ANTIBODY-DRUG CONJUGATE AND ITS ROLE IN CANCER RESEARCH

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ABSTRACT- In the age of precision medicine, new developments and proof-of-concept formats are rapidly enhancing antibody-based treatments. Antibody-drug conjugates (ADCs) have completely changed the way cancer chemotherapy is practiced over the past couple of decades. ADCs use monoclonal antibodies (mAbs) to precisely bind tumor-associated target antigens and release a highly effective cytotoxic agent, in contrast to traditional therapies that cause damage to healthy tissues during dose escalation. An incredibly effective class of anti-cancer medications with an already sizable and expanding clinical pipeline has been created by the synergistic combination of mAbs conjugated to small-molecule chemotherapeutics via a stable linker.

This paper's main goal is to summarise the most recent research and breakthroughs in the field of ADCs. Following intravenous injection, ADCs bind to their target antigens and are internalized by receptor-mediated endocytosis. This makes it easier for the cytotoxin to be released later, which eventually causes the cancer cell to undergo apoptosis. The mAb, linker, and cytotoxin—the three parts of an ADC—affect the conjugate's toxicity and efficacy. Ten ADCs have thus far received FDA approval for oncological uses, and many more are presently undergoing clinical and preclinical testing. Brantuximab vedotin and trastuzumab emtansine (T-DM1), the two ADCs currently on the market, have each been shown to be effective against solid and hematological cancers. Future ADCs will be successful if they can pick targets better, make cytotoxins more potent, create creative linkers, and overcome drug resistance. ADCs are anticipated to be incorporated into the next generation of targeted cancer therapies as more research is done to address these problems.

KEYWORDS: Cancer, chemotherapy, monoclonal antibodies, Trastuzumab emtansine, antibody-drug conjugates, and Brentuximab vedotin

INTRODUCTION-

CANCER- Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Normally, human cells grow and multiply through a process known as cell division, to form new cells as the body needs them. After a certain time, cells will go through a process called programmed cell death called APOPTOSIS. But in the case of cancer cells, this apoptosis process will not be there and the absence of this leads to uncontrollable growth of the cell cycle; another important factor is the inhibition or mutation in the Tumour suppressor gene that also regulates the cell cycle.

TYPE OF CANCER	CANCER NUMBERS YEARS			TOTAL NUMBER
	2019	2020	2021	
Breast Cancer	1,510	1,167	1,133	3,810
Lung Cancer	353	329	283	965
Ovarian Cancer	211	340	134	685
Lymphoma Cancer	257	212	172	641
Prostate Cancer	272	166	192	630

TYPES OF CANCER AND NUMBER OF PATIENTS REPORTED AND THE RISK PROBABILITY HAS GIVEN IN THE TABLE

	Male			Female			Both Sexes		
Site	Patients	CR	Cum Risk	Patients	CR	Cum Risk	Patients	CR	Cum Risk
All sites	679,421	94.1	1 in 9	712,758	103.6	1 in 9	1,392,179	98.7	1 in 9
Oral cavity and pharynx	139,018	19.2	1 in 41	49,951	7.3	1 in 112	188,969	13.4	1 in 60
Tongue	39,902	5.57	1 in 147	13,870	2.0	1 in 401	53,772	3.8	1 in 215
Mouth	57,380	7.9	1 in 103	22,483	3.3	1 in 241	79,863	5.7	1 in 144
Pharynx	3,029	0.4	1 in 1,793	1,102	0.2	1 in 5,475	4,131	0.3	1 in 2,701
Other oral cavity	38,707	5.4	1 in 137	12,496	1.8	1 in 476	51,203	3.6	1 in 213
Digestive system	163,845	22.7	1 in 32	11,137	16.0	1 in 50	273,982	19.4	1 in 39
Esophagus	32,622	4.5	1 in 159	20,206	2.9	1 in 264	52,828	3.7	1 in 198
Stomach	32,713	4.5	1 in 160	17,430	2.5	1 in 319	50,143	3.6	1 in 213
Small intestine	2,155	0.3	1 in 2,492	1,451	0.2	1 in3,901	3,606	0.3	1 in 3,044
Colon	20,572	2.8	1 in 260	15,685	2.3	1 in 348	36,257	2.6	1 in 298
Rectum	21,915	3.0	1 in 244	14,985	2.2	1 in 372	36,900	2.6	1 in 295
Anus, anal canal	2,897	0.4	1 in 1,865	2,028	0.3	1 in 2,682	4,925	0.3	1 in 200
Liver and intrahepatic bile duct	26,678	3.7	1 in 189	10,732	1.6	1 in 514	37,410	2.7	1 in 277
Gallbladder and other biliary	12,385	1.7	1 in 422	19,510	2.8	1 in 284	31,895	2.3	1 in 340
Pancreas	11,908	1.6	1 in 429	8,110	1.2	1 in 657	20,018	1.4	1 in 519
Respiratory system	103,552	14.3	1 in 48	32,480	4.7	1 in 165	136,032	9.6	1 in 74

Larynx	27,146	3.8	1 in 184	3,316	0.5	1 in 1,633	30,462	2.2	1 in 331
Lung and bronchus	71,788	9.9	1 in 68	26,490	3.9	1 in201	98,278	7.0	1 in 101
Other respiratory organs	4,618	0.6	1 in 1,273	2,674	0.4	1 in 2,156	7,292	0.5	1 in 1,600
Bones and joints	8,115	1.1	1 in 1,013	5,840	0.8	1 in 1,370	13,955	1	1 in 1,162
Soft tissue	8,047	1.1	1 in 842	6,590	1.0	1 in 1,052	14,637	1	1 in 936
Skin (excluding basal and squamous)	11,203	1.6	1 in 510	8,962	1.3	1 in 640	20,165	1.4	1 in 568
Melanoma of the skin	3,003	0.4	1 in 1,904	2,3645	0.3	1 in 2,281	5,367	0.4	1 in 2,075
Other nonepithelial skin	8,200	1.1	1 in 695	6,598	1.0	1 in 890	14,798	1.4	1 in 781
Breast	5,377	0.7	1 in 1,022	205,424	29.9	1 in 29	210,801	15.4	1 in 56
Genital system	51,994	7.2	1 in 105	155,630	22.6	1 in 36	207,624	14.7	1 in 54
Uterine cervix	-	-	-	75,209	10.9	1 in 75	75,209	10.9	1 in 75
Uterine corpus	-	-	-	26,514	3.9	1 in 190	26,514	3.9	1 in 190
Ovary	-	-	-	43,886	6.4	1 in 133	43,886	6.4	1 in 133
Vulva	-	-	-	2,838	0.3	1 in 2,459	2,138	0.3	1 in 2,459
Vagina and other genital, female	-	-	-	7,570	1.1	1 in745	7,570	1.1	1 in 745
Placenta	-	-	-	313	0.0	1 in 30,912	313	0.0	1 in 30,912
Prostate	41,532	5.7	1 in 125	-	-	-	41,532	5.7	1 in 125
Testis	4,352	0.6	1 in 2,095	-	-	-	4,352	0.6	1 in 2,095
Penis and other genital, male	6,110	0.8	1 in 916	-	-	-	6,110	0.8	1 in 916
Urinary system	33,260	4.6	1 in 158	11,265	1.6	1 in 502	44,534	3.2	1 in 240
Urinary bladder	20,470	2.8	1 in 250	5,403	0.8	1 in 1,014	25,873	1.8	1 in 402
Kidney and renal pelvis	12,362	1.7	1 in 442	5,657	0.8	1 in 1,038	18,020	1.3	1 in 620

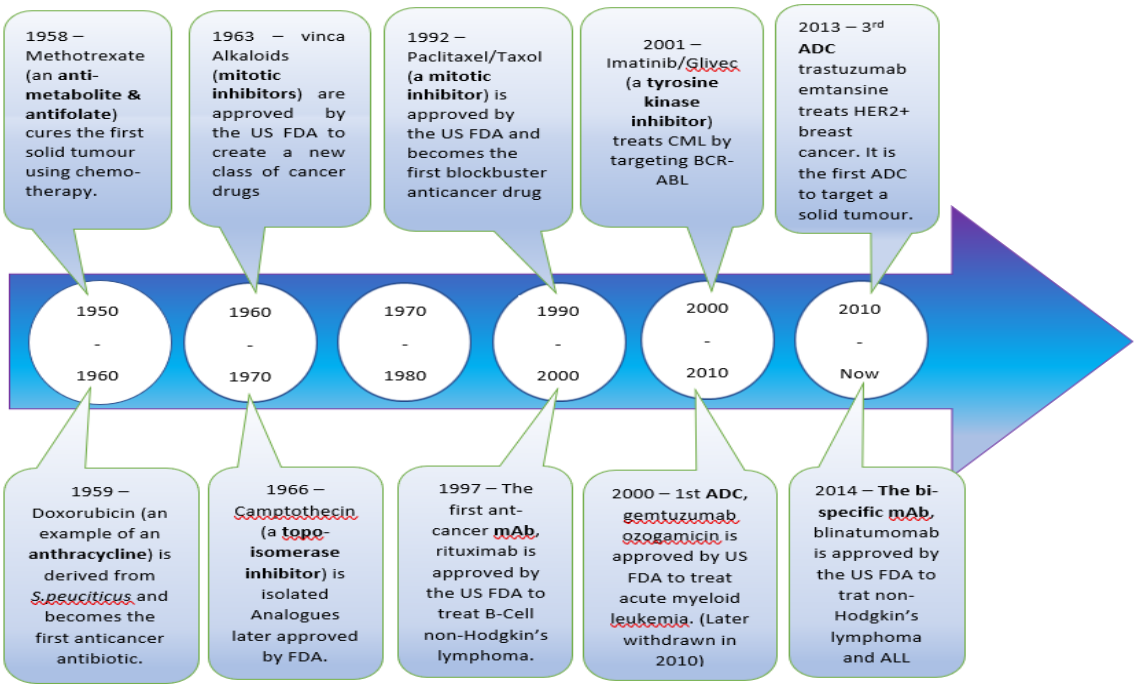
Over the past half-century, cancer management has improved significantly along with the advancement of chemotherapy. Using the cytotoxic agent in the field of chemotherapy opens major treatment options, the following treatment can also be used for the treatment- surgical

removal, radiation, targeted therapies using small molecules or monoclonal antibodies, and, more recently, immunotherapy.

Antibody-drug conjugates (ADCs) are mostly used as therapeutic techniques for treating cancer. It can easily destroy the tumor cells from healthy tissues with the help of monoclonal antibodies conjugated with a specific drug. (10) It is a new technique in the field of cancer treatment. Having the backbone of Antibody Drug Conjugate the following components - antibody, linker, and payload technologies have improved potency and serum half-lives, it also reduces the immunogenicity and improved specificity for cancer cells compared to other techniques(1). Approving ten first-generation ADC agents by FDA increases the spur of candidates at the clinical and preclinical levels (2). Some ADC products are in the last stage of the clinical trial including brentuximab vedotin (SGN-35; Seattle Genetics) for the treatment of CD30-positive malignancies such as Hodgkin's lymphoma, inotuzumab ozogamicin (CMC-544; Pfizer) for CD22-positive B cell malignancy such as non-Hodgkin lymphoma and trastuzumab emtansine (T-DM1; Genentech/Roche/immunogen) for human epidermal growth factor receptor 2 (HER2) positive metastatic breast cancer (2).

In Antibody Drug Conjugate antibody is conjugated with drug with the help of a linker molecule and this drug will act on the specific target cells and shows a synergistic activity this is also called cytotoxic payload monoclonal antibody combination therapy. The mechanism of action of mAb's is the following: (a) induction of apoptosis by abrogation, (b) T-cell function modulation through complement-dependent cytotoxicity (CDC), complement-dependent cell-mediated cytotoxicity (CDCC), or antibody-dependent cellular cytotoxicity (ADCC), and (c) effort of inhibitory impacts on tumor vasculature and stroma.18-20 A combination of humanized or non-humanized mAb and potential small molecules as chemotherapeutic payloads introduced a new class of cancer therapy, antibody-drug conjugates (ADCs)(3).

BRIEF HISTORY OF ANTIBODY-DRUG CONJUGATE-



In 1913 German physician and scientist Paul Ehrlich discovered the concept of selective delivery of toxic agents to target cells that are causing the disease to the tissues of different cells. (4). Ehrlich come up with the idea of a zauberkugel ("magic bullet") that would allow the selective targeting of pathogenic microbes without harming the human body.ADC is a targeted therapy technique that was first demonstrated after forty-five years of paul ehrlich's targeted therapy concept in the form of methotrexate conjugated to leukemia cell-targeting antibody (Mathe et al., 1958)(5). Earlier chemotherapy drugs were being used for cancer therapy but In recent years, an enhanced understanding of cancer biology has shifted the focus of cancer treatment from traditional chemotherapy to targeted cancer therapies that take advantage of the differentiating features of tumor cells to provide a framework for drug development. Advanced techniques are based on the concept of ADC and recently it was found that cancer cells express a different kind of tumor-specific antigen that is targeted by different kinds of drugs that specifically bind onto this antigen and suppress the activity of that antigen and prevent cancer & that's how the evolution of chemotherapeutic techniques or drugs happen.

STRUCTURE ANALYSIS OF ANTIBODY-DRUG CONJUGATE(ADC)-

Antibodies or immunoglobins are the proteins that act against the protein antigen and terminate their activity.

In the ADC year by year, different kinds of antibodies are being used example- a monoclonal antibody derived from mice, a chimeric antibody that is a mixed kind of antibody constant part has been taken from humans and a variable part has been taken from mice, and vice versa and finally after a couple of years monoclonal antibody that is fully derived from human are being used because it gives greater specificity than the other types.

In ADC except for monoclonal antibodies, other structures are also present like Linker and Payload or the drug.

The linker links the monoclonal antibody and the payload structure together and this payload can be attached to the linker on the constant region of the monoclonal antibody. The role of this linker plays a vital role in the function of the ADC because how tightly it is bound to that structure based on the delivery of the drug

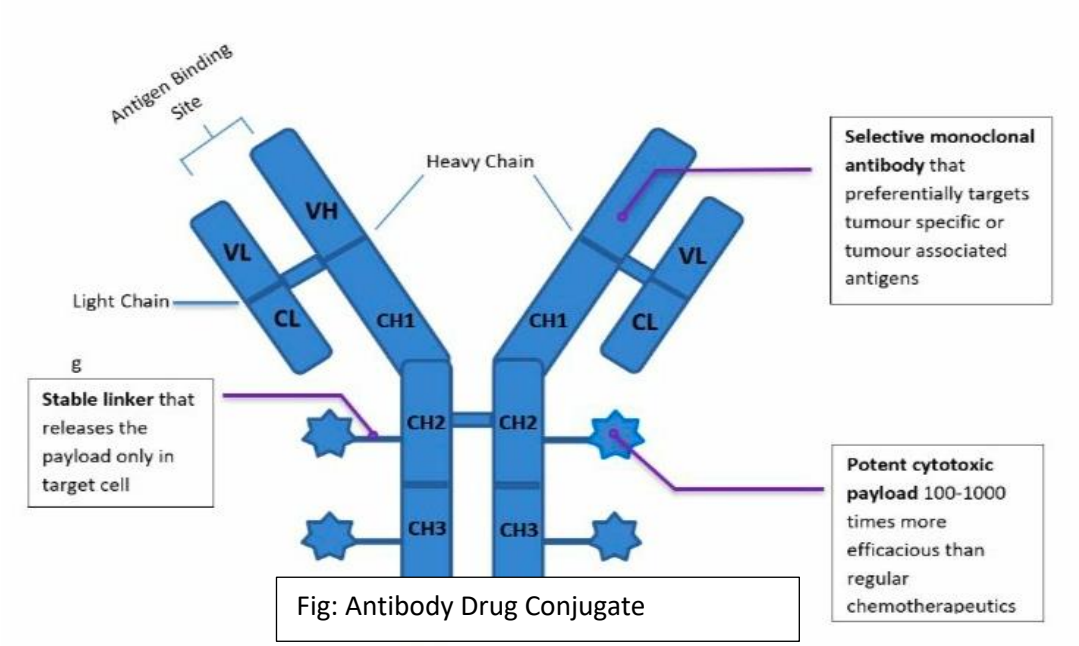
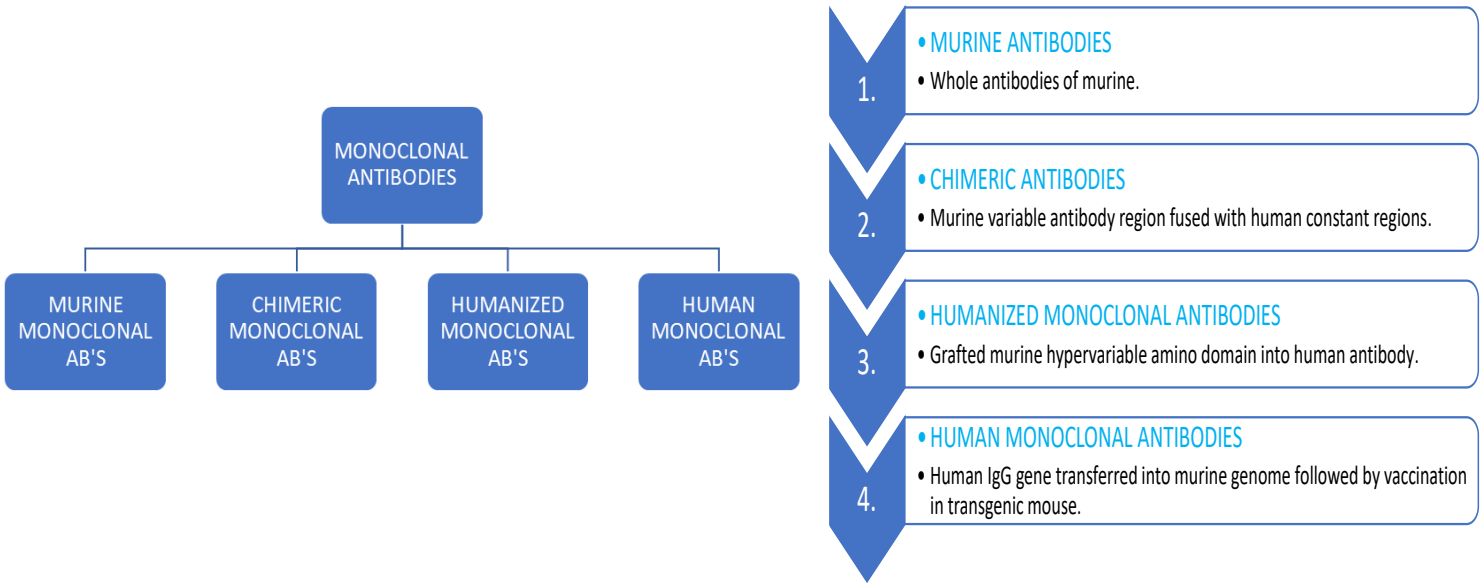


Fig: Antibody Drug Conjugate

to its proper site is dependent. Payload acts as a drug in ADC that is attached to the antibody via the linker molecule. After getting internalized inside the body by the action of lysosome this will get released from the structure and gets attached to its target site and interfering with the microtubule formation process and also mediating the apoptosis process.

ANTIBODY USED FOR ANTIBODY-DRUG CONJUGATE-

As already mentioned in the introduction in the first decade of this concept mouse monoclonal antibodies are being used so now monoclonal antibody; Monoclonal antibody are the artificial antibody produced in the lab by fusing b cells and myeloma cells. There are different types of monoclonal antibodies.



CHOOSING OF MONOCLONAL ANTIBODY-

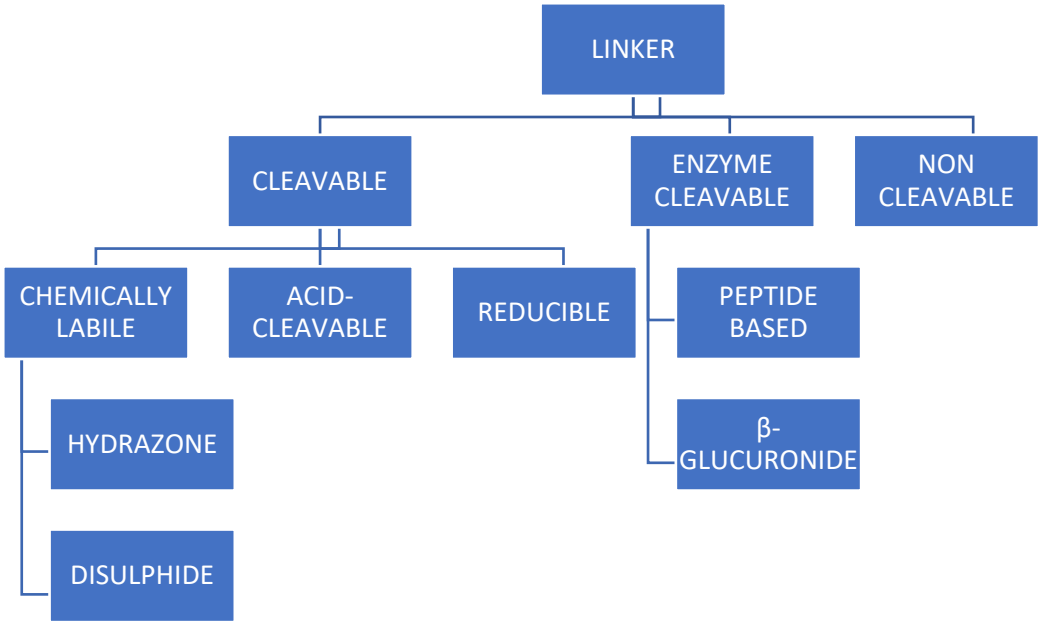
ADC map shows high target-specificity, target affinity, and prolonged drug exposure at the tumor site. Due to these features of antibodies, it will show minimal cross-reactivity with healthy tissues, sub-nanomolar affinity to the target antigen, and a long pharmacokinetic half-life combined with minimal immunogenicity (6). Humanized immunoglobulins have a good role in reducing the immunogenicity of tumor cells. These antibodies interact better with both immune cells and the complement system(7). IgG1 is more effective in making monoclonal antibodies than the other subclasses of IgG. IgG1 has more affinity toward the FC gamma receptor and it also induces antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). This high affinity or tight binding promotes proper internalization and efficient payload delivery. The CH3 region of IgG4s is responsible for the formation of hybrid antibodies and can be replaced with the CH3 region of IgG1 mAbs. IgG2 antibodies are favorable for therapeutic use because they tend to form covalent dimers that support antibody–antibody associations. These associations have the potential to enhance the affinity and/or internalization of the mAb but may also cause the ADC to aggregate and become ineffective. IgG2 offers the theoretical possibility to conjugate more payloads because it contains four interchain disulfide bridges compared with two in IgG1 and IgG4. Due to the high complexity in the cysteine-rich hinge region of the igG2 and igG4, it is very difficult to develop an ADC with those types of antibodies. Due to the less complexity present in the IgG1, it is very easy to develop an ADC with the help of this antibody. (5,7)

Earlier ADC used murine mAbs shows its strong, acute immune response in humans that leads to the rapid formation of human anti-mouse antibodies within 2 weeks of a single dose. Later, chimeric IgG antibodies came into action that has a human constant region and a murine variable region. Another alternative is the humanized IgG antibodies, it has a human variable sequence except for the portion responsible for antibody-antigen complementarity. Nowadays

ADCs use either humanized or fully human antibodies for treatment. Brentuximab vedotin (an anti-CD30 ADC) and BT062 (an anti-CD138 ADC) both incorporate chimeric mAbs.(6,8). There are two types of ADC bispecific and barotropic ADC biospecific ADC recognize two different antigens and barotropic ADC recognizes two different epitopes on the same antigen, conferring a more target-specific drug delivery compared to monospecific ADCs. There are more than 100 formats or patterns of bispecific ADC that have been reported in the literature. Due to robust selectivity, bsADCs improve the safety profile of conventional ADC formats and upgrade their applicability. These monoclonal antibodies are very target specific they don't attach to the adjacent healthy tissues. The bsADCs enhance the internalization process and promote lysosomal trafficking and degradation(7).

LINKER-

One of the biggest challenges in the development of ADCs is the selection of a suitable linker with which to conjugate the cytotoxic payload to the mAb. Various ADC properties depend on the linker composition including toxicity, specificity, stability, and potency, and thus a wide range of possible linker structures have been investigated(9). One of the important parts of the ADC is Linker because of its specialty in holding the payload to the monoclonal antibody and its ability to release at a specific target site. Depending on this specificity first drug that was approved by FDA in 2000 later it was disapproved in 2010 – GEMTUZUMAB OZOGAMICIN this is the name of this drug, the reason behind the disapprovement is the looseness of the linker towards the payload. Because of this problem, it delivers the drug to the healthy tissues despite delivering it to the cancer cell antigens.



NON-CLEAVABLE LINKER-

Non-cleavable linkers depend on the process of lysosomal degradation. Non-cleavable linkers are a modified version of first-generation linkers that is having more plasma stability than many cleavable linkers. Non-cleavable linkers do not have a definable payload dispersion mode.

With the help of this strategy, the non-cleavable linker can be able to deliver the medication attached to the conjugation amino acid within the antibody. With the help of an enhanced therapeutic index of non-cleavable linkers, it amplified the plasma stability of that ADC(10).

CLEAVABLE LINKER-

The majority of the ADCs contain cleavable linkers. The various environmental factors that lead to distinguishing the cleavable linker such as pH, redox potential, and enzymatic reaction, for example, hydrolysis of acid-labile bonds, enzymatic cleavage of ester, or amide bonds. (11). The three types of enzyme cleavable linkers are:

- Chemically labile linkers,
- Acid-cleavable linkers, and
- Reducible linkers.

CHEMICALLY LABILE LINKER-

So the fracture, augmenting the acidity of the endosomal–lysosomal route along with the absorption of glutathione within cells can be chemically labile linkers utilized in ADCs. (10-11)

THIS TYPE IS ALSO SUBDIVIDED INTO TWO MORE CATEGORIES-

1**. HYDRAZONE LINKER-** It is the first type of chemically labile linker that depend on the low pH within lysosomes to undergo acid hydrolysis and release the cytotoxic drug. (8,10).

2**. DISULPHIDE LINKER-** It is an alternative to a hydrazone linker. Their mode of action is based on the thiol group present in the tumor-containing cancer cell as they are involved in promoting cell survival and tumor growth and are produced during cell-stress conditions such as hypoxia. (8,10,11).

ACID-CLEAVABLE LINKER-

Hydrazones are also examples of acid-cleavable linkers. These linkers are specially engineered so that it is only active at acidic ph and at the acidic ph it will hydrolyze and release the cytotoxic drug inside the cell and also it is stabilized at the ph-7 in the blood ph so that it maintains the specificity in the drug release.

REDUCIBLE LINKER-

Disulfides or reducible linkers depend on the difference in reduction potential in the intracellular array versus plasma. The low level of glutathione expressed in the tumor cytoplasm is greater than in normal cytoplasm. Additionally, tumor cells express enzymes of the protein disulfide isomerase family, that influence the reduction of the disulfide bond in the cellular environment. Disulfide bond linkers stabilize the conjugates and keep them undamaged

throughout cardiovascular circulation and make them carefully bound by the glutathione abundant in high concentrations, dispensing the cytotoxic payload at the neoplasm cells. (12).

ENZYME CLEAVABLE LINKER-

Enzyme-cleavable linkers are chemically-cleavable linkers that depend on the presence of hydrolytic enzymes in the cell. These linkers may be peptide-based or beta-glucuronide linkers.

1**. PEPTIDE-BASED LINKER-** Peptide linkers offer improved control of drug release by attaching the cytotoxic drug to the mAb via a dipeptide linkage. The proteases can break the peptide bond that is only active in low pH environments, the cytotoxic drugs are released in the pH-neutral environment of the blood. The dipeptide linkage is cleaved in the acidic environment within lysosomes by lysosomal proteases, such as cathepsin-B and plasmin. (11-12).

2. **β -GLUCURONIDE LINKER-** In antibody-drug conjugates, the formation of β-glucuronide linker relies on cleavage by the enzyme β-glucuronidase, which is overexpressed in the lysosome of many tumor cells. It shows the hydrophilic properties, that promote the solubility of the ADC when it is compared to dipeptide-based ADCs. Due to their hydrophilic properties, β-glucuronide linkers promote the solubility of the beta-glucuronide antibody-drug conjugates (12-13).

CYTOTOXINS OR PAYLOAD-

The cytotoxic drug or "small molecule, payload or warhead" is a critical factor influencing ADC activities and characteristics. Cytotoxic drugs used for ADC development it must meet several requirements, including strong cell toxicity, the appropriate modified site from where the conjugate releases the original drug in the tumor cell, preserved potency after conjugation, acceptable aqueous solubility, stability in the aqueous formulation, and physiological conditions, and a definite action mechanism. (14).

Payloads used in ADCs tend to be cytotoxic compounds that are highly toxic to be used as anticancer drugs on their own. Only a small fraction of the administrated ADC therapy will likely reach the tumor site. (14-15). Typically, the cytotoxins used in ADCs are 100–1000 times more effective than regular chemotherapeutics and it is having sub-nanomolar potency also. (11,14).

These payloads are also subdivided into two more types based on their mechanism-

1. Microtubule inhibitors

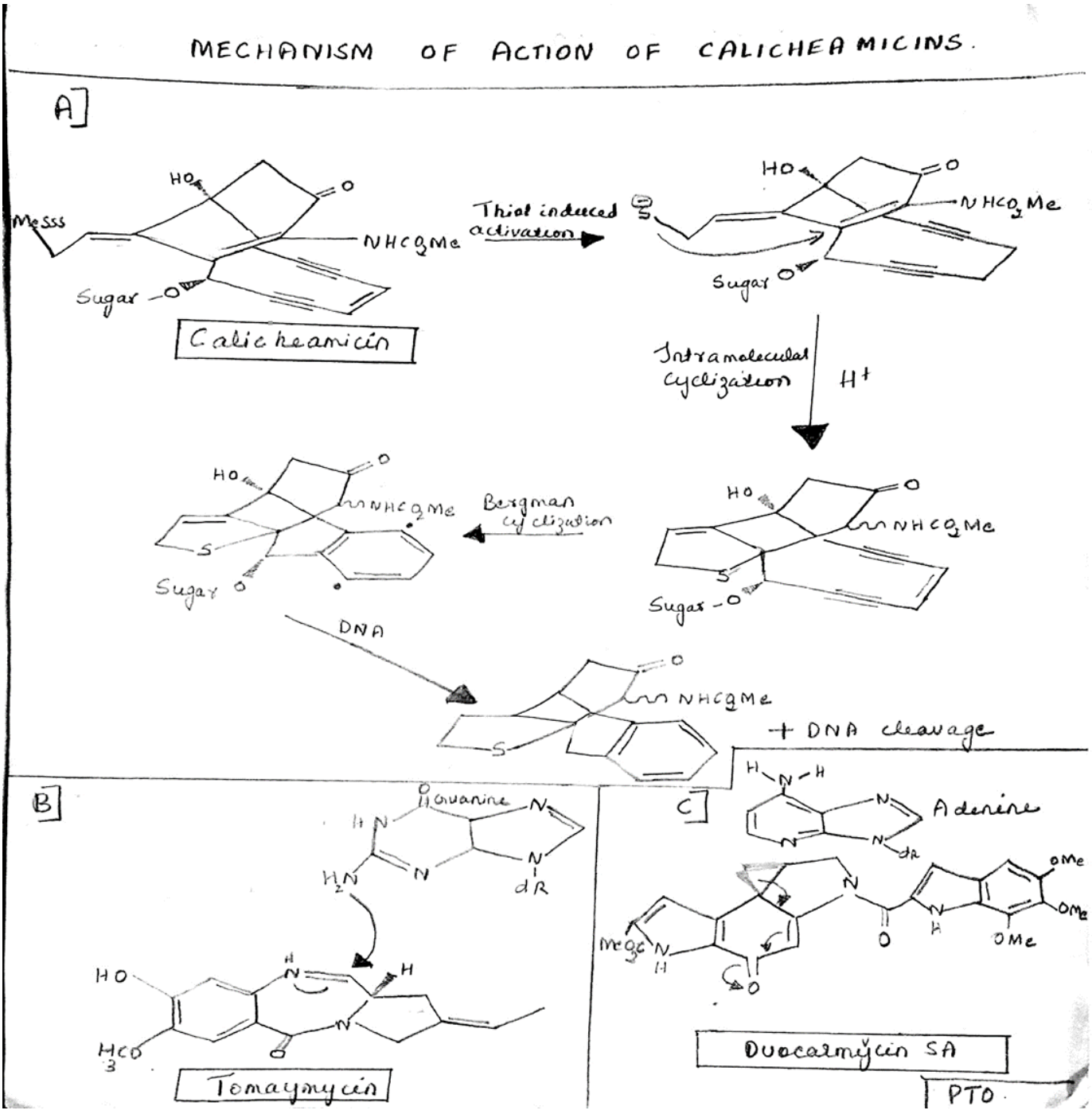
2. DNA-damaging agents

Other small molecules, such as α-amanitin (a selective RNA polymerase II inhibitor), are also under investigation.

Currently available ADCs contain payloads belonging to three major groups of cytotoxins; the **calicheamicins** (e.g., the calicheamicin γ1 derivative found in Mylotarg), the auristatin (e.g., monomethyl auristatin E (MMAE) in Adcetris) and the **maytansinoids** (e.g., DM1 in Kadcyla). Recently, there has been interest in using additional classes of highly potent antimitotic

compounds example-**duocarmycins, amanitins**, and **pyrrolobenzodiazepines.** Each of these classes is discussed in more detail below. (14)

CALICHEAMICINS- Calicheamicin was identified in a search for new DNA-damaging agents in the 1980s. It was isolated from the bacterium *Micromonospora echinospora*. The calicheamicins were recognized as the most potent antitumor agents ever discovered. Calicheamicin γ_1^I is the most important member of this family, which is also used to construct ADCs.



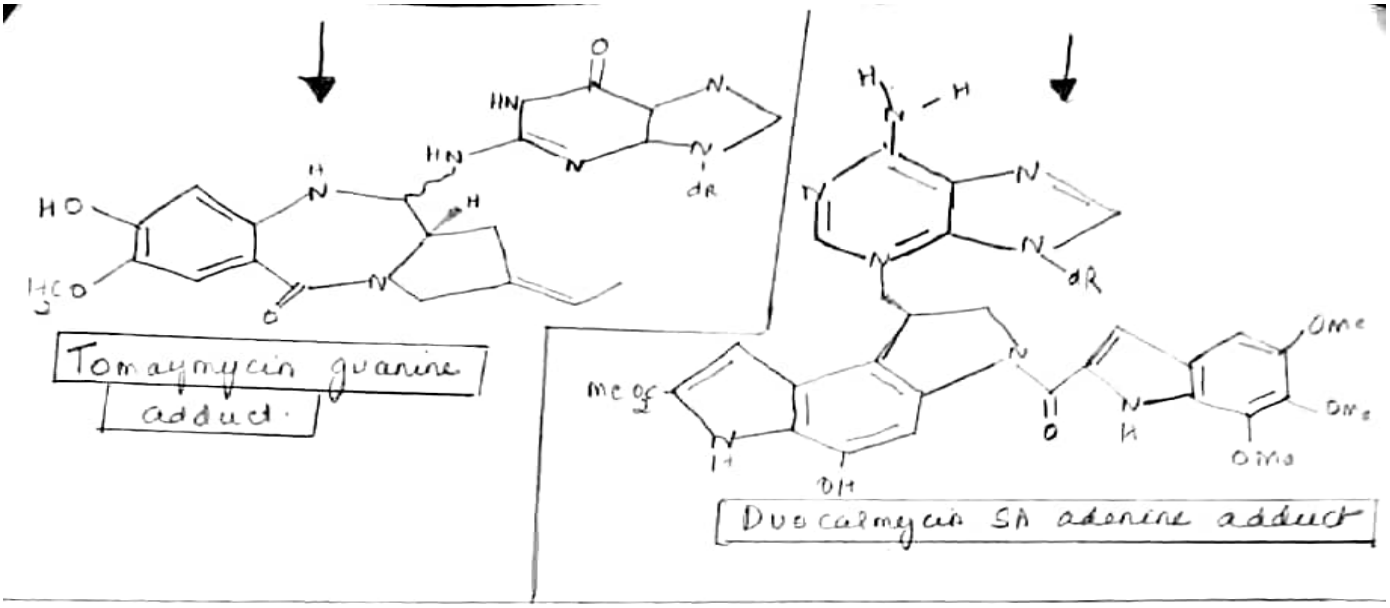


FIG: MECHANISM OF ACTION OF CALICHEAMICINS

Pyrrolobenzodiazepines-

In the 1960s, **pyrrolobenzodiazepine (PBD)** monomers were isolated from Streptomyces bacteria. They were highly potent antibiotic and antitumor activity. PBDs mode of function is through the N10-C11 imine/carbinolamine functionality with the amino group that is located in the C2 position of guanine residue that forms a DNA adduct.(12)

AURISTATINS-

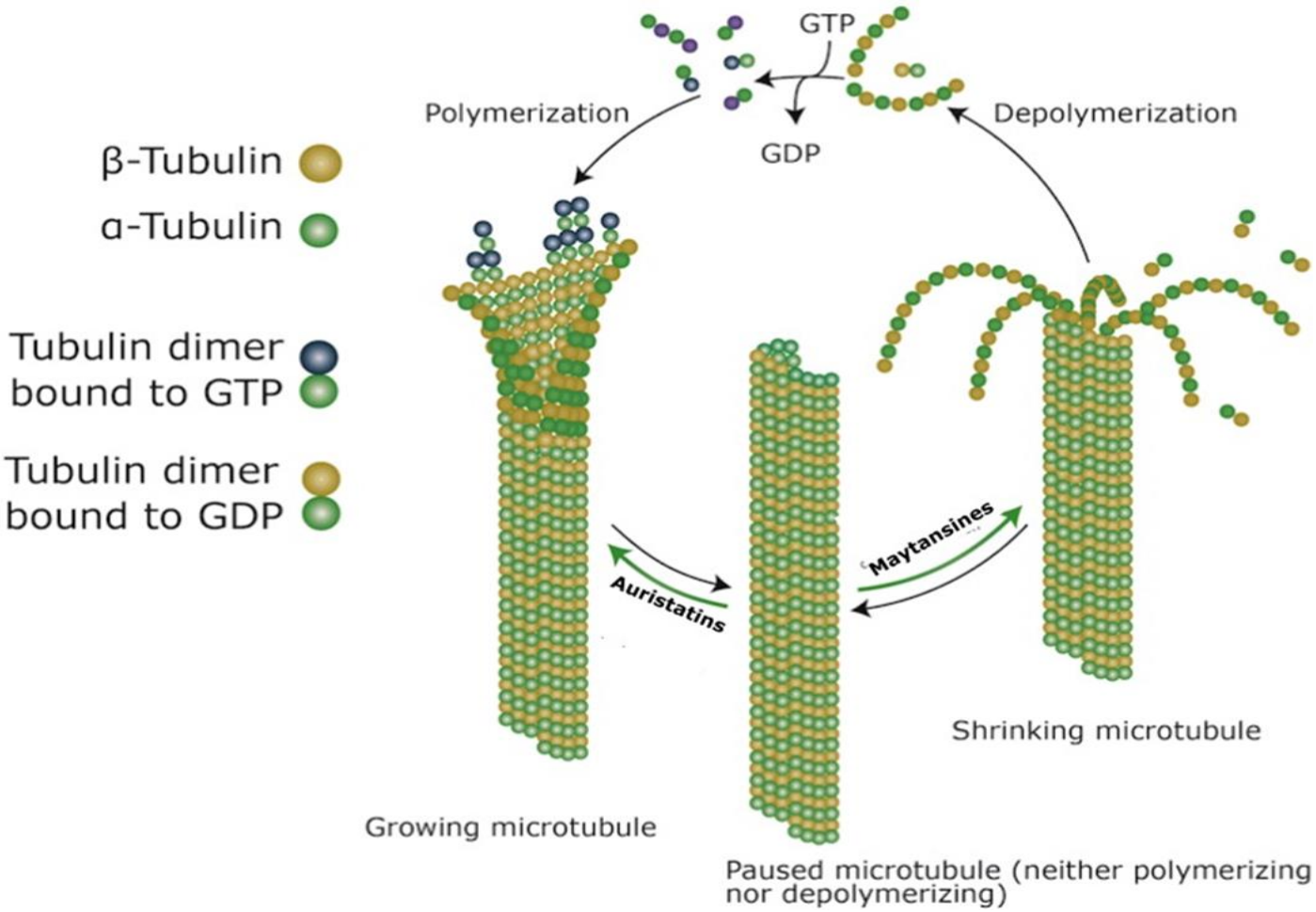
The first known auristatin, dolastatins 1 and 2, were originally isolated from the wedge sea hare Dolabella auricularia. This family of tubulin-inhibiting cytotoxins binds at the alkaloid binding domain of tubulin, which leads to metaphase arrest. The structure of dolastatin 10 was used for derivatives monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), both of which have an N-terminal secondary amine (rather than the tertiary amine present in dolastatin 10), that allows for straightforward linker attachment. MMAE easily crosses the cell membrane than MMAF because it is having C-terminal carboxylic acid group, on the other hand, MMAF is also more hydrophilic and is having a lesser tendency to aggregate and it is having lower systemic toxicity than MMAE. The marketed ADCs Adcetris, Padcev, and Policy all contain MMAE payloads. (1,15)

MAYTANSINES- Similar to auristatin, maytansines, the derivatives of which are known as maytansinoids (DMs), interfere with microtubule assembly but it is mechanistically similar to vinca alkaloids. Maytansines promote the capping of the 'plus' end of the growing microtubule and it blocks the polymerization of tubulin dimers and prevents the formation of mature microtubules. GTP molecule will hydrolyze which is bound to the beta-tubulin, it promotes the disassembly of the existing microtubule that again freezes the cell in metaphase preventing cell division. Of the two DMs, DM1 and DM4, the former is used as the active drug in T-DM1 (16).

Fig: Effect of auristatin and maytansines on microtubule formation

MECHANISM OF ACTION OF ADC-

One of the important features of ADC is its mechanism, To understand the feature of different parts of the ADC first we have to know about the general mechanism of ADC. To be an ideal ADC monoclonal antibody should have the killing capacity and selectivity properties, and it



should be able to release the cytotoxic drug in quantities large enough to kill tumor cells. Each of the steps has its challenges that create complexity to design the ADCs. These are illustrated

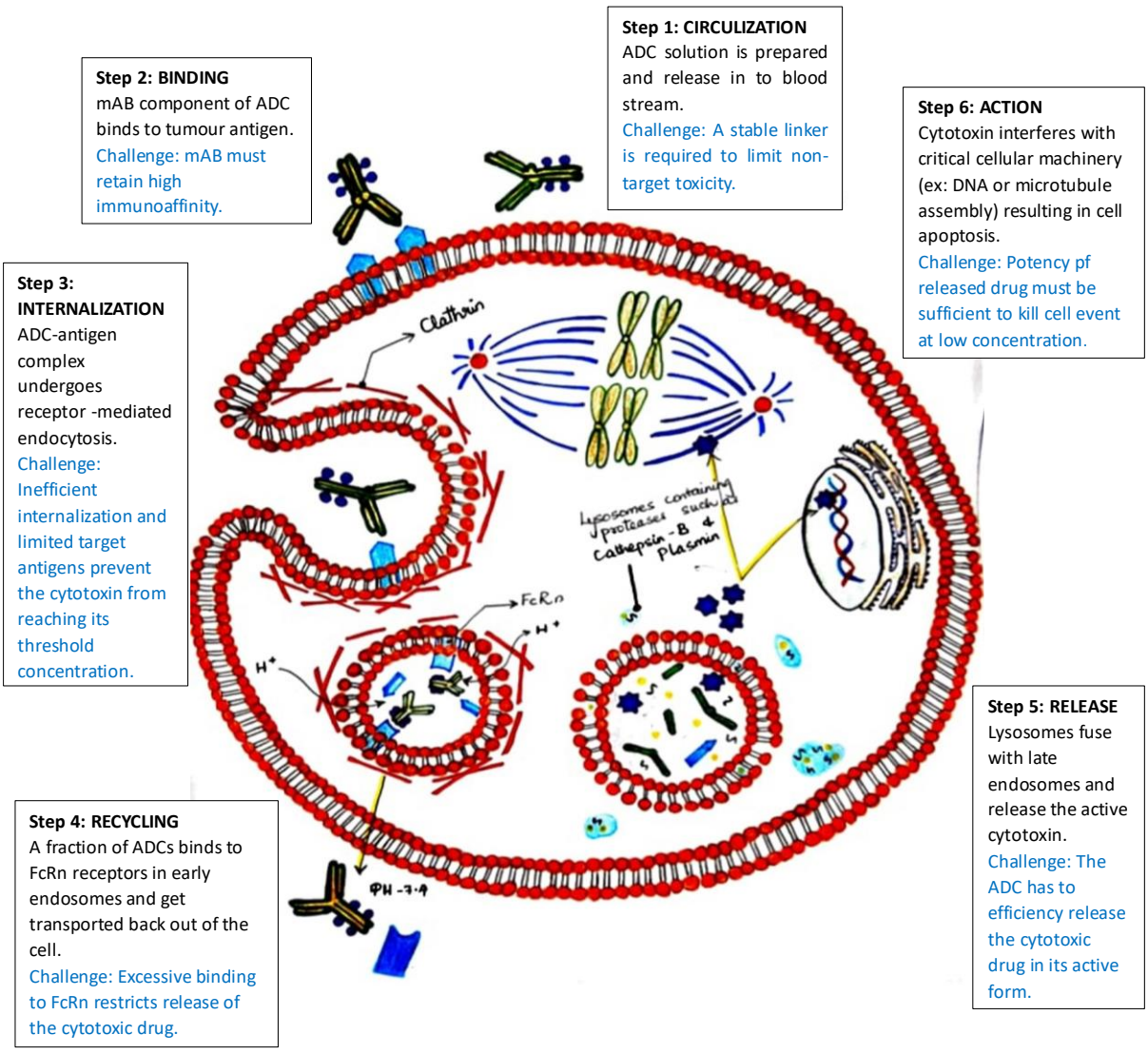
in the fig below challenges that create complexity to design the ADCs. These are illustrated in the fig below

STEPS:-

1. **CIRCULATION:-**

ADCs are administered intravenously because gastric acids and proteolytic enzymes that are present in our body have the property to degrade the monoclonal antibody of the ADCs.

CHALLENGE:- To prevent the unwarranted release of the cytotoxin and maximize drug delivery to cancer cells the linker should be very stable when ADCs are administrated into the bloodstream.



2. **BINDING:-**

The mAb component of ADC first will circulate in the bloodstream and search for its target tumor-specific / tumor-associated cell surface antigen that is present on the target cancer cells, when it finds it will bind over there tightly.

CHALLENGE:- Monoclonal antibodies must retain high immunoaffinity and they should be able to release the cytotoxic drug when it is required.

3. **INTERNALIZATION:-**

After binding of monoclonal antibody component of the ADC to its specific antigen, it will form ADC-ANTIGEN COMPLEX. That complex gets internalized via the receptor-mediated endocytosis process. Next clathrin molecule will come and it will coat the early endosome containing the ADC-ANTIGEN COMPLEX.

CHALLENGE- Cytotoxins can't be able to reach their threshold concentration because of inefficient internalization and the presence of less number of target antigens.

4. **RECYCLING:-** An influx of H+ ions inside the endosome leads to creating an acidic environment that will lead to the binding of the mAb component of non-specifically bound ADC to the FcRns (Human Neonatal Fc Receptors) that forms the ADC-FcRns complex. After that, the ADC- FcRns complex will be transported outside the cell having a ph of 7.4, which will promote the dissociation of the ADC from the FcRns.

CHALLENGE:- Excessive binding of ADC to the FcRns might inhibit the release of cytotoxic drugs from ADC. FcRns function can only be seen inside the endosome.

5. **RELEASE:-**

ADC that remains inside the endosome in an unbound form with FcRns which is called a late Endosome. Lysosome-containing proteases such as cathepsin- B and Plasmin will fuse with the late endosome and will release the degrading enzymes inside the late endosome that will degrade the ADC complex and releases the active cytotoxic drug inside the cytoplasm.

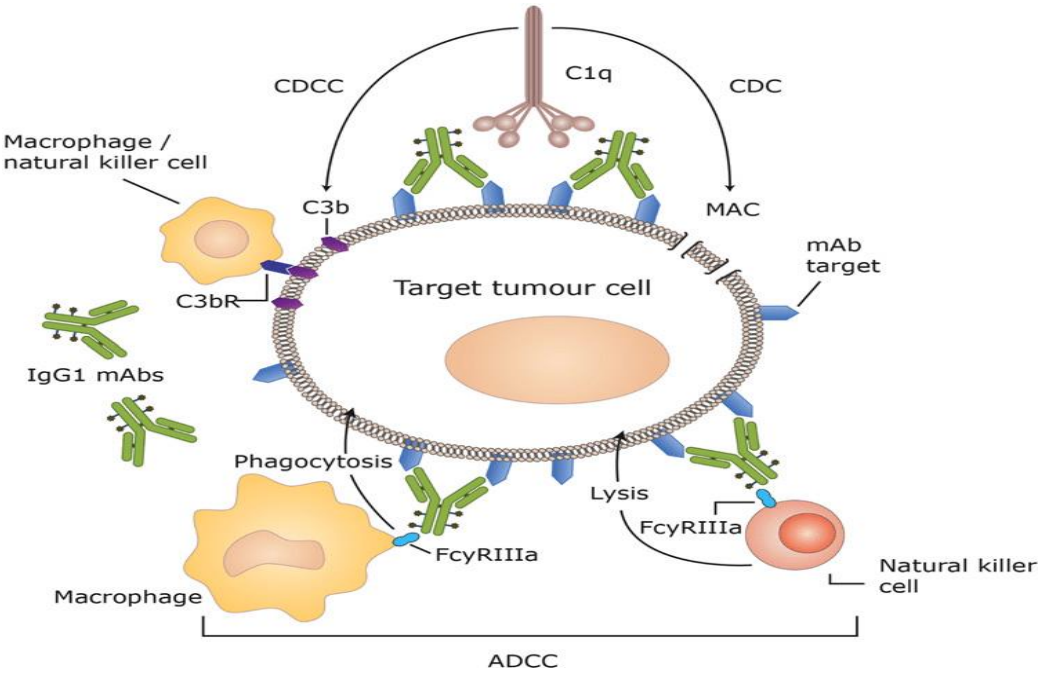
CHALLENGES:- The ADC has to release the active cytotoxic drug in its active form and it should be present in a sufficient concentration within the cancer cell for the destruction to be guaranteed.

6. **ACTION:-**

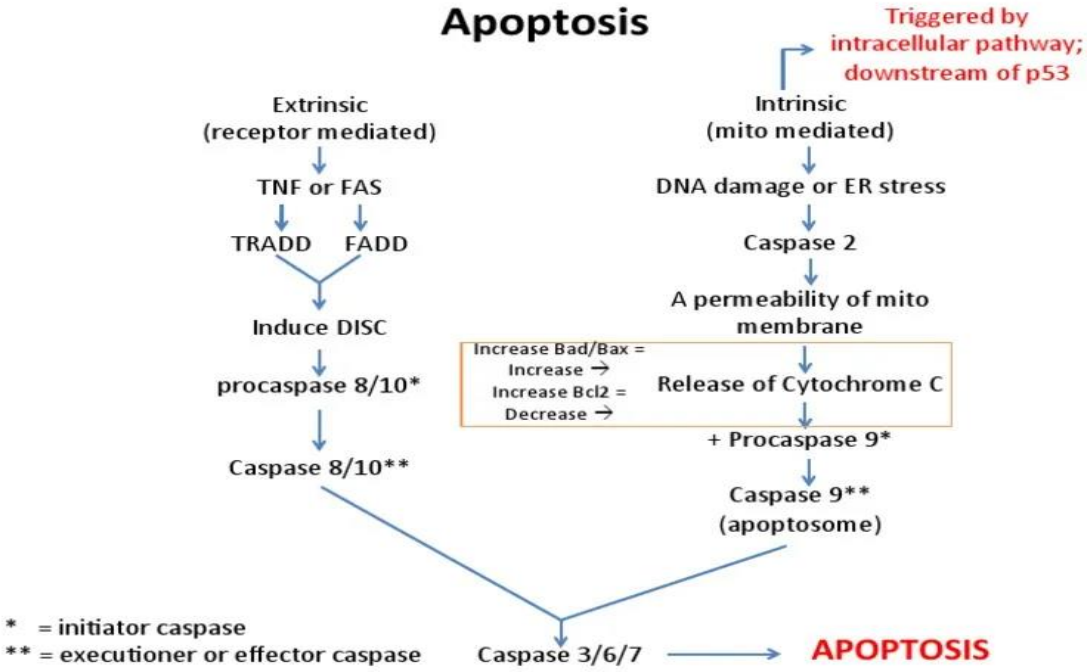
Based on the different cytotoxic drugs, the mechanism of action will be different. The most common mechanisms are – By inducing apoptosis and by direct killing cancer cells (ADCC, CDC-etc)

CHALLENGES:-

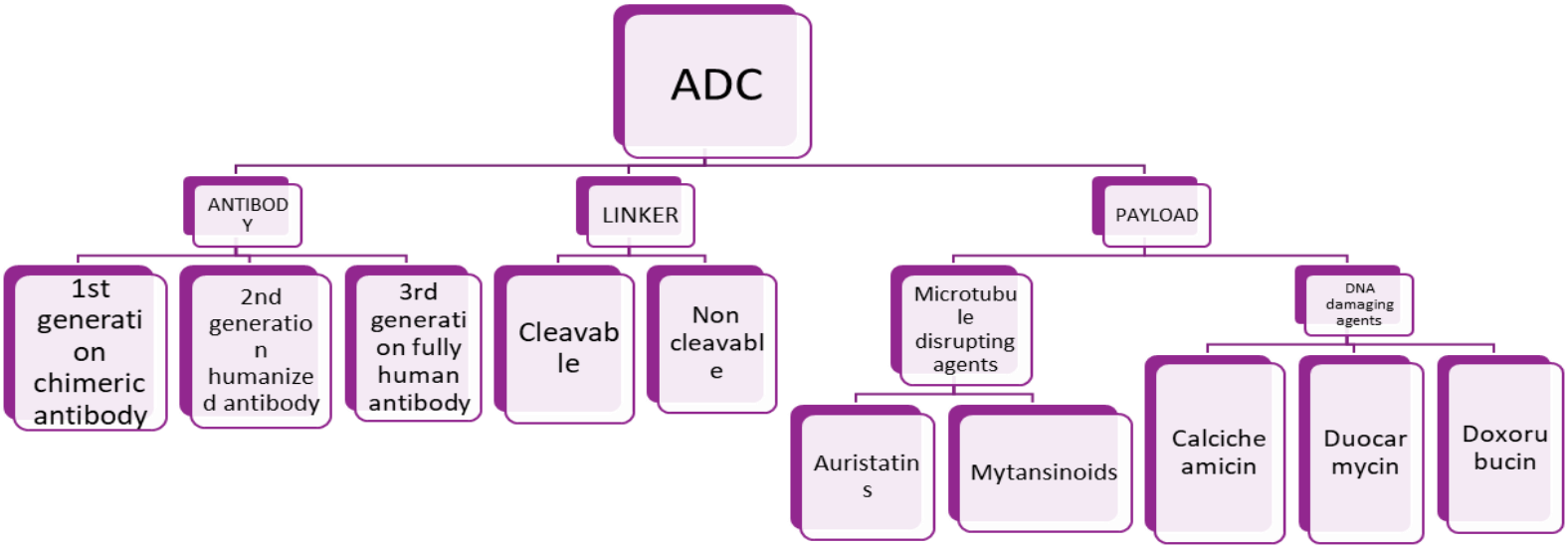
Only 1-2% of the administered drug will reach the targeted tumor cells, thus the cytotoxic drug is required to be highly efficacious at very low concentrations.



The Mechanism of the action of cytotoxic drugs is given below:-



DIFFERENT COMPONENTS OF ADC:-



APPLICATION OF ADC IN ONCOLOGICAL AND NON-ONCOLOGICAL CONDITIONS: -

1. Application of ADC in oncological therapeutics has shifted the treatment era from conventional therapy to molecularly targeted medicine. mAb is attached to the cytotoxic drug that will help to deactivate the cancer cells. By attaching radioactive isotopes to the cytotoxic drug, mAb with a stable linker helps to discriminate between the cancer cell and healthy tissue. It has big applications in the different fields of cancers like solid malignancies, prostate cancer, gastric cancer, pancreatic cancer, and hepatocellular carcinoma. There are some examples of ADC and their particular target site,manufacturer,year of approval,target antigen,all of the components

ADC	Manufacturer	Year of Initial FDA Approval	Indications	Target	Antibody	Payload	Linker	DAR	Common Adverse Events (>10%)
Gemtuzumab Ozogamicin (Mylotarg®, CMA-676)	Pfizer/Wyeth	2000, Withdrawn 2010, Re-approved 2017	Newly diagnosed (de novo) CD33+ AML in adults (as a monotherapy or combined with chemotherapy) and pediatric patients 1 month and	CD33	Humanized IgG4	Calicheamicin derivative	Acid-labile hydrazone linker	~2-3	Infection, hemorrhage, thrombocytoperia, hyponatremia, rausea, vomiting, elevated ALP, elevated aminotransf

			older (combined with chemotherapy) and relapsed/refractory CD33+ AML in adults and pediatric patients ≥ 2 years of age						erase, fatigue, febrile neutropenia, constipation, abnormal pain, pyrexia, mucositis
Brentuximab Vedotin (Adcetris®, SGN-35)	Seattle Genetics, Millennium/ Takeda	2011	Previously untreated Stage 3/4 cHL at high risk of relapse or progression as post-auto HSCT or after failure of2 prior chemotherapy regimens, previously untreated sALCL, replaced peripheral cutaneous ALCL or CD30+ MF	CD30	Chimeric IgG1	MMAE	Protease-cleavable dipeptide (Val-Cit) linker	~4	Neutropenia, peripheral sensory neuropathy, fatigue, upper respiratory tract infection, nausea, diarrhea, anemia, thrombocytopenia, pyrexia, rash, abdominal pain, vomiting, arthralgia, myalgia, pruritus, peripheral motor neuropathy, headache, constipation, dizziness, lymphadenopathy, dyspnea,

									back pain, anxiety
Ado-trastuzumab Emtansine (T-DM1, Kadcyla®)	Genentech Roche	2013	Unresectable locally advanced or metastatic HER2+ breast cancer, previously treated with trastuzumab and a taxane, adjuvant treatment for HER2+ early breast cancer with residual invasive disease after neoadjuvant taxane and trastuzumab	HER2/ERB2	Humanized IgG1	DM1	Thioether (non-cleavable linker)	3.5	Nausea, constipation, diarrhea, vomiting, abdominal pain, dry mouth, stomatitis, headache, peripheral neuropathy, dizziness, epistaxis, cough, dyspnea, fatigue, musculoskeletal pain, arthralgia, myalgia, pyrexia, thrombocytopenia, anemia, increased aminotransferases, insomnia, rash, hypokalemia
Inotuzumab Ozogamicin (Besponsa®, CMC-544)	Pfizer/Wyeth	2017	Relapsed or refractory CD22= B-cell precursor ALL in adults	CD22	Humanized IgG4	Calicheamicin derivative	Acid-labile hydrazone linker	~4	Thrombocytopenia, neutropenia, infection, anemia, leukopenia, nausea, fatigue, pyrexia, elevated

									transaminases, febrile neutropenia, elevated gamma-glutamyltransferase, Lymphopenia, headache, abdominal pain, diarrhea, constipation, vomiting, stomatitis, elevated ALP
Polatuzumab Vedotin-piiq (Polivy®, DCDS4501A, RG7596)	Genentech Roche	2019	Relapsed or refractory diffuse large B-cell lymphoma (combined with bendamustine and rituximab) in adult patients after ≥ 2 prior therapies	CD79b	Humanized IgG1	MMEAE	Protease-cleavable dipeptide (Val-Cit) linker	3.5	Neutropenia, thrombocytopenia, anemia, leukopenia, lymphopenia, febrile neutropenia, peripheral neutropenia, dizziness, diarrhea, vomiting, infusion-related reactions, pyrexia, decreased appetite, fatigue, pneumonia, upper respiratory tract

									infection, decreased weight, hypokalemia
Enfortumab Vedotin (Padcev®, AGS-22M6E, AGS-22CE)	Astellas/ Seattle Genetics	2019	Locally advanced or metastatic urothelial cancer in adult patients who had received prior treatment with a PD-1/L1 inhibitor and platinum based chemotherapy in neoadjuvant/adjuvant setting	Nectin4	Fully human IgG1	MMAE	Protease-cleavable dipeptide (Val-Cit) linker	~3.8	Peripheral neuropathy, dysgeusia, fatigue, decreased appetite, rash, alopecia, dry skin, dry eye, vomiting, constipation, nausea.
Fam-trastuzumab deruxtecan- nxki (Enhertu®,DS-8201a, T-DXd)	AstraZeneca/ Daiichi Sankyo	2019	Unresectable or metastatic HER2+ breast cancer in adult patients who have previously received ≥ 2HER2 blocked regimens in the metastatic setting, locally advanced or metastatic HER2+ gastric or gastroesophageal	HER2/ERB2	Humanized IgG1	DXd (exatecan derivative)	Protease cleavable tetrapeptide (Gly-Gly-Phe-Gly) linker	7-8	Nausea, vomiting, constipation, diarrhea, abdominal pain, stomatitis, dyspepsia, fatigue, alopecia, rash, decreased appetite, hypokalemia, anemia, neutropenia, leukopenia, thrombocytopenia, cough,

			adenocarcinoma after trastuzumab based treatment						dyspnea, epitaxis, headache, dizziness, upper respiratory tract infection, dry eye

Except for applications in the oncology field, it is also been tried in the non-oncology field. There are different types of payload for non-oncological implications that vary from-glucocorticoid receptor modulators and kinase inhibitors to antibiotics and siRNA(16,17)

CONCLUSION- ADCs are approaching an exponential development phase as a result of extensive study over the previous ten years. A growing body of clinical and preclinical knowledge will direct the creation of medicines with greater potency and a wider therapeutic window than parental compounds. The limitations of first-generation ADCs are anticipated to be solved in the oncological context by promising techniques including bispecific antibodies and dual-drug ADCs. Nevertheless, this cautious optimism is anticipated to be expanded in a number of other non-oncological diseases as a result of ADC's early technological contributions beyond the oncological field.

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