading to apoptosis.

4.7. The Approved Enediyynes for Use as Anticancer Drugs

4.7.1. Enediyne Derivative and Conjugate in Clinical Use

<table>
<thead>
<tr>
<th>Enediyne Derivative</th>
<th>Approved</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMANCS</td>
<td>Japanese Government in 1993</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Mylotarg (gemtuzumab ozogamicin)</td>
<td>FDA in 2000 but withdrawn in 2010.</td>
<td>Acute myeloid leukemia</td>
</tr>
</tbody>
</table>

4.7.2. Neocarzinostatin (NCS)

NCS is the first enediyne antitumor antibiotic that is in clinical use for the treatments of leukemia, gastric carcinoma and pancreatic adenocarcinoma (Figure 3). In clinical studies NCS is shown to be active against acute leukemia. Studies revealed that NCS give remedy of 9 out of 51 patients completely and of 9 a partial remedy. NCS also is found to effective against hepatoma and hematologic malignancies.

Both continuous and intermittent intravenous infusion was the process of NCS administration to patients with a variety of malignant diseases. Leukemic patients on intermittent therapy showed greater changes in bone marrow cellularity than those treated by continuous infusion.
Anorexia, nausea, and vomiting were the most frequent side effects of NCS administration. In phase I and phase II evaluations, dose limiting toxicity was late myelosuppression. Myelosuppression was more pronounced in patients who had received previous chemotherapy. Gastrointestinal side effects were mild. Three patients had a severe acute reaction resembling anaphylaxis. Allergic reactions were more frequent with intermittent than with continuous infusions. Therefore, the clinical trials of NCS were obstructed by anaphylactic responses.

4.7.2.1. Polymer-Neocarzinostatin Conjugate (SMA-NCS)

NCS is actually a prodrug that requires sulfhydryl activation for the activity (Figure 3), which results in lower selectivity and cytotoxic activity. However, initial clinical trials were hindered by anaphylactic responses due to its non-covalently bound protein component. It was observed that if NCS could be made immunologically inert then this allergic action can be removed. This was exactly done by coupling to a maleic acid-based polymer (SMANCS, Figure 4). The SMANCS is thus clinically used primarily in the treatment of hepatocellular carcinoma. Hepatocellular carcinoma (HCC, also called malignant hepatoma) is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of hepatic cirrhosis).

A principal advantage in the use of SMANCS is the tumor targeting mechanism through an enhanced permeability and retention effect. Use of SMANCS has resulted in potential reduction or elimination of toxicity as is generally accounted in bone marrow toxicity associated with the use of NCS.

![Figure 4. The SMA-NCS conjugate.](image)

SMANCS was used for the treatment of hepatoma, gastric carcinoma and lung cancer. This was approved in Japan. Since then, hepatic arterial infusion of a SMANCS/Lipiodol emulsion has been used as one of the practical treatments for advanced or recurrent hepatocellular carcinoma.

**Phase I Clinical Evaluation:** In Phase I, SMANCS was found to decrease the concentrations of α-fetoprotein in 86% of patients and decrease the tumour size in 95% patients.

**Phase II Clinical Evaluation:** Phase II clinical study with primary hepatoma also showed a relatively high response rate of 36~40%.
The most successful use of SMANCS was seen when it was administered to an individual patient. In this treatment the SMANCS was administered as dose per tumour size and follow-up treatments were given on a need basis.

Arterial infusion therapy with SMANCS/Lipiodol was effective for large renal cell carcinoma without metastases in conjunction with surgery. In surgical patients without metastases administration of SMANCS/Lipiodol infusion has lead to the 5- and 10-year survival rates of 83.0% and 75.2%, respectively.

4.7.3. Mylotarg

4.7.3.1. Introduction

Because of lack of tumor-cell specificity and extreme cytotoxicity, the direct application of the enediyne antibiotics as antitumor drugs is generally limited. It is that need which drew attention of scientists to generate modified enediyne compounds with improved specificity and pharmacological properties.

Mylotarg (CMA-676, gemtuzumab ozogamicin), the combination of Calicheamicin (CAL) with an antibody that binds to CD33 antigen, was approved by the FDA in 2000 to treat acute myeloid leukemia (AML), a bone marrow cancer. It is the first in a new class of antibody-targeted chemotherapy for the treatment of patients 60 years and older in first relapse with CD33-positive acute myeloid leukemia (AML) (Figure 5).

Mylotarg ® composed of a recombinant humanized IgG4, kappa antibody conjugated with a cytotoxic enediyne antitumor antibiotic, calicheamicin. The antibody portion of Mylotarg binds specifically to the CD33 antigen. CD33 antigen is a sialic acid-dependent adhesion protein that is found on the surface of leukemic blasts and immature normal cells of myelomonocytic lineage. But it is not present in normal hematopoietic stem cells.

The anti-CD33 hP67.6 antibody is produced by mammalian cell suspension culture using a myeloma NS0 cell line. This is then purified to remove or inactivate viruses. Three separate and independent steps are involved in retrovirus inactivation and removal (in the purification) process and thus to get purified hP67.6 antibody. These steps include- (a) low pH treatment, (b) DEAE-Sepharose chromatography, and (c) viral filtration.
Mylotarg contains amino acid sequences of which approximately 98.3% are of human origin. The constant region and framework regions contain human sequences while the complementarity-determining regions are derived from a murine antibody (p67.6) that binds CD33. This antibody is linked to N-acetyl-γ-calicheamicin via a bifunctional linker. Gemtuzumab ozogamicin has approximately 50% of the antibody loaded with 4-6 moles calicheamicin per mole of antibody. The remaining 50% of the antibody is not linked to the calicheamicin derivative. Gemtuzumab ozogamicin has a molecular weight of 151 to 153 kDa.

Mylotarg is a sterile, white, preservative-free lyophilized powder containing 5 mg of drug conjugate. The drug is light sensitive and must be protected from direct and indirect sunlight and unshielded fluorescent light during the preparation and administration of the infusion. The inactive ingredients are: dextran 40; sucrose; sodium chloride; monobasic and dibasic sodium phosphate.

Gemtuzumab interferes with the growth of cancer cells and slows their growth and spread in the body. Gemtuzumab is usually given to people who are at least 60 years old and have a relapse of their disease and who cannot receive other cancer medications.
4.7.3.2. Mechanism of Action of Mylotarg

Gemtuzumab ozogamicin binds to the CD33 antigen. This antigen is expressed on the surface of leukemic blasts in more than 80% of patients with acute myeloid leukemia (AML). CD33 is also expressed on normal and leukemic myeloid colony-forming cells, including leukemic clonogenic precursors. But it is not expressed on pluripotent hematopoietic stem cells or on nonhematopoietic cells.

Mylotarg (Gemtuzumab ozogamicin) is first directed against the CD33 antigen expressed by hematopoietic cells. Then the anti-CD33 antibody portion of Mylotarg binds with the CD33 antigen. After infusion, near complete saturation of CD33 antigenic sites by Mylotarg was reached for AML blasts, monocytes, and granulocytes, whereas Mylotarg did not bind to lymphocytes. This binding event results in the formation of a complex that is internalized. Upon internalization, the calicheamicin derivative of Mylotarg is released inside the lysosomes of the myeloid cell. As soon as the enediyne anticancer antibiotic calicheamicin is released, it migrates to the cell nucleus and binds to the minor groove of leukemic DNA. Then the calicheamicin cleaves DNA double strand that ultimately lead to cell death.

![Mechanism of Action of Mylotarg](image-url)
4.7.3.3. Clinical Pharmacology of Mylotarg

The patients treated with Mylotarg exhibited far less toxicity than patients who received standard chemotherapy. In contrast to standard chemotherapy, Mylotarg therapy did not result in hair loss, severe oral mucositis, or damage to the intestinal mucosa. Gemtuzumab ozogamicin is cytotoxic to the CD33 positive HL-60 human leukemia cell line. Gemtuzumab ozogamicin produces significant inhibition of colony formation in cultures of adult leukemic bone marrow cells. In preclinical animal studies, gemtuzumab ozogamicin demonstrates antitumor effects in the HL-60 human promyelocytic leukemia xenograft tumor in athymic mice.

4.7.3.4. Human Pharmacokinetics of Mylotarg

After administration of the first recommended (9 mg/m²) dose of gemtuzumab ozogamicin, the elimination half lives of total and unconjugated calicheamicin were found to about 41 and 143 hours, respectively, in 2 hour infusion. After the second dose of 9 mg/m² the half life of total calicheamicin was found to increase to about 64 hours when the area under the concentration-time curve (AUC) was about twice that in the first dose period. The AUC for the unconjugated calicheamicin increased 30% after the second dose. Age, gender, body surface area (BSA), and weight did not affect the pharmacokinetics of Mylotarg.

There is no evidence that reducing Mylotarg dose will reduce the underlying risk of veno-occlusive disease (VOD). Metabolic studies indicated that the calicheamicin derivative is released hydrolytically from gemtuzumab ozogamicin. Many metabolites of this derivative were found in human liver microsomes and cytosol, and in HL-60 promyelocytic leukemia cells.

4.7.3.5. Possible side effects of gemtuzumab (Mylotarg)

Administration of gemtuzumab injection into the vein produces side reaction which again varies from patient to patient.

Following are the serious side effect may come during the course of treating with gemtuzumab:

- pain in your upper right stomach, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes);
- swelling, rapid weight gain;
- feeling like you might pass out;
- pale skin, easy bruising or bleeding (such as nosebleeds), purple or red pinpoint spots under your skin;
- fever, chills, body aches, unusual weakness, flu symptoms;
- white patches or sores inside your mouth or on your lips;
- chest pain or tightness, feeling short of breath;
- lower back pain, blood in your urine;
- increased thirst, fruity breath odor, increased urination;
- urinating less than usual or not at all;
- numbness or tingly feeling around your mouth;
• muscle weakness, tightness, or contraction, overactive reflexes;
• fast or slow heart rate, weak pulse; or
• confusion, uneven heart rate, leg discomfort, muscle weakness or limp feeling.

Less serious side effects of administration of Mylotarg are:

• nausea, vomiting;
• diarrhea or constipation;
• headache;
• dizziness, anxiety, depressed mood; or
• sleep problems (insomnia).

4.7.3.6. Withdrawal of Mylotarg (gemtuzumab ozogamicin)

FDA notified healthcare professionals that results from a recent clinical trial raised new concerns about the product’s safety, and the drug failed to demonstrate clinical benefit to patients enrolled in trials.

Mylotarg (gemtuzumab ozogamicin) was approved in May 2000 under the FDA’s accelerated approval program for treatment of acute myeloid leukemia (AML), a bone marrow cancer. A post approval clinical trial was begun by Wyeth (now Pfizer) in 2004. The trial was designed to know whether adding Mylotarg to standard chemotherapy demonstrated an improvement in clinical benefit i.e. survival time to acute myeloid leukemia patients. The trial was stopped early when no improvement in clinical benefit was observed. It was also found that a greater number of deaths occurred in the group of patients who received Mylotarg compared with those receiving chemotherapy alone. Therefore, it is now recommended that Mylotarg will not be commercially available to new patients.
4.8. Enediynes Under Clinical Investigation

4.8.1. Lidamycin Chromophore (LDM)

Lidamycin strongly inhibits DNA and RNA synthesis (Figure 7). It shows extremely potent cytotoxicity toward various cancer cells. The IC\textsubscript{50} value of LDM indicated its 1000-fold more potency than that of mitomycin C and adriamycin in human cancer cells. LDM exhibits inhibition of transplantable tumors in mice, such as leukemia L1210, P388, ascites hepatoma H22, sarcoma 180 and melanoma Harding-Passey. The inhibition rate of tumor growth by LDM is found to be higher than that of mitomycin C at the tolerated dose.

Figure 7. Structure of Lidamycin chromophore.

LDM is highly potent in suppressing angiogenesis at a minimum effective dose. LDM blocked bFGF binding to its receptor, and inhibited the formation of the bFGF-receptor immune complex. With such potent activity LDM may be a new potent antiangiogenesis agent with markedly anti-metastatic activity. LDM also shows synergetic effects in combination with cisplatin on the proliferation of BEL-7402 cells, and produced internucleosomal DNA fragmentation with a decrease of Bcl-2 expression. LDM was also found to show enhanced apoptosis and cytotoxicity with amplification of DNA cleavage when administered with Distamycin A. Therefore, a combination of LDM with an anticancer agent, DNA-binding ligand and MAPK inhibitor represents a new strategy for cancer chemotherapy.
4.8.2. The Esperamicins

The esperamicins are among the most powerful antitumor agents known (Figure 8.). They exhibit a forceful activity against a number of murine tumor models. Despite the number of side effects, esperamicin A1 has successfully passed Phase I and is now in Phase II clinical trials.

![Esperamicin A1](image)

Figure 8. Structure of Esperamicin A1.

4.8.3. Dynemicin A

Dynemicin A is currently in Phase III clinical trials. Prodrugs of dynemicin A are much more interesting than dynemicin A alone, because of the possibility to activate them by aldolase antibody 38C2 specifically inside tumor cells (Figure 9).

![Dynemicin A](image)

Figure 9. Structure of Dynemicin.
4.9. Immunoconjugates

**Immunoconjugates**: Immunoconjugates are antibodies conjugated to a biologically active molecule, usually a toxin, radioisotope or label used in immunotherapy. These are used to develop monoclonal antibody therapy as a targeted form of chemotherapy. Thus they are often known as antibody-drug conjugates (Figure 10).

![Figure 10. Schematic presentation of immunoconjugates.](image)

**Antibody**: An *antibody* (Ab), also known as an *immunoglobulin* (Ig), is a large Y-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. The antibody recognizes a unique part of the foreign target, called an antigen (For details see Bioorganic Chemistry Course).

![Figure 11. The structure of monoclonal antibody (mAb).](image)
**Toxin:** A toxin is a poisonous substance produced within living cells or organisms; man-made substances created by artificial processes are not toxins. Toxins can be small molecules, peptides, or proteins that are capable of causing disease on contact with or absorption by body tissues interacting with biological macromolecules such as enzymes or cellular receptors. Toxins vary greatly in their severity, ranging from usually minor and acute to almost immediately deadly. Immuno-toxins constitute highly cytotoxic agents that are conjugated to mAbs and tested for antitumour therapy efficacy.

In chemotherapy low-molecular-mass chemical substances are also used. However, toxins that are used in chemotherapy are generally the enzymes. The toxins exert their cytotoxic activity inside the cell. In most cases one single molecule in the appropriate intracellular compartment is sufficient to kill the cell. Toxins are generally modified to remove their binding sites for targets expressed in normal tissue for their use in anticancer therapy. Moreover, toxins are undergone for deglycosylation to avoid rapid clearance by liver cells expressing mannose receptors. Toxins can be targeted to the tumour cells by conjugation to targeting moieties, such as monoclonal antibodies (mAbs; called immunotoxins) or growth factors, cytokines and peptide hormones (called fusion protein toxins). For therapeutic efficacy, the toxin-conjugates have to be internalized into the cell upon binding to their respective ligands. The cell-killing potency of immunotoxins depends on several biochemical properties, such as antigen-binding affinity, internalization rate, intracellular processing and intrinsic toxin-domain potency.

**Immunotherapy:** Immunotherapy is a medical term defined as the "treatment of disease by inducing, enhancing, or suppressing an immune response". Immunotherapies designed to elicit or amplify an immune response are classified as **activation immunotherapies**, while immunotherapies that reduce or suppress are classified as **suppression immunotherapies**.

**Cancer immunotherapy:** Cancer immunotherapy is the use of the immune system to reject cancer. The main premise is stimulating the patient's immune system to attack the malignant tumor cells that are responsible for the disease. This can be either through immunization of the patient (e.g., by administering a cancer vaccine, such as Dendreon's Provenge), in which case the patient's own immune system is trained to recognize tumor cells as targets to be destroyed, or through the administration of therapeutic antibodies as drugs, in which case the patient's immune system is recruited to destroy tumor cells by the therapeutic antibodies. Cell based immunotherapy is another major entity of cancer immunotherapy. This involves immune cells such as the Natural killer Cells (NK cells), Lymphokine Activated killer cell(LAK), Cytotoxic T Lymphocytes(CTLs), Dendritic Cells (DC), etc., which are either activated in vivo by administering certain cytokines such as Interleukins or they are isolated, enriched and transfused to the patient to fight against cancer.

Since the immune system responds to the environmental factors it encounters on the basis of discrimination between self and non-self, many kinds of tumor cells that arise as a result of the onset of cancer are more or less tolerated by the patient's own immune system since the tumor cells are essentially the patient's own cells that are growing, dividing and spreading without proper regulatory control.
In spite of this fact, however, many kinds of tumor cells display unusual antigens that are either inappropriate for the cell type and/or its environment, or are only normally present during the organisms' development (e.g. fetal antigens). Examples of such antigens include the glycosphingolipid GD2, a disialoganglioside that is normally only expressed at a significant level on the outer surface membranes of neuronal cells, where its exposure to the immune system is limited by the blood–brain barrier. GD2 is expressed on the surfaces of a wide range of tumor cells including neuroblastoma, medulloblastomas, astrocytomas, melanomas, small-cell lung cancer, osteosarcomas and other soft tissue sarcomas. GD2 is thus a convenient tumor-specific target for immunotherapies.

Other kinds of tumor cells display cell surface receptors that are rare or absent on the surfaces of healthy cells, and which are responsible for activating cellular signal transduction pathways that cause the unregulated growth and division of the tumor cell. Examples include ErbB2, a constitutively active cell surface receptor that is produced at abnormally high levels on the surface of breast cancer tumor cells.

The use of some agents can lead to the re-activation of latent tuberculosis (TB) and this must be assessed for before those agents are used therapeutically.
4.10. Antibody–Drug Conjugates

4.10.1. Origins of Monoclonal Antibody Therapy

Traditional chemotherapy is the pillar for cancer treatment. However, cytotoxic agents are not tumour specific. The rapidly proliferating cells are more prone to the cytotoxic effect of these drugs. Therefore, increased toxicities of such chemotherapeutic cytotoxic agents against normal tissues are major drawbacks of Traditional chemotherapy of cancer. As a consequence, anticancer chemotherapeutics are often given at suboptimal doses.

Therefore, searching for an alternative tumor specific agents to kill tumor cell gave rise to the concept of immunotherapy which takes advantages of the immune systems. The immune system responds to the environmental factors it encounters on the basis of discrimination between self and non-self. Tumor cells are not specifically targeted by one's immune system since tumor cells are the patient's own cells. Tumor cells, however are highly abnormal, and many display unusual antigens that are either inappropriate for the cell type, its environment, or are only normally present such as fetal antigens during the organisms' development.

Other tumor cells display cell surface receptors that are rare or absent on the surfaces of healthy cells, and which are responsible for activating cellular signal transduction pathways that cause the unregulated growth and division of the tumor cell. Examples include ErbB2, a constitutively active cell surface receptor that is produced at abnormally high levels on the surface of approximately 30% of breast cancer tumor cells. Such breast cancer is known as HER2 positive breast cancer.

Immunotherapy developed as a technique with the discovery of the structure of antibodies and the development of hybridoma technology, which provided the first reliable source of monoclonal antibodies (mAb) which are a key component of the adaptive immune response and playing a central role in both in the recognition of foreign antigens and the stimulation of an immune response to them. The advent of monoclonal antibody technology has made it possible to raise antibodies against specific antigens presented on the surfaces of tumors, thereby allowing specific targeting of tumors both in vitro and in vivo. Initial research on malignant neoplasms found mAb therapy of limited and generally short-lived success with malignancies of the blood. Furthermore treatment had to be specifically tailored to each individual patient, thus proving to be impracticable for the routine clinical setting.

Throughout the progression of therapeutic monoclonal antibody drug development there have been four major antibody types developed: murine, chimeric, humanised and human. Initial therapeutic antibodies were simple murine analogues, which contributed to the early lack of success. It has since been shown that these antibodies have: a short half-life in vivo (due to immune complex formation), limited penetration into tumour sites, and that they inadequately recruit host effector functions. To overcome these difficulties the technical issues initially experienced had to be surpassed. Chimeric and humanized antibodies have generally replaced murine antibodies in modern therapeutic antibody applications. Hybridoma technology has been replaced by recombinant DNA technology, transgenic mice and phage display.
In the past few years, more than 30 immunoglobulins (IgGs) and their derivatives have been approved for use in various disease indications. Many antibody-based drugs have been approved and marketed to treat diseases such as cancer and inflammatory diseases that are affecting large numbers of patients. They are also being used for more specialized indications owing to special regulatory procedures for rare medical conditions like paroxysmal nocturnal haemoglobinuria. Currently, 26 antibodies are in Phase III clinical trials (35%) and 9 out of them have got orphan drug designation.

A detailed knowledge of antibody structure and activity allows researchers to engineer them on a more rational way for improving their *pharmaceutical properties, target specificity, stability, and functional potency. Therefore, many* clinically useful unconjugated monoclonal antibodies (mAbs) have been developed which can selectively recognize antigens that are preferentially expressed on or near tumor cells. These antibodies exert their cytotoxic effects through mechanisms such as cell signaling, antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis, and complement-dependent cytotoxicity. However, the majority of these mAbs are used in combination with chemotherapy, and many others have demonstrated insufficient clinical activity. Therefore, significant efforts have been put forth to gear up mAbs through various modifications towards enhancing their functional diversity, ability to kill tumor cell with high potency and target specificity. One approach by which the activities of these mAbs have been enhanced is through conjugation with cytotoxic drugs, generating antibody-drug conjugates (ADCs) capable of antigen-specific delivery of highly potent cytotoxic drugs to tumor cells.

In summary, anti-neoplastic drugs like doxorubicin, daunomycin, vinca-alkaloids, and taxoids etc. have demonstrated their ability to kill cancer cells but generally with limited selectivity and high toxic effects on normal cells yielding marginal therapeutic indices. On the other hand, approved naked antibody drugs like rituximab, trastuzumab, cetuximab, bevacizumab, panitumumab, alemutuzumab, and ofatumumab have demonstrated their therapeutic utility in malignancies. However it is observed that these mAbs often show significant clinical efficacy when linked with small cytotoxic drugs. Therefore, covalent conjugation of mAbs and drugs with synthetic chemical linkers to create antibody-drug conjugates is though an older concept (In the 1960s ADCs was used in animal models and in the 1980s, clinical trials with murine IgG-based ADCs were conducted) but now a day widely used for treating tumor.
4.10.2. Defining Antibody-Drug Conjugates: ADC Technology

Antibody Drug Conjugates (ADCs) are therapeutic agents designed to target the delivery of a cytotoxic drug specifically to tumor cells (Figure 12). In ADCs cytotoxic agents are linked to monoclonal antibodies that bind to tumor cell-specific antigens or to antigens that are overexpressed on the surface of tumor cells. The antibody acts as a sort of GPS system and it increases delivery of potent cell-killing drugs to the tumor cell and reduces the exposure of normal cells.

![Schematic drawing of antibody-drug conjugates.](Image)

4.10.3. Elements of ADCs

ADCs are made up of three components:

(a) a monoclonal antibody: An important factor in the successful development of ADCs is the selection of well-characterized antigens to serve as the target for the antibody. The full expression pattern of the antigen throughout the body and on both healthy and tumor cells must be taken into consideration in order to avoid unwanted toxic side effects. Likewise, the expression of tumor-specific antigens within the tumor itself may be heterogeneous, with some cells displaying the antigen and others not, and this can affect the efficacy of the ADC (Figure 13).

Generally, B- and T-cell surface proteins are frequently chosen as target antigens since they are widely expressed on the surface of malignant B and T cells in these types of cancer.

Targets include B-cell surface proteins such as CD20, CD22, CD40, and CD79, and T-cell surface proteins such as CD25 and CD30, as well as proteins that are overexpressed on carcinoma cells, including the human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), prostate-specific membrane antigen (PSMA), and Cryptic family protein 1B (Cripto).
(b) a cytotoxic drug: Three main types of drugs are used as the cytotoxic component of ADCs: calicheamicin, maytansinoids, and auristatins. They fall into two classes, according to the mechanism via which they lead to cell death, with the two latter types causing the unraveling of structural fibers in the cell, and calicheamycin causing irreparable DNA damage. In order to maximize killing potential, the cytotoxic agent needs to be highly potent. Using highly toxic drugs that are more potent than standard chemotherapy agents ensures that what little gets into the cell has the maximum effect (Figure 13).

(c) a linker: The linker joins the antibody and drug together. The linker region is extremely important to ADC design, as a delicate balance must be struck between stability in the bloodstream (to ensure the toxin is not released prematurely) and efficient release inside the cell once it entered.

Cleavable or releasable linkers that are broken down inside the cell are chosen to allow drug release inside the cell. (i) Initially, hydrazone linkers (-C=N-NH-) which are broken down only in the acidic environment of the cell were used. However, these often suffer from poor stability in the blood. Thus, the antibody falls off and the ADC fails to strike multiple targets around the body. Hence poor stability of linker renders the ADC ineffective or causes toxic side effects (Figure 13).

(ii) A range of linker that improves the stability of the ADC, while still allowing efficient drug release in the tumor cell has been development. These include disulfide-based linkers (-S-S-) that are selectively broken down inside the tumor cell where the concentration of thiols is higher than in the blood and peptide linkers (-NH-CO-) that are
broken down by enzymes found inside the tumor cell.

(iii) Non-cleavable linkers like thioether (-S-) linkers are being used now a days after the discovery that the cleavage of linker is not necessary in order for the drug to become activated. It is now discovered that the linker can be degraded by the cellular protein degradation machinery, leading to drug activation.

(iv) Another linker type that was recently described is the maleimidyl-based hydrophilic linker (PEG4Mal linker), which is designed to target drug-resistant tumor cells as it cannot be pumped through multidrug resistance channels on the cell surface.

To make an effective ADC the following key factors must be kept in mind:

- A target antigen, which is cancer specific
- A monoclonal antibody, diabody or ScV fragment that displays a high binding affinity to the antigen
- A cytotoxic agent that is highly potent
- A linker designed to allow ADCs to remain inactive when in the blood

4.10.4. How the Antibody-Drug Conjugate Works?

The antibody guides the ADC to target tumor cells, where it binds to cell surface antigens. The ADC is then (internalized) taken into the cancer cell and the active drug is released into the target cancer cell and perform its cell-killing function.

- An ADC comprises of an antibody linked to a cytotoxic drug via a linker (2–8 drugs).
- The ADC is delivered intravenously and localizes to target tumour cells by binding to tumour specific antigens.
- Internalization of the ADC.
- Proteases and hydrolases digest the antibody and linker to release free drug.
- The drug then binds to its molecular target, leading to apoptosis.

The following steps are involved in the generalized mechanism of action of antibody-drug conjugates (ADCs) (Figure 14):

(a) The ADC binds to the antigen (which is found on the surface of cells but is over-expressed in cancerous cells) on the target cell.
(b) The antigen-ADC complex is internalized through receptor-mediated endocytosis and, in most cases, is transported from early endosomes to lysosomes.
(c) In the lysosome, internal conditions may destabilize the linker or mAb backbone, causing the cytotoxic component to dissociate. Sometimes, the linker can be cleaved by enzymes called cathepsins, which are active at low pH, only available in the lysosome of the cell. This ensures that the drug is released inside the cancerous cell and the drug circulates freely in the cytoplasm with minimal toxicity to surrounding tissue.
(d) Most cytotoxic components of current ADCs either bind to the minor groove of DNA and induce strand breakage or bind to tubulin, resulting in microtubule disruption (e).
(f) Finally, both the processes lead to apoptosis.

Figure 14. Mechanism of action of antibody-drug conjugates.
**Internalization of antibody–drug conjugates:** To regain their cytotoxic activity, the cytotoxic agent has to be cleaved from the chemo-immunoconjugate. Uptake of antibodies predominantly occurs via the clathrin-mediated endocytosis pathway. After binding the respective antigen associated with coated pits, antibody–drug conjugates will be readily endocytosed, from where they transit through several stages of transport and endosomal vesicles and finally end up in a lysosome. There, linkers and antibody will be cleaved releasing the cytotoxic agent which — after exit from the lysosomal compartment — exerts its cytotoxic effect (**Figure 15**).
4.10.5. Advantages of ADC

Antibody drug conjugates discriminate between diseased and normal tissue. Advances in coupling antibodies to cytotoxic agents allow control over drug pharmacokinetics and significantly improve delivery of a cytotoxic agent to cancer cells.

The main advantages of the ADC Technology includes:

- A highly targeted therapy, delivering extremely potent drugs
- Broad therapeutic window with efficacy at low doses
- Reduction of classic chemotherapy side-effects
- Active in more than one cancer disease class
- Addresses an unmet need in cancer therapy

4.11. Targeted Chemotherapy

Targeted therapy includes a wide variety of different strategies- (a) direct or (b) indirect approaches.

(a) In direct approaches targets are tumour-associated or tumour-specific proteins. This approach uses binding of monoclonal antibodies (mAbs) to the relevant antigens to alter their signalling or uses small-molecule drugs to interfere with these proteins (tumour-associated or tumour-specific proteins).

(b) Indirect approaches rely on tumour-associated proteins expressed on the cell surface that serve as a target device for fusion proteins containing different kinds of effector molecules.

In this indirect approach, targeting of drugs to tumours can be efficiently achieved by means of tumour-specific mAbs or ligands binding to receptors that are present on tumour cells. Furthermore, the target must be so chosen as to allow internalization of the construct to the cell surface. The targeting-moiety–drug constructs must be non-immunogenic. This property must be introduced into mAbs by engineering humanized or human mAbs to avoid rapid inactivation by humoral immune responses. Moreover, engineering mAbs also allows one to alter the antigen-binding affinity and the molecular architecture of the mAb. This process then optimizes the targeting device.
4.11.1. Antibody-Targeted Chemotherapy

Antibody-targeted chemotherapy: It involves the use of a cytotoxic agent (toxin) chemically linked to a monoclonal antibody (mAb) that specifically recognizes a tumor-associated antigen. Monoclonal antibody (mAb) specifically delivers the cytotoxic agent to tumor cells, and maximizes its antitumor effect and minimizes its normal tissue exposure, resulting in an improved therapeutic index.

The therapeutic effectiveness of chemotherapeutic drugs or toxins targeted to the tumour cells relies on the factors such as-(a) binding to the target, (b) the toxin-conjugates have to be internalized into the cell upon binding to their respective ligands.

These antibody-toxin conjugates would therefore predominantly kill tumour cells that present the respective antigen, although it has been reported that an antibody–maytansine conjugate completely destroyed tumours which expressed the antigen heterogeneously. For therapeutic efficacy, the cell-killing potency of immunotoxins depends on several biochemical properties, such as (a) antigen-binding affinity, (b) internalization rate, (c) intracellular processing and (d) intrinsic toxin-domain potency.
Below are examples of approved therapeutic antibodies (Table 4).

Table 4: FDA approved therapeutic antibodies for cancer treatment

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Type</th>
<th>Isotype/conjugate</th>
<th>Target</th>
<th>Indication</th>
<th>Year of Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoclonal antibodies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rituximab (Rituxan)</td>
<td>Chimeric</td>
<td>IgG1</td>
<td>CD20</td>
<td>Low-grade B-cell NHL</td>
<td>1997</td>
</tr>
<tr>
<td>Trastuzumab (Herceptin)</td>
<td>Humanized</td>
<td>IgG1</td>
<td>HER2/neu</td>
<td>Metastatic breast cancer</td>
<td>1998</td>
</tr>
<tr>
<td>Alemtuzumab (Campath)</td>
<td>Humanized</td>
<td>IgG1</td>
<td>CD52</td>
<td>CLL</td>
<td>2001</td>
</tr>
<tr>
<td>Bevacizumab (Avastin)</td>
<td>Humanized</td>
<td>IgG1</td>
<td>VEGF</td>
<td>Metastatic CRC</td>
<td>2004</td>
</tr>
<tr>
<td>Cetuximab (Erbitux)</td>
<td>Chimeric</td>
<td>IgG1</td>
<td>EGF receptor</td>
<td>Metastatic CRC</td>
<td>2004</td>
</tr>
<tr>
<td><strong>Immunoconjugates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin (Mylotarg)</td>
<td>Humanized</td>
<td>IgG4/calicheamicin</td>
<td>CD33</td>
<td>AML (Withdrawn 2010)</td>
<td>2000</td>
</tr>
<tr>
<td>Inotuzumab ozogamicin</td>
<td>Humanized</td>
<td>IgG4/calicheamicin</td>
<td>CD22</td>
<td>NHL</td>
<td>Phase III</td>
</tr>
<tr>
<td>Ibritumomab tiuxetan (Zevalin)</td>
<td>Murine</td>
<td>IgG1/90Y</td>
<td>CD20</td>
<td>Relapsed or refractory NHL</td>
<td>2002</td>
</tr>
<tr>
<td>Tositumomab and tositumomab (Bexxar)</td>
<td>Murine</td>
<td>IgG2a/131I</td>
<td>CD20</td>
<td>NHL refractory to Rituximab and relapsed following chemotherapy</td>
<td>2003</td>
</tr>
</tbody>
</table>

AML = acute myeloid leukaemia; CLL = chronic lymphocytic leukaemia; CRC = colorectal cancer; EGF = epidermal growth factor; NHL = non-Hodgkin’s lymphoma; VEGF = vascular endothelial growth factor.

Antibody-directed enzyme prodrug therapy (ADEPT): It is a targeted therapy that aims to expand the antitumour effect of a drug/ligand/toxin towards cells. These drugs/ligands are not expressed in the cell. In ADEPT, a weakly toxic prodrug is selectively activated into a toxic agent at the tumour site by an enzyme. The selective activation at the tumor site is done by a tumour-specific antibody.

In contrast to the antibody–drug conjugates, the antibody–enzyme conjugate in efficient ADEPT has to remain on the cell surface after binding the respective antigen. However, to prevent toxicity the enzyme–antibody conjugate also has to be cleared rapidly from the circulation. This is induced by mannose glycosylation of the targeting moiety. The enzymes that are used for ADEPT can be divided into three classes: (a) class I: enzymes of non-mammalian
origin with no mammalian homologues; (b) class II: enzymes of non-mammalian origin with a mammalian homologue and (c) class III: enzymes of mammalian origin.

Members of each class have specific advantages and disadvantages. For example, prodrugs that are cleaved by class I enzymes have to be designed in such a way that the endogenous enzymes are unable to cleave, thereby avoiding toxicity against normal cells. However, because they are of non-mammalian origin, class I enzymes evoke a strong immune response.

By contrast, class III enzymes are only weakly immunogenic, but endogenous enzymes can cleave prodrugs that are designed for class III enzymes at inappropriate sites, causing less specific cytotoxicity.

Several preclinical studies proved the feasibility of ADEPT. However, clinical data on ADEPT are limited and largely restricted to Phase I trials. The preclinical data demonstrating high concentrations of cytotoxic drugs in the tumour which will kill antigen negative tumour cells without severe systemic toxicity. Therefore, though, the clinical efficacy of this approach remains to be established, the preclinical data demonstrates its efficiency and warrants further testing of ADEPT (Figure 16).
4.11.2. Working Concept of Antibody-Targeted Chemotherapy

(a) Targeting monoclonal antibodies to the tumour can result in the destruction of the tumour cells by antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity. (b) Targeting cytokines or immune-modulatory molecules either by bispecific scFv or antibody–ligand fusion proteins to the tumour modulates the immune response against the tumour. Moreover, antibody–ligand fusion proteins can induce apoptosis to targeted cells as well as bystander cells by presenting FasL. (c) A more direct approach to kill the targeted cell is the conjugation of cytotoxic drugs (D), toxins (T) or radionucleotides to the monoclonal antibodies. The direct targeting approach requires the homogenous expression of antigen in the tumour cell population; depending on the radionucleotides used. However, radioimmunoconjugates can exert bystander effects and kill surrounding cells which do not express the antigen. (d) The antibody-directed enzyme prodrug therapy (ADEPT) approach specifically aims at causing bystander effects by targeting enzymes to the tumour cell and delivering a prodrug that is converted to a chemotherapeutic by the targeted enzyme.

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**Figure 16a. Working principle of antibody-targeted chemotherapy.**
4.12. **Antibody-Enediyne Conjugates under Clinical Investigation**

From the above discussion it is clear that the antibody-targeted chemotherapy involves the use of a cytotoxic agent chemically linked to a monoclonal antibody (mAb) that specifically recognizes a tumor-associated antigen. mAb specifically delivers the cytotoxic agent to tumor cells and maximizes its antitumor effect and minimizes its normal tissue exposure. This results in an improved therapeutic index of the drug/cytotoxic agent. Reaching the tumor cell the drug kills them. This concept is also applied for using enediyne class of antitumor antibiotics to kill tumor cells. Therefore, various mAb-enediyne conjugates were designed that showed clinical promise and success in targeted cancer chemotherapy.

<table>
<thead>
<tr>
<th>Enediyne Derivative</th>
<th>Molecular Target</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>3G11-LDM</td>
<td>type IV collagenase</td>
<td>hepatoma, colorectal carcinoma</td>
</tr>
<tr>
<td>Fab-LDM</td>
<td>tumor-specific antigen</td>
<td>hepatoma BEL-7402</td>
</tr>
<tr>
<td>VH-LDP-AE</td>
<td>type IV collagenase</td>
<td>sarcoma HT-1080</td>
</tr>
<tr>
<td>CMC-544 (inotuzumab ozogamicin) IgG4/calicheamicin</td>
<td>CD22</td>
<td>B-cell lymphoma (NHL); Phase III clinical trial.</td>
</tr>
<tr>
<td>huCTM01-calicheamicin γ</td>
<td>MUC1 antigen</td>
<td>breast, ovarian carcinoma</td>
</tr>
<tr>
<td>hu3S193CalichDMH</td>
<td>Lewis(γ) antigen</td>
<td>gastric, colon, prostate carcinomas</td>
</tr>
<tr>
<td>138H11-Camtheta</td>
<td>γ-glutamyltransferase</td>
<td>metastasized renal cell carcinoma</td>
</tr>
<tr>
<td>A7-NCS</td>
<td>tumor-specific antigen</td>
<td>human gastric, colorectal, pancreatic carcinoma</td>
</tr>
<tr>
<td>chA7Fab-NCS</td>
<td>tumor-specific antigen</td>
<td>human pancreatic carcinoma</td>
</tr>
</tbody>
</table>
4.12.1. Monoclonal Antibody-Lidamycin Conjugate (mAb-LDM)

- mAb-LDM conjugates have high potency against tumors
- It possess low toxicity
- It shows exceptional promise as “warhead” drug candidates.
- The conjugate 3G11-LDM against type IV collagenase displays extremely potent cytotoxicity compared with free LDM
- 3G11 showed positive immunoreactivity in most cases of colorectal carcinoma, and negative immunoreactivity in the adjacent non-malignant tissues.
- 3G11-LDM remarkably suppresses the growth of hepatoma 22
- 3G11-LDM increases the survival time of tumor bearing mice.
- The antitumor efficacy and the survival time of the conjugate are higher than those of free LDM or 3G11
- Two immunoconjugates of LDM were reported-
  - (a) a direct conjugate: linking LDM to MAb directly;
  - (b) an assembled conjugate: apoprotein was conjugated to mAb and the chromophore was added to the mAb-apoprotein conjugate. The cytotoxicity of the assembled conjugate is much stronger than that of the direct conjugate. In vivo the assembled conjugate selectively and highly inhibited tumor growth.

- The Fab-LDM conjugate also showed selective cytotoxicity against cancer cells.
- **Assembled fusion protein scFv-LDM:** To reduce the molecular size of the immunoconjugate, a recombinant fusion protein of single-chain Fv and LDM apoprotein (scFv-LDP) conjugate was prepared through genetic engineering. Then the chromophore was added to it to give final “Assembled fusion protein scFv-LDM”. scFv-LDM significantly reduced the activity of type IV collagenase and inhibited cell invasion in highly metastatic human lung carcinoma PG cells. scFv-LDM also showed extremely potent cytotoxicity in PG cells.
- LDM is currently undergoing clinical trials in China.
4.12.2. Monoclonal Antibody-Calicheamicin Conjugate (mAb- CAL)

- Mylotarg is the first clinically approved (but now withdrawn) cytotoxic immunoconjugate for the treatment of elderly patients with relapsed acute myeloid leukaemia.
- Since then, a number of tumour-targeted mAb-CAL conjugates against various tumor targets are being explored for their therapeutic applications.
- A similar conjugate CMC-544 was evaluated in clinical trials in patients with B-cell non-Hodgkin's lymphoma. CMC-544 is a CD22-specific immunoconjugate of CAL and mAb that binds human CD22 with high affinity and causes potent cytotoxic activity against malignant CD22+ B cells.
- Several experimental results support the clinical application of CMC-544 as an antibody-targeted chemotherapy in the treatment of CD22+ B-lymphoid malignancies.
- The conjugate CMB-401 consisted of an engineered mAb hCTM01 against the MUC1 antigen, expressed on many solid tumors of epithelial origin, covalently bound to CAL. CMB-401 showed targeted killing of MUC1-expressing cells in vitro and produced pronounced doserelated antitumor effects over an 8-fold dose range against a MUC1-expressing, ovarian xenograft tumor (OvCar-3).
- CMB-401 also gave good antitumor effects at similar doses with a cisplatin-resistant MUC1-expressing cell line. The conjugate hu3S193-CalichDMH of CAL and humanized antibody hu3S193 recognizing Lewis(y) (Le(y)) antigen could eliminate Le(y) expressing carcinomas.
- hu3S193-CalichDMH caused selective tumor growth inhibition.
- In vitro the efficacy of hu3S193-CalichDMH was qualitatively dependent on the expression of Le(y) and on the sensitivity of the tumor cells to CAL. In vivo hu3S193-CalichDMH inhibited tumor growth in three separate models, causing regression and growth arrest of gastric carcinoma N87, prostate carcinoma LNCaP and colon carcinoma LOVO xenografts. Thus, the selectivity and efficacy of hu3S193-CalichDMH against Le(y)+ tumors supported additional evaluation of this conjugate for clinical application.
- The effective treatment of metastasized renal cell carcinoma (RCC) remains one of the major challenges in urological oncology, because RCC has been resistant to all conventional experimental therapeutics. The conjugate of MAb 138H11 and CAL (Camγ) was highly toxic to the Caki-1 RCC cells. In vivo 138H11-Camγ was very effective in reducing tumor size and preventing or significantly delaying the regrowth of residual tumor cells, in contrast to the controls. This tumor-inhibitory effect was due to specific targeting by 138H11 to the RCC. In addition, the MAb itself also stimulated the local immune response. 138H11-Camγ holds promise for treatment of RCC in small metastases and residual tumor cells.
4.12.3. Monoclonal Antibody-Neocarzinostatin Conjugate (mAb-NCS)

- **Monoclonal antibody-neocarzinostatin conjugate** is A7-NCS wherein NCS is linked with monoclonal antibody mAb A7 by a disulfide linkage. This conjugate is designed and effective against human colonic and gastric cancers.
- The anticancer effect of A7-NCS is stronger than that of free NCS.
- In clinical trials, A7-NCS decreased liver metastasis in size and survival rate for the A7-NCS treated patients was higher than that of patients treated with conventional chemotherapy.
- A7-NCS appeared to be superior to conventional chemotherapy and allowed for a longer survival time for patients with liver metastasis from colorectal cancer.
- However, Human anti-mouse antibody (HAMA) was detected in all the A7-NCS-treated patients. In order to decrease HAMA, A7-NCS was replaced by Fab fragments of chimeric mab A7 conjugated to NCS (chA7Fab-NCS).
- chA7Fab-NCS was administered to seven patients with colonic cancer. The results showed that chA7Fab-NCS was more rapidly cleared than A7-NCS.
- chA7Fab-NCS did not elicit HAMA in two of seven evaluated patients. chA7Fab-NCS NCS elicited low levels of HAMA in the other five patients.
- In contrast, A7-NCS elicited high levels of HAMA in all patients tested. Anti-isotype HAMA was not seen in seven evaluated patients tested with chA7Fab-NCS, while A7-NCS elicited high levels in all patients tested.
4.13. Future Prospect and Recent Advances in Enediyne Research

The enediyne antitumor antibiotics are the masterpiece of nature’s ingenuity. They share a common mechanism for producing DNA lesions wherein a diradical species is generated which abstracts hydrogen from sugar-phosphate backbone situated in the minor groove of DNA. Enediyne anticancer antibiotics and their derivatives are thus considered to be lead candidates in combating one of the major diseases, cancer today. Calicheamicin seems to be leading most directly to clinical studies, compared to the other enediynes. Enediynes also block cell cycle progression and induce apoptotic cell death. Enediyne class of antibiotics showed extreme cytotoxicity to various human cancer cell lines. They have attracted a special interest for the development of antitumor agents because of their potent anticancer activity, the attractive mechanism of action, and the sequence specificity. Several of the naturally occurring members of the enediyne family of antibiotics have entered clinical trials as is revealed from the sections discussed in this module. As for an example, LDM is currently undergoing clinical trials in China.

In practical application with the enediyne antitumor antibiotics it is very difficult to prevent tumor cell growth without causing nonspecific side effects, particularly for the common solid tumors. To direct the enediyne drugs more precisely to tumor cells and away from sites of toxicity, and/or to maintain enediyne drugs at a therapeutic concentration over long periods of time, innovative drug-delivery systems are being developed. Therefore, efforts were underwent to attach enediyne drugs with a clinically suitable delivery systems such as polymer and/antibody. The polymer-conjugated and antibody-linked enediyne drugs such as SMANCS and Mylotarg exhibited to improve an in vivo half-life of biological activity that was longer than those of the necked enediyne drugs. It is interesting that such drugs get accumulate in the tumor much more than the accumulation of the necked enediyne compounds, and thus exhibited greatly improved antitumor properties in animals and humans. Recently, polymer protein conjugates and immunoconjugates are being used routinely as anticancer therapeutics, and are being developed as components of combination therapies. Calicheamicin linked to various drug antibody conjugates seem suitable for clinical trials. Inotuzumab ozogamicin (CMC-544) which is an antibody conjugate of calicheamicin has entered into Phase III clinical trial. The advent of ADC technology makes calicheamicin, lidamycin and many other enediynes as future important antitumor drug of interest.

At the same time, genes that govern the production of enediynes are disclosed. This finding allows improvement in the production and development of new enediyne and related compounds with DNA cleaving ability.
4.14. Selected References

7. http://users.ox.ac.uk/~path0116/tig/new1/mabth.html