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RESEARCH ARTICLE

THE NEW AGE SAVIOUR FOR COMBATING CANCER: ANTIBODY DRUG CONJUGATES.

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Abstract

Traditional techniques opted to treat cancer were found to be extremely harmful to the tissues when given at a slightly higher dose. ADCs (Antibody Drug Conjugates) have transformed the field of cancer chemotherapy. ADCs use monoclonal antibodies (mAbs) to explicitly tie tumour-related target antigens and convey an exceptionally powerful cytotoxic agent. The synergistic mix of mAbs conjugated too little molecule chemotherapeutics, through a stable linker, has given rise to an adequate class of anti-cancer drugs with an effectively extensive and quickly developing clinical pipeline. Antibody drug conjugates (ADCs) are an important division of therapeutics that allows the antigen-selective ability of mAbs to deliver highly potent cytotoxic drugs at the site of antigen-expressing tumor cells. The utilization of mAb coordinated delivery can present a high therapeutic index to exceptionally strong cytotoxic drugs, expanding both the efficacy and level of safety of the treatment. In other words, to achieve the goal of highly improved therapeutic efficacy and reduced toxicity, each component of an ADC i.e. the mAb, linker and the drug needs to be considered in context of targeted antigen. Furthermore, the mechanism of ADCs, characteristics of targets, methods of preparation, linker drugs being used in ADC design and regulatory requirements for new drug approval are discussed.

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Introduction:-

The most grievous disease in today's world is cancer which is spreading its claws and engulfing a major part of human population irrespective of their age. Every country is facing the emergence of new types of cancer every year and in this fight against cancer new methods to combat it are being developed continuously.

We are aware of chemotherapy which is the most traditional method of treatment of cancer. Every cancer patient is suggested to take a series of chemotherapy sessions in which their body is exposed to certain radiations which in turn kill the cancer cells. These sessions are mostly followed by surgery in which these harmful, continuously dividing and proliferating cells are removed from the body of the individual. Although chemotherapy is thought of as the pinnacle of cancer treatment but it also causes a lot of side effects. These side effects occur because the chemotherapeutic drugs are not able to distinguish between the normal cells and the tumour cells. So, while killing

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the cancer cells they also kill some normal cells and lead to inefficient functioning of certain tissues which are manifested in the form of side effects. These side effects range from nausea to hair loss and are highly undesirable.

As cancer is a serious threat nowadays new technologies and techniques are continuously being devised which can prove helpful in treating this deadly disease. One of these new age methods includes the use of antibodies which are coupled with potent cytotoxic payloads.

The antibodies have an ability of distinguishing between the cancer cells and the normal body cells and hence there is no or very less chance of undesirable side effects to occur. They recognise the cancer cells on the basis of the antigen present on their cell surface and thereafter the payloads that are attached to them release their cytotoxic drugs and kill the tumour cells.

The entire system is hence called “Antibody Drug Conjugates” as it has a conjugation of a particular monoclonal antibody with drug delivery system. They act like a target drug delivery system and have high specificity and efficacy.

A lot of ADCs are now being developed and clinical trials are going on in order to declare them fit for the market and human use. The ADC that has recently come into the market is Brentuximab vedotin which is used to treat Hodgkin lymphoma, Trastuzumab emtansine which is used to treat metastatic breast cancer etc.

Antibody Drug Conjugates:-

We are aware of the fact that certain antibodies play a crucial role in combating and destroying the cancer cells by activating the immune system against them. This process is known as Antibody dependent cell mediated cytotoxicity.

The antibodies are so specific to the cancer cells that the normal cells are unaffected by them and hence they can be a useful alternative to the chemotherapy which is unable to distinguish between the normal and cancer cells and thus have side effects. But this method of treatment involving just antibodies and their ability of signalling the immune system against the cancer cells is not very affective in combating cancer at a large scale.

So, a new class of cancer medicines are now emerging which uses the monoclonal antibodies in combination to cytotoxic payloads. These cytotoxic payloads are highly potent and act like a drug delivery system. The selectivity of antibodies and the cytotoxicity of the drugs are incorporated together into a single system. Both these entities are linked with a labile biodegradable covalent bond. This entire association is known as the Antibody drug conjugates (ADC).

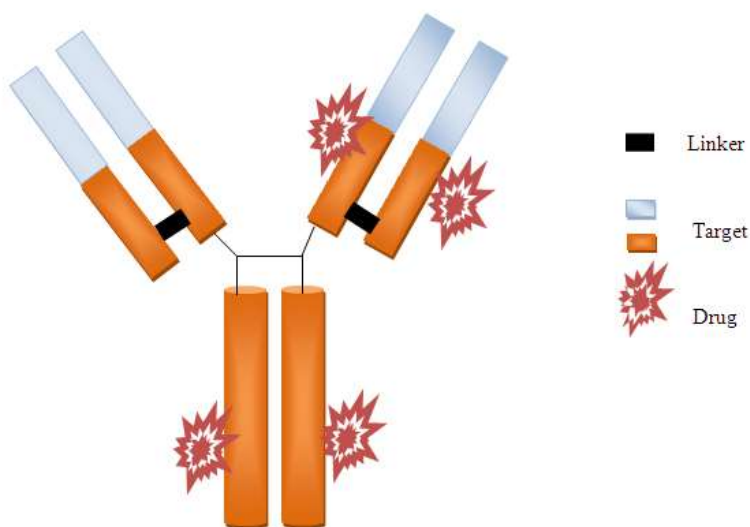


Figure1:- Structure of an ADC

In order to understand this term a lot of other things need to be understood. The following section will discuss different attributes of this simple yet complex conjugate system which is also represented as mAb-Linker-Drug.

Target Antigens:-

An antigen is a molecule that is capable of eliciting an immune response or production of antibodies.

The ADCs recognise these antigens and then attach to them. That is why they are known as the target antigens, the drugs are then released after internalising the ADC complex into the tumor cell. In order to ensure the optimal functioning of the ADCs the target antigen should be selected by taking into consideration the following characteristics:

- There should be no or minimal antibody shedding to prevent the faulty binding within circulation.
- The target antigen should be expressed in limited homogeneity to prevent the faulty binding of antibody. .
- The receptor mediated endocytosis should have the capability to effectively internalise the antibody.
- After the treatment with ADC the target antigen should not be downregulated [24].
- Binding affinity of a particular target antigen should be taken into consideration as it would decide how effectively it would attract an ADC.
- The epitope on target antigen should also be taken into consideration as it decides the internalisation rate of the ADC.
- The numbers of target antigens present on the tumor cells have to be taken into consideration because the antigen-tumor density has a prerequisite minimal value to ensure the efficacy of the ADCs.
- During the analysis of activity of the TDM1 using biomarker it was found that it was more active in cases of tumor where HER-2 subgroups show high expression, although ADCs can also function well with respect to target antigens with low expressivity [34].

Challenges Faced:-

There are a lot of problems that were recognized while administrating the ADCs regarding the interaction of the target antigens and antibody. Some of them being:

- High interstitial tumor pressure
- Downregulation of antigens
- Kinetic and physical barriers
- The bystander effect which states that the drug moves to neighbouring cells after being cleaved from the antibody due to membrane permeability and hence causing their death leading to off target systemic toxicity [24] [34].

To overcome these limitations different approaches are carried out:

One of these includes targeting the antigens in stroma and vasculature,

Another includes targeting the tumour initiating cells (TICs) or cancer stem cells.

Antibody Selection:-

Monoclonal Antibodies are monospecific moieties that are synthetically or naturally produced as a single clone from unique parent cells or cell lines, they have similar primary structure but the secondary and tertiary structure may vary due to the post translational modifications.

The most commonly employed antibody format is human IgG1 isotope of human IgG [34].

After becoming a part of the ADC the antibody can retain their original properties hence they still have the ability to activate the immune system against cancer cells and carry out antibody dependent cellular cytotoxicity (ADCC) [34]. The antibodies are hence independent which has its own advantages as well as side effects. Being independent helps in achieving the complement dependent cytotoxicity while it can lead to a decrease in tumour localisation and increase in toxicity.

Components of an ADC:-

1.1 Monoclonal Antibodies:

It is a single pure antibody which is produced by only one clone of cells. They can be readily formed inside the laboratory in large quantities and thereby used as therapeutic agents. Kohler and Milstein developed a Hybridoma

technique which is widely utilized for the formation of mAbs where Hybridoma is a hybrid cell which is formed by the fusion of B cells with the tumor cells.

1.1.1 Hybridoma Technology

Principle: The technique incorporates the basic properties of both, the B cells (Antibody production) as well as the tumor cells (Ability to divide continuously).

PROCEDURE:

1. Isolation of B Cells

- Immunize the mice (2-4 weeks old) with the antigen against which the mAb is to be produced by sub-cutaneous injections.
- Isolate the B- cells from the spleen and the tumor cells from the bone marrow of the mice. [Tumor cells used are -ve HGPRT, HGPRT mutant cells that are raised by mutations utilizing 8-azaguanine].

2. Selection of Hybrid cells using HAT Medium

- In HAT-rich medium, the cellular synthesis of purines and pyrimidines from simple sugars is blocked by Aminopterin i.e. the de-novo pathway is blocked.
- Cells survive by using HGPRT from Salvage pathway. [HAT= Hypoxanthine Aminopterin Thymidine medium; HGPRT= Hypoxanthine Guanine Phosphoribosyl Transferase].

Working of HAT medium:-

Aminopterin blocks the de-novo pathway and the cells become deficient in HGPRT.

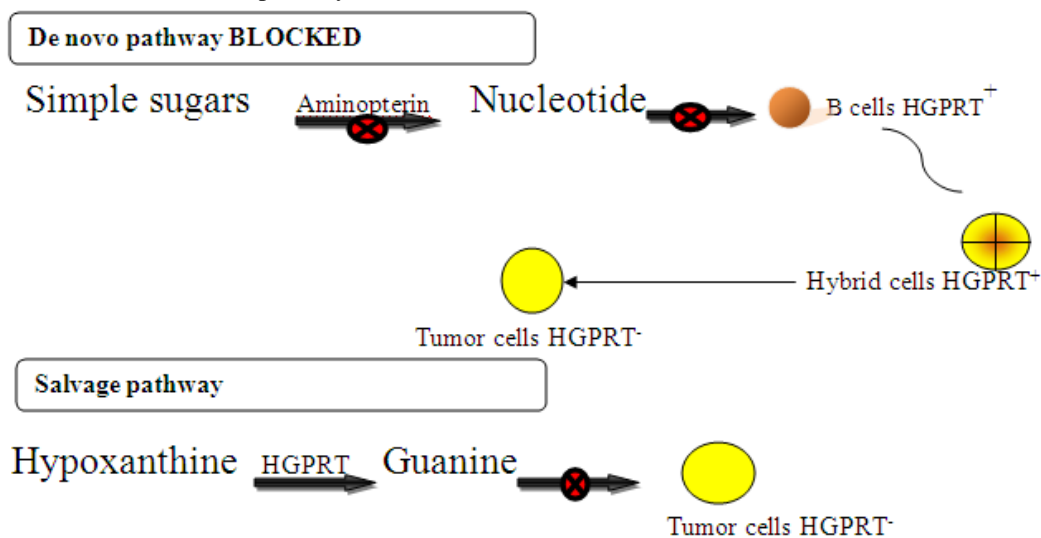


Figure2:- Working of HAT medium.

B- Cells are HGPRT⁺ and could thrive in the HAT medium. After few divisions, B- cells die. Hybrid cells contain HGPRT from the B- cells so only they thrive in the HAT medium.

3. Production of mAb_s

The mAbs are produced in suspended cell cultures in huge fermenters under certain specific conditions.

Types of monoclonal antibodies:-

1. Naked mAb_s: These are the most common type of mAbs which has no drug or radioactive material attached to it and it works by itself. The 3 different ways in which mAbs work are:

- They themselves get attached to the cancer cells and elicit the immune response by acting as a marker to destroy the cancer cells. Eg. Alemtuzumab (Campath[®]) used to treat Chronic Lymphocytic Leukemia (CLL). This gets attached to antigen CD52 on the lymphocytes.
- They may even enhance the functioning of immune system by targeting the checkpoints of immune system.

- While some other may actually bind to and block the antigens that promote the growth of cancer cells. Eg. Trastuzumab (Herceptin[®]) HER2 protein present on the surface of the cells of stomach and breast which helps them grow extensively. Trastuzumab binds to this protein and causes its inactivation.

2. **Conjugated mAbs:** These are attached to a drug or a radioactive material and acts as a carrier to take the drugs to the cancer cells. They are also regarded as “labeled, loaded or tagged” mAbs.

- Radio labeled antibodies: conjugated mAbs which have a radioactive material attached to them. Eg. Ibritumomab tiuxetan (Zevalin[®]) which is found on B cells and is used to treat some of the non-Hodgkin lymphoma.
- Chemolabeled antibodies: these have potent chemotherapeutic drugs attached to them. Eg. Brentuximab vedotin (Adcetris[®]) which is attached to the drug MMAE and it targets CD30 antigen (found on the lymphocytes). It is used to treat Hodgkin lymphoma and anaplastic large cell lymphoma.

3. **Bispecific monoclonal antibodies:** these can attach to 2 different proteins at the same time because they are made of parts of 2 different mAbs. eg. Blinatumomab (Blincyto) one part of which attaches to CD19 (found on some leukemia cells) protein whereas the other attaches to CD3 (found on T cells). This binds to the cancer cells and immune cells together and invokes the immune system to attack the cancer cells.

Linkers:-

The linkers as the name suggests help linking the antibodies to the cytotoxic drugs or payloads. They play an instrumental role in the functioning of the ADCs and influencing their efficacy. Highly specific linkers are chemically prepared in order to make the ADCs functional, they are biodegradable and decide the release profile of the cytotoxic drug. Using the right kind of linker is very important in order to maintain the antibody-drug stability, and hence they are now being given more importance than earlier after showing failed performance in the previously performed clinical trials. The linkers are now chosen more critically and are thus being restricted to just a few choices namely hydrazones, peptides disulphides and thioethers.

Table1:-Different chemical linkers.

Linker	Release Mechanism
HYDRAZONE	Degradation in acidic compartments due to low Ph
PEPTIDE	Lysosomal protease induced hydrolysis
DISULPHIDE	Cleaved by the exchange between disulphide and intracellular thiol like glutathione
THIOETHER	Intracellular proteolytic degradation

Linkers are further of two types cleavable and non-cleavable:

- Non-Cleavable linkers follow ADC lysosomal degradation; in this case the cytotoxic payload is active even when it's attached to the linker and an amino acid residue. It helps in avoiding non-specific discharge of drugs and also facilitates in altering the chemical properties and hence affinity of small molecules. Eg. T-DM1 [41].
- Cleavable linker on the other hand involves the possibility of bystander effect. They function on low pH in lysosomes and hence are **acid sensitive**, so the linker is digested in the acidic environment inside the lysosome or endosome and the drug is delivered.

Another type is called **lysosomal protease sensitive** linkers which have the chemical constitution such that the lysosomal proteases can digest them and hence release the drug. Third type is called the **glutathione sensitive**

linkers which operate by taking advantage of the high glutathione concentration in the liver [39] [40]

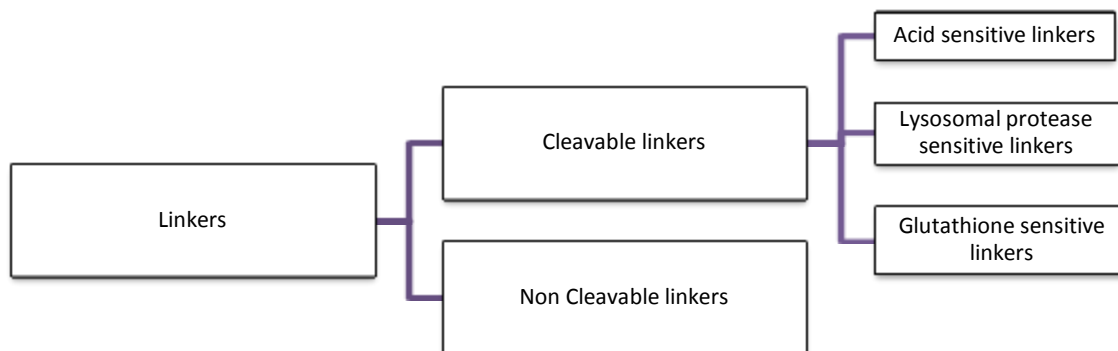


Figure3:- Different types of linkers.

3.2 Cytotoxic Payloads

Microtubule inhibitors	DNA Damaging drugs		Radio-immunoconjugates
Arrest the cell at G2/M phase and cause its death.	They are active throughout the cell cycle and attack the genetic framework of the cell.		Radionucleotides are linked to monoclonal antibodies as payloads.
Eg. (i) Maytansinoids like DM1 and DM4 (phase II trials) (ii) Tubulysins, the tubulising is conjugated with trastuzumab to produce a stable and potent ADC. [11] (iii) Auristatin like monomethyl auristatin E.	Eg. MEDICINE (i) Duocarmycin (ii) Calicheamicin	MECHANISM OF ACTION alkylating compound. (phase I trial) [49] Disrupt the double strand of DNA causing cell death. [16] [40]	Eg. (i) Yttrium-90-ibritumomab tuxetan. (ii) Iodine-131-tositumomab.

Table2:- Different types of cytotoxic payloads and their examples.

The drugs that were earlier used in association with the antibodies were the traditional chemotherapeutic drugs [41] but then due to potency constrains three new classes were discovered namely microtubule inhibitors, DNA damaging drugs and radio-immunoconjugates.

Drug Antibody Ratio (DAR):-

There are three factors on which the working of an ADC work:

1. Pharmacokinetics: branch of pharmacology that deals with the movement of drugs.
2. Therapeutic index or ratio: amount of agent that causes therapeutic effect to the amount of agent that causes toxicity i.e. $\frac{\text{amount causing therapeutic effect}}{\text{amount causing toxicity}}$
3. Efficacy: Producing the desired result.

The DAR plays an important role in regulating all of the above parameters,

If the drugs attached are less in number then the efficacy would be reduced. On the other hand, if the drugs attached are more in number than required the complex would become unstable and the pharmacokinetics would be affected as its half-life would be reduced and systemic toxicity increased [33].

After a lot of experimentations and studies it has been found that the desired DAR should be near 4.

Due to advances in the field of understanding the optimal DAR, production of homogenous ADCs can now be done using pre specified DAR which is calculated using different techniques like non canonical amino acid incorporation or modification of peptide tags [33] [3].

Mechanism of Action:-

The antibody drug conjugates as complicated as they sound have a simple mechanism of action.

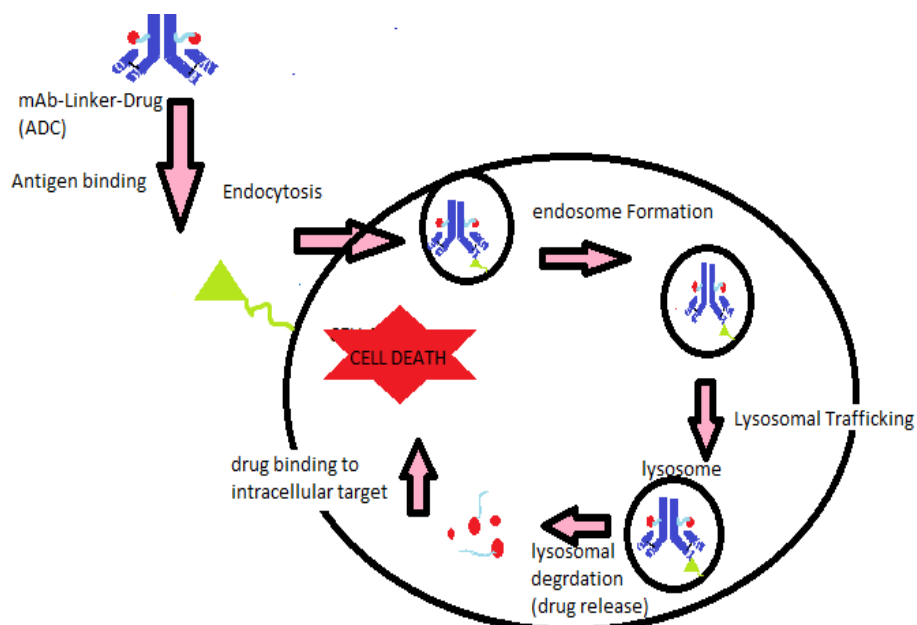


Figure4:- Mechanism of action of ADCs.

The above diagram explains the mechanisms of action of the ADC which is summarized below:

1. The mAb-Linker-drug first binds to the antigen that is present at the surface of the cell. (antigen = green triangle)
2. After binding to the antigen internalisation of the ADC takes place.
3. After entering the cancer cell endocytosis is initiated through the endosome-lysosome pathway.
4. The drug is finally released when due to the acidic medium inside the lysosomes the linkers dissolve and the monoclonal antibodies release the payloads.
5. The drug henceforth released kills the cell.

Sometimes the drug can also kill the neighbouring cells if it is membrane permeable, this is sometimes useful as it kills surrounding tumor cells as well but when the surrounding cells are normal it may cause off target systemic toxicity.

Potent Antibody Drug Conjugates for Cancer Therapy:-

Earlier, the field of antibody drug conjugates (ADCs) clinically approved drugs and mouse monoclonal antibodies (mAbs) only were prominent that too just for the delivery of cytotoxic drug to the tumour with high selectivity. After having studied the behavior of the antigen-antibody complex, various factors influencing ADCs activity, tolerability and success were listed. They were: Addition of highly potent drugs, linkers, target antigen, mAb carriers, methods of attachment of drugs to mab, etc. [42]. The study also listed few of the marketed products (adcs) such as:

A) Name: Gemtuzumab ozogamicin [13]

Trade name: Mylotarg®

Developer name: Pfizer/ Wyeth

Approved in: 2001

Used to: treat acute myelogenous leukemia

It was withdrawn from the market in 2010 after a request from FDA (Food and Drug Administration).

B) Name: Brentuximab vedotin [9]

Trade name: Adcetris®

Marketed by: Seattle Genetics and Millennium/Takeda

Approved: 2013

C) Name: Trastuzumab emtansine

Trade name: Kadcyla

Marketed by: Genentech and Roche

Used for: HER2-positive metastatic breast cancer (mBC) [12].

ADCs currently in the market

Name of ADC	Target antigen	Antibody	Linker	Drug class	Stage	Tumor indications	Developer
Brentuximab vedotin	CD30	Ch IgG1	Valine-citrulline	Auristatin MMAE	Approved	HL/ALL	Seattle Genetics
Inotuzumab ozogamicin	CD22	Hz IgG4	Hydrazone	Calicheamicin	Phase III	NHL	Pfizer
Gemtuzumab ozogamicin SAR3419	CD33	Hz IgG4	Hydrazone	Calicheamicin	Phase II	Relapsed AML	Pfizer
BT062	CD19	Hz IgG1	SPDB	Maytansine DM4	Phase II	NHL	sanofi
BT062	CD138	Ch IgG4	SPDB	Maytansine DM4	Phase II	MM	Biotest
RG7593/DC DT2980S	CD22	Hz IgG1	Valine-citrulline	Auristatin MMAE	Phase I	NHL	Genentech/Roche
RG-7596	CD79b	Hz IgG1	Valine-citrulline	Auristatin MMAE	Phase I	NHL	Genentech/Roche
Milatuzumab -doxorubicin	CD74	Hz IgG1	Hydrazone	Doxorubicin	Phase I	MM	Immunomedics
Trastuzumab -emtansine	HER2	Hz IgG1	SMCC	Maytansine DM1	Phase III	Breast Cancer	Genentech/Roche Celldex
Glembatumab vedotin	GPNMB	Hu IgG2	Valine-citrulline	Auristatin MMAE	Phase II	Breast Cancer	Therapeutics
Anti-PSMA ADC	PSMA	Hu IgG1	Valine-citrulline	Auristatin MMAE	Phase II	Prostate Cancer	Progenics
Lorvotuzumab mertansine	CD56	Hz IgG1	SPP	Maytansine DM4	Phase I/II		Immunogen
AGS-5ME	SLC44A4	Hu IgG2	Valine-citrulline	Auristatin MMAE	Phase I	Pancreatic, Prostate Cancer	Astellas
SAR566658	CA6	Hu IgG1	SPDB		Phase I	Solid Tumors	Sanofi

BAY 79-4620	CA-IX	Hu IgG1	Valine-citrulline	Auristatin MMAE	Phase I	Solid Tumors	Bayer
BAY 94-9343	Mesothelin	Hu IgG1	SPDB	Maytansine DM4	Phase I	Solid Tumors	Bayer
SGN-75	CD70	Hu IgG1	Maleimido caproyl Phenylalanine	Auristatin MMAF	Phase I	RCC, NHL	Seattle Genetics
Labestuzumab-SN-38	CD66e/CEACAM5	Hu IgG1	lysine	CPT-11 SN38	Phase I	CRC	immunomedics
ASG-22ME	Nectin-4	Hu IgG1	Valine-citrulline	Auristatin MMAE	Phase I	Solid Tumors	Astellas

Table3:- [Ch: chimeric; Hz: humanized; Hu: fully human; MMAE: monomethyl auristatin E; MMAF: monomethyl auristatin F; GPNMB: Glycoprotein NMB; PSMA: prostate specific membrane antigen; HL: Hodgkin's Lymphoma; ALCL: Anaplastic Large Cell Lymphoma; NHL: Non Hodgkin's Lymphoma; AML: Acute Myelogenous Leukemia; MM: Multiple Myeloma; RCC: Renal Cell Carcinoma; CRC: Colorectal Carcinoma.

Includes ADCs with disclosed targets currently listed on Clinicaltrials.gov

Source: Clinicaltrials.gov: December 2012.] [32].

Conjugated Antibodies for Targeting Tumor Cells:-

In spite of extensive efforts, use of mAb technology has had just a little achievement in enhancing treatment results in patients with strong tumors. Improving the tumor cell-killing movement of antibodies through conjugation to exceedingly potent cytotoxic "payloads" to make ADCs offers a methodology for creating medications that are hostile to tumors [20].

Early ADCs showed reaction profiles like those of "established" chemotherapeutic agents and their execution in clinical trials in tumor patients was by and large poor. Be that as it may, the current clinical advancement of ADCs that have exceptionally intense tubulin-acting agents as their payloads have significantly changed the viewpoint for ADC innovation. Twenty-five such ADCs are in clinical advancement [21].

Role of Antibodies in targeting cancer cells:-

Other chemotherapeutic agents like Paclitaxel, etc. are not as potent as they should be because they are more or less non-selective. They fail to discriminate between tumor cells and normal cells which lead to the chances of cytotoxicity in almost every cell they come across. Moreover, to reduce the chances of indiscriminate cytotoxicity, they are given in really small doses which are ineffective most of the times and make the tumor cells resistant to them as the time progresses. So, there was a need to look into the prospects of immunology which could enhance the targeting capacity of the chemotherapeutic agent [4].

Antigens which are specific to tumor cells are known as – "Tumor specific antigen" whereas the antigens which are put up prominently by the cancer cells are known as - "Tumor associated antigens". These antigens can be targeted by antibodies which may result in the building up of a particular cytotoxic molecule. Non-antibody ligands such as arginine-glycine-aspartate (RGD), folate, trasferin, etc. have also been used as a targeting agent but they pose certain disadvantages. These are present equally on the non-tumor cells too so, the drug bound to the non-antibody agent may even bind to the normal cell leading to its death. Hence, till date antibodies are the best solution for the targeting of cancer cells owing to their specificity and high selectivity.

There are 4 antibodies present in the serum namely IgG, IgA, IgM, IgE. Out of these 4, the one used for diagnostic and therapeutic purpose is IgG. It is a Y-shaped structure with its 2 arms containing the antigen- recognition sites and its stem with the Effector activity. Monoclonal antibodies can either be utilized completely or its fragments may be used [1]. If used fully, its affinity to antigens increases many fold because of the presence of two arms with antigen recognition sites. Its zeal is high too. But, it has a disadvantage too; it may trigger immunogenicity in

human beings. But, with the recent advancements in the field of anti body engineering, chimeric or human antibodies are being formed which have a little or no immunogenic response on the human body [19].

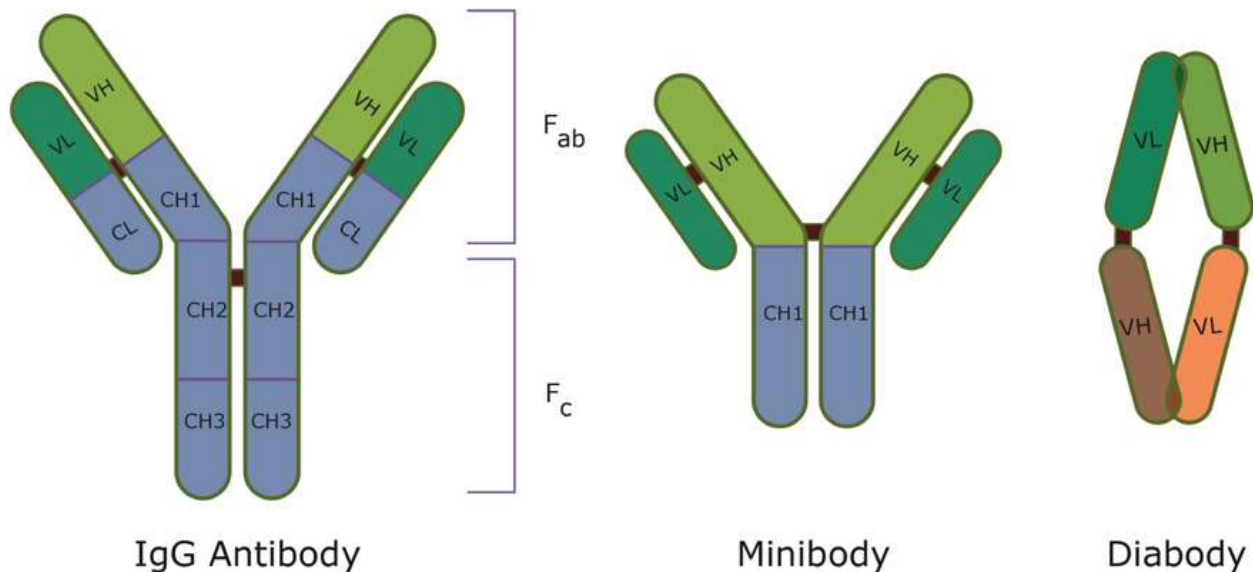


Figure5:- Schematic diagram of a unit of immunoglobulin G antibody (Smaglo, et al.).

Immunogenicity may further be reduced by using the fragment F_c of the molecule of antibody. The F_c part of the antibody binds to the F_c of the receptor on the macrophages and elicits an immunogenic response. So, if the antibody molecule is used without its F_c part, the immunogenic response would be far lesser. It has a disadvantage too. Using fragments leads to lesser avidity or zeal but this can be solved by engineering the fragment of antibody such that it has multivalent binding sites. For this, the fragments are first cleaved using pepsin or papain and then joined together by RDT techniques to give rise to monovalent or bivalent fragments [17].

Few Experiments Performed:-

3.1 Targeting cancer cells using PLGA nanoparticles surface modified with monoclonal antibody [35]

The specificity of delivery utilizing nanoparticles was at first a fortuitous property, active targeting has now turned into a focal idea in therapeutic research. This idea has been produced into practical application with the development of a variety of immuno-conjugates, likewise known as drug-attached antibodies (Abs) [17] [23] [15] [30] [32]. Certain mAbs appeared to have started specific signalling cascades, which can potentiate the therapeutic impact of the attached drug [21] [46]. The last has been affirmed for chemotherapeutic drugs and tumor-focusing on antibodies [21]. Be that as it may, the quantity of drug molecules that can be joined to an antibody molecule is normally the constraining element for such a technique [40].

Material required: Poly (lactic-co-glycolic acid) (PLGA), 50:50, Resomer RG 503H), polyvinyl alcohol (PVA, Mowio 4-98), ethyl acetate, EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide), 546-labelled goat anti-mouse immunoglobulin and Blue Cell Tracker, bovine serum albumin (BSA) and fluorescein, Coomassie Plus reagent.

8.2 Preparation of monoclonal antibody and PLGA NP_s

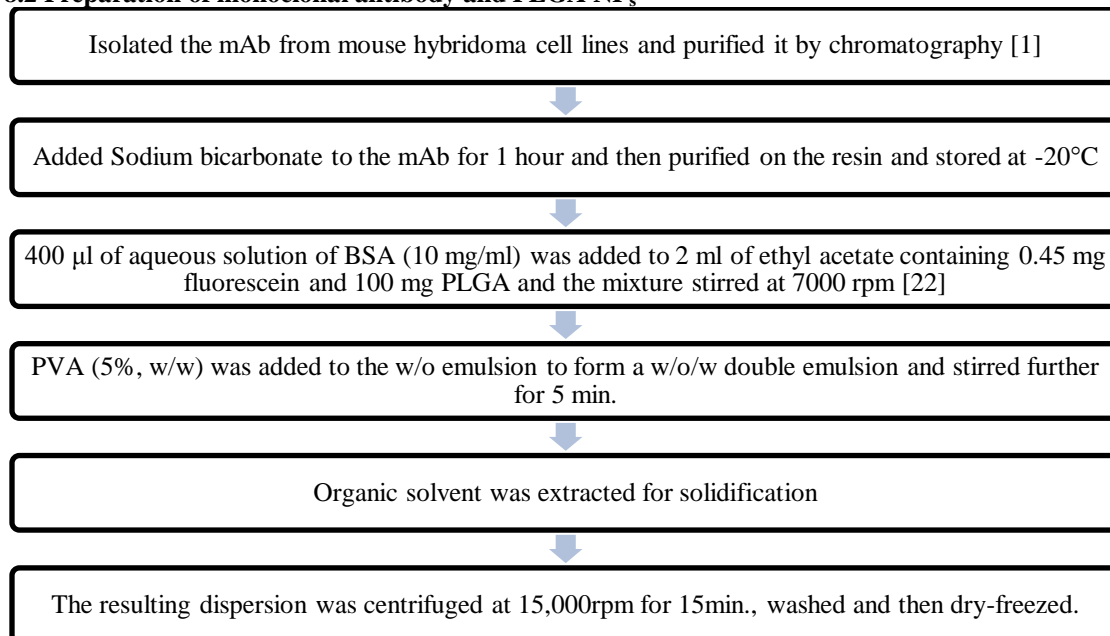


Figure 6:- Preparation of monoclonal antibody and PLGA NP_s

8.3 Advancement of strong monoclonal antibody auristatin conjugates for tumor therapy

The *in vitro* and *in vivo* properties of monoclonal antibody(mAb)- drug conjugates comprising of the synthetic dolastatin 10 analogs Auristatin E (AE) and MonoMethylAuristatin E (MMAE), connected to the chimeric mAbs cBR96 (particular to Lewis Y on carcinomas) and cAC10 (particular to CD30 on hematological malignancies) was studied.

The linkers utilized for conjugate formation included an acid labile hydrazone and protease-delicate dipeptides, causing substituted conjugates that proficiently discharged active drugs in the lysosomes of antigen-positive (Ag⁺) tumor cells.

The peptide-connected mAb-valine-citrulline-MMAE and mAb-phenylalanine-lysine-MMAE conjugates were a great deal steadier in buffers and plasma than the conjugates of mAb and the hydrazone of 5-benzoylvaleric acid AE ester (AEVB). Therefore, the mAb-Val-Cit-MMAE conjugates showed more noteworthy *in vitro* specificity and lower *in vivo* poisonous quality than comparing hydrazone conjugates.

4. Patent on Antibody Drug Conjugates

Eli Lilly and Company assigned the project of devising the suitable antibody, drug and linkers. This invention came as a major breakthrough in the field of organic chemistry, immunology and pharmaceutical industry.

The antibody drug conjugates or immunoconjugates comprise of a monoclonal antibody represented as Ab which recognises a target antigen and delivers the drug there. This invention provides a physiologically acceptable formula of ADC. The β alanine derivatives are used in the preparation of ADCs.

Different components of ADCs are explained below:

Antibody	Drug	Intermediates
Immunoglobulins like IgG, IgA, IgM etc are used. Antigen binding molecules are produced by recombinant technologies. Genetically engineered antibodies with redefined specificity are also being used.	The preferred family of drugs are the daunomycin family, the vinca drugs, the mitomycins, the bleomycins etc.	The β alanine derived linkers were used. They act as the intermediates because they are the one which join the antibody to the drug. The linker involves the methylidene linking mechanism to link the antibody to the reactively available amino,

Hybridoma technology is used to produce monoclonal antibodies	hydroxy or thiol functional group.
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Table4:- Components of ADCs

The method of synthesis of β alanine intermediates and hence the immunoconjugates:

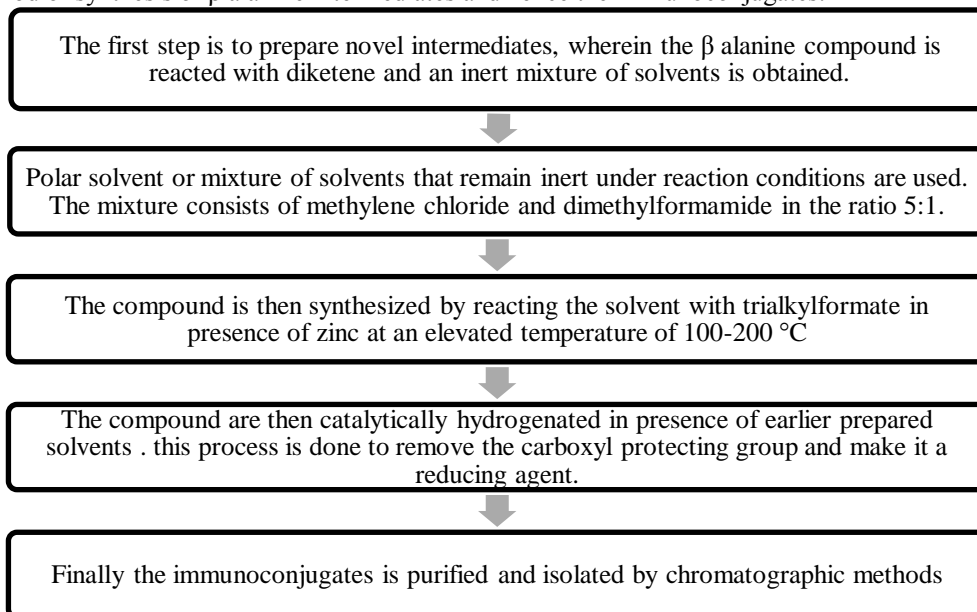


Figure 7:- The method of synthesis of immunoconjugates

10. Advantages of ADCs:

The ADCs have come as a major breakthrough in the field of medicine. It has a number of advantages over the traditional chemotherapy in treating cancer.

(i) It has a larger therapeutic window i.e. the minimum dosage that is required is lesser than the dosage required for chemotherapeutic drugs while the maximum dosage that can cause serious harm is also higher. Hence, it works in a wider window than the earlier drugs used to.

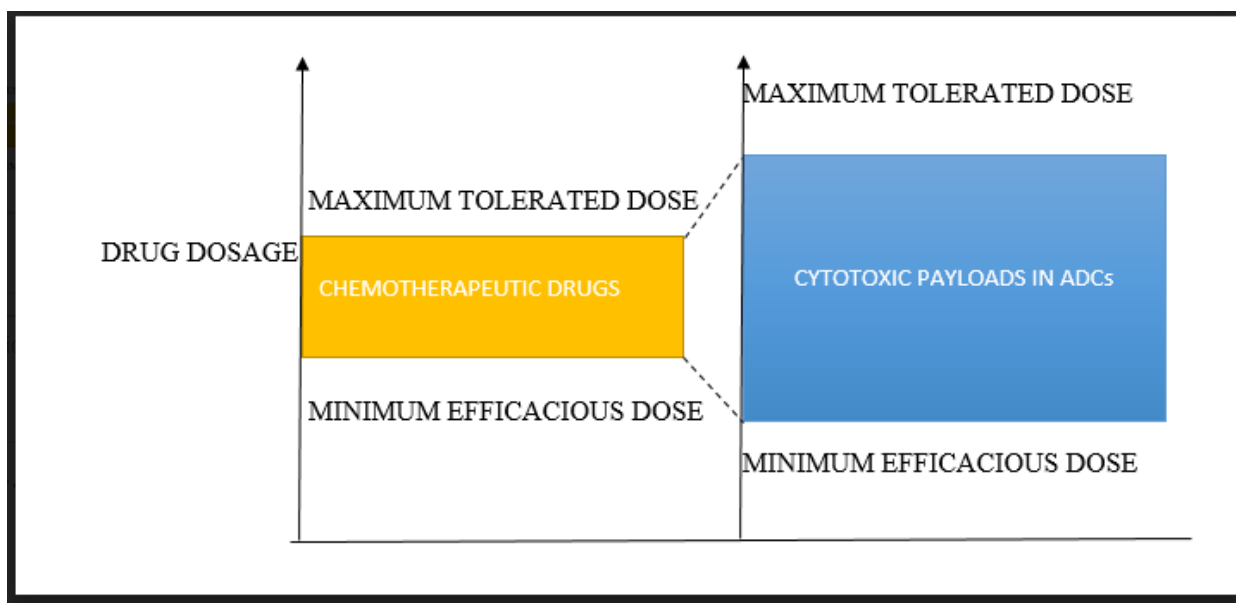


Figure8:- Therapeutic windows comparison between ADCs and chemotherapy.

- (ii) It has higher specificity and the release of drug can be controlled.
 (iii) The drug is released only when the conjugation reaches the target organ and hence the amount of drug required is also less.

11. Drawbacks of adc:

Everything that has advantages also pose some disadvantages, the drawbacks of the ADCs are as follows:

- Due to common knowledge, the monoclonal antibodies chosen for making ADCs were of high affinity but after recent studies it has been found that the mAb with low affinity have high penetrance with respect to solid tumour cells.
- The target antigens are always not exclusively present on the tumour cells and hence unwanted toxicity towards other normal cells cannot be prevented.
- The multi drug resistance pumps (MDRP) in our cellular systems actively resist the ADCs and hence they cannot perform their function effectively as the cell develops a resistance to them.
- The drug antibody ratio i.e. DAR has to vary with consideration to different properties like DOP (Distribution of payload). The DAR is mostly increased in order to produce the desired therapeutic effect which increases the cost of the treatment.

12. Challenges And Future Prospects

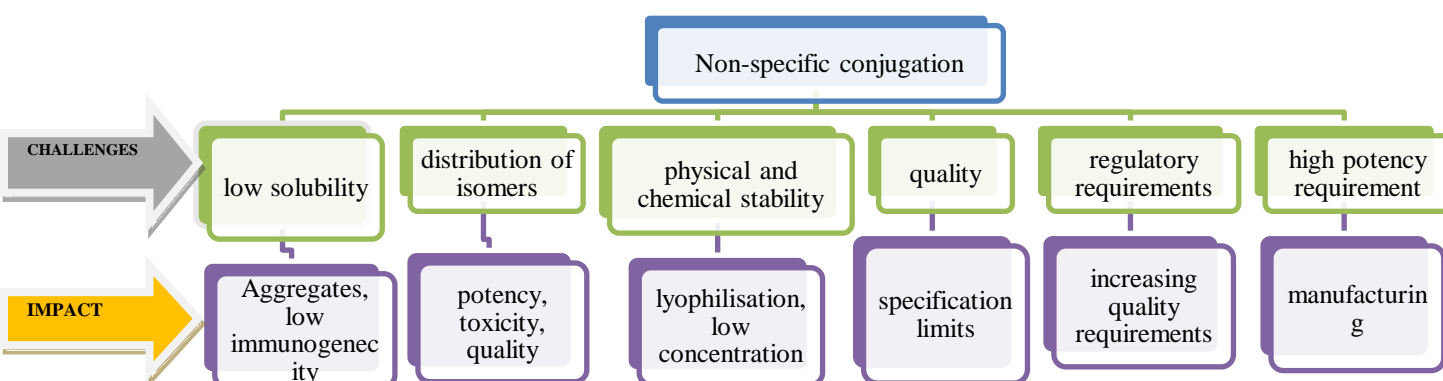


Figure9:- Challenges

Besides, this class of drugs (ADCs) gives another chance to rethink the future and possibly safe cytotoxic treatment. The mix of enhanced and upgraded potency with better tolerability offers hope to cure more life threatening tumors and, for those malignancies that can't be completely destroyed, guaranteeing an expanded treatment and an enhanced quality of life for these patients. Besides, this class of drugs (ADCs) gives another chance to rethink the future and possibly safe cytotoxic treatment. The mix of enhanced and upgraded potency with better tolerability offers hope to cure more life threatening tumors and, for those malignancies that can't be completely destroyed, guaranteeing an expanded treatment and an enhanced quality of life for these patients. A century after Paul Ehrlich, his test has been taken up by another era of researchers who are working perseveringly to enhance the specificity and action of growth chemotherapy. In spite of the fact that ADCs have quite recently come up in the limelight, the development of the field is quickly spreading and the effect on cancer care is probably going to be worth the wait in the years to come.

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