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Preclinical Experimental Screening Models For The Assessment Of Anti Cancer Drugs

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ABSTRACT :

This study aimed to explore the correlation between durability and tumor petrification in patient with metastatic interreelated cancer who had received cetuximab combined with chemotherapy. This study was single center retrospective analysis that has been enlistment the 111 patients who had received the therapy in between April 2011 and October 2016**.**Tumor petrification and regimen efficacy were appraised sovereign by radiologists on the basis of computed tomography scans. Associated between tumor petrification and clinical characteristics, tumor replication rate and patient durability were analyzed. Among the 111 enrolled patients ,27 had tumor calcification ((27/111)(24.3%)).In the past decade, TumorFlare Reaction (TFR) was considered as a new side effect associated with Immunomodulatory agents and as a condition of chroniclymphaticleukemia(CLL) The incidence of TFR in CLL ranged from 28 to 58%umor response in patient treated beyond progression was reported in 97% with prebrolizumab and in renal cell carcinoma 69% with nivolumab**.** Rare life -threatening or fatal causes were reported, symptoms were usually mild. Therefore, the recognition of suitable clinical benefit criteria in prospective trials for a better perception is compulsory. One of the most well known method of inducing spontaneous regression of cancer is the application of Coley's toxin(Heat -killed streptococcus pyrogens and secratia marcescens) ,which has been used for the success full treatment of sarcoma**,** carcinomas**,** lymphomas**,** myelomas**,** melanomas. use of Bacillus Chalmette Guerin vaccine is the treatment of urinary bladder cancer. Bacteriolytic **,**Bactofection therapy or treatment and bacteria directed enzyme therapy are the some of potential cancer treatment modalities that use microorganisms.

Key words: Anticancer, experimental models, Tumour flame reaction, coleys toxin, bacteria directed enzyme therapy.

INTRODUCTION :

Cancer is defined as 'Generic terminology for a group of diseases that are characterized by abnormal proliferation of cells'. This pathological expansion of cells can permeate to adjoining tissues and remote organs. Various types of models to summarized in this. It is important to keep in consideration. These experimental screening models not directly 100% correlate to human cancer. On the models may or may not be active in a similar manner on humans in clinical trails. Also, cancer advancement has much reductant. This vigorous and changeable nature of human cancer and the variation in the rate of advancement of cancer in animal models when contrast to humans must be regarded during the evaluation of these observations of these experimental models correlate with human cancer 1-5 properly .preclinical experimental screening models are used to identify the new therapeutics agents in combination with stradads approved[1,2] .convenventional experimental screening models have been attrition rate of 95% that is, the success rate of 1 in20 for oncology drugs, and are also time consuming. Such attrition rates are because of limited Strength, efficacy ,and predictive capability of various preclinical screening designs and models have led to higher attrition rates. These screening methods are newer Efficient, it is help to screen the cancer [1,3] .

MATERIALS AND METHODS:

❖ In these various experimental screening models are involved:

➢ **INVIVO MODELS:**

1. Carcinogenic induced tumour models
2. Xeno graft models with established tumour lines
3. Hallow fibre assay(HFA)
4. Genetically engineered mouse models(GEMM)

➢ **INVITRO MODELS :**

1. enzymatic assays

1.1 Tetrazolium salt assays

- MTT Assay
- XTT Assay
- MTS assay

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1.2. Sulforhodamine B assay
2.Membrane integrity assay
2.1.Tryptan blue exclusion dye test
2.2.H-Thymadineincorporation assay.

IN-VIVOMODELS:

1. Carcinogen induced tumour models:

This is the one of the preclinical screening model, it is widely used and this is thematriarchmodel. This carcinogen induced tumour model also called as Autochthonous tumor model. In clinical trails this model has high translation and high correlation and helps to elucidate the various step carcinogenesis and the mechanism of cancer or tumour development[1,4].In the year of 1918,yamagiwa and Ichikawa inject the tumour cell on rabbit skin and cancer cell were inject into the mice for evaluation of NCE for combined with anti cancer property for the reduction of size of tumour .promotersarethe multiple exposures to other agents ,promoters are acts as stimulating inflammatory response and proliferation of DNA damage [5].
Some of the carcinogens are majorly used those are DMBA (7,12-dimethyl-benz(a) anthracene), AOM (Azoxymethane), NMU (N-nitro-N-methylurea), MNNG (N-methyl-N-nitro-N-nitrosoguanidine). some tobacco-smoke related carcinogens and nitrosamines like DEN (diethyl-nitrosamine), NNK (4- methyl – nitrosamino - 3 - pyridyl - 1 - butanone).
This model have some merits related to the clinical relevance. Carcinogens are induce lesions those are organ specific and reproducible and have a high correlation with human cancer inas much as molecular, biochemical similarities and histopathological. Induced tumour have capability to metastasizing to other organ ssimilarities,Apart from the benefits it have some restraints and blemish or imperfections. In this major draw back that is ,it requires for the carcinogen to introduce a tumour and lack of immune system it takes much time .Time of incubation of tumour depends on that provided dose, dose timing, rodent strains and dose frequency .coming to demerit, it is a time dependent manner, explain the various steps of initiation and progression of tumor development.

2. Xenograft models with established tumour lines: Tumour cell licensor tissues are isolated from the same or different species of rat and mouse and also isolated from the Various human cancer cell lines those lines are implanted in mouse and rat that is immune compromised .Amice which are immunodeficient in that mice tumour cells are implanted. A normal mouse's have adaptive immune system and also humoral immune system will induce an immunogenic response lead to cause apoptosis of the implanted tissue/cells ,those cells are rejected in the time of results,nouse if the tumour has to be induced in that time. But on the other way, tumour induction is much more effective in immunocompromised mice because the implanted tissue/cells are not easilyrejected.[8,9].The evaluation of the potential system is based on the change in the size of the tumour ,after the introduction of the measured compound in the tumor-induced mice. Based on the region of transplant, xenograft models [1] divided into two types:
a) **Ectopic xenograft model -** In this model the tissue/cell is implanted on a non-analogous region from which it was obtained. Majorly in mice, subcutaneous injection which allows the tumour to promote on the surface of the mice.Themost prominent advantage of ectopic xenograft model is it makes the visual observation and quantification or measurement of the tumour grow on the surface of the mice/rat over time relatively easy. Tumour growth characters are different from the origin of tumour [10,11].
b) **Orthotropic xenograft model** - It allows for the tumour tissue/cell to develop in the region which is analogous to the origin of the primary tumour. Coming to benefits of this model ,this model has high correlation and tumour growth is takes place in the analogous region.

Coming to demerits, Difficult to visual observation and tracking of tumour growth or metastasis and much animals are requires for this model It is very Expensive and complicated techniques are also an option if sacrificing is not feasible like using cancer cell lines with express markers those marks are related to the fluorescence or luciferase which then can be observed using optical imaging, computerized tomography . Other disadvantages include the requirement of well-trained experts with surgical skills for the surgeries for transplanting the tumour tissue/cell to the orthotropic region. Also, the endpoint for determining the effects of therapy are very complex and most often, survival is the only feasible and practical endpoint[10,11].

3. Hollow Fibre Assay (HFA): This assay was developed by the Hollingshead et. al at NCI and it is composed of 2 CM tubes filled with tumour cell lines[12].
HFA is not a self -organized or not a standalone model and It is replacement for a secondary model. HFA also compute the activity of anticancer drugs abutting cancer-induced animals via various cancer cell lines on immunodeficient mice, equitable the method of initiation and site of initiation are the variables that have changed. The method of induction and site of induction in HFA is what sets it apart and makes it very reproducible, efficient, and easy to observe and can not be used as standalone model because which have low correlation and does not simulates the biological activity of cancer growth [13].Hallowfibre assay model is depend on the n tumour cell's ability to form a tumour in hollow tubes ,those hallow

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tube consisting of Polyvinylidenefluoride.. The inner layer of the hollow tubes is merged into a layer of living cells that are in associated with necrotic cell or death cell. Tumour cell lines are cultured until they reach the log phase growth stage, After some time, which the cells are introduced inside hollow fiber tubes some parameters must be follow, those parameters are 1 mm diameter and 2 cm length aftertime of use of hallow fibre tubes .Those tubes are cultured, that tubes are incubated for 24 to 48 hours. After 24-48 hrs, incubated hollow fiber tubes are Implanted either subcutaneously / via intraperitoneal route into an immune-deficient mice strain, to reduce /reduction to usage of much animals, three implantation is done in each animal which animal is used in this model[14].After 3-4 days, the test drug is administered viaintraperitonealfor the next four more days while the implant is still inside of the mice. On the 5th or 6th day, the tubes are removed, and the cell viability assays are performed like MTT Assay to count the viable cells and contrast correlate the control, test, and standard groups and consequently , the percent inhibition can be stubborn.Cell cycle analysis, induction of apoptosis can also be determined [14].Similarly, the drug is tested for subcutaneous injection and after it passes both intraperitoneal and Subcutaneous routes .One more demerit Is left, Hallow fibreassay model has the limitation of space for the tumour to grow, those tumour growth is limited by the diameter of the hollow tube. One more drawback is tubes are allow to permeation of particles upto 500 kDa.
This assay is valuable, rapid model system with predictive values[15-17.]

INVITRO MODELS:

Various approaches in-vitro methods have emerged, which rely mainly on cell-based screening to evaluate potential anticancer drugs for their efficacy. An in-vitro assay is a mechanism based approach that is, it targets the molecular malignancy, which is either established the reason for cancer[18,19.] In vitro models can be performed on a small scale but preferably on a commercial scale that is, it is performed via HTS means High Throughput Screening. HTS is a process of screening a large number of compound against a number of targets per unit time, which generates more hits in less time and then subsequently generating more products in less time. HTS can screen up to 1,00,000 compounds in a day and above 1,00,000 is called ultra HTS[20,21]. In vitro models are either cell based assays or bio chemical assays. Cell-based assays are types of in vitro assays techniques in which the test compounds are tested against living cells. Examples of responses from a living cell can be cell viability, cell death, motility, proliferation, toxicity and change in morphology eg. Cell wall disruption .Depending upon the response or property which is being required on the living cell upon the action of the drug, there are different types of cell based assays are

❖ **Phenotypic assay**: It is used to evaluate the cellular processes like cell motility, cell proliferation, cell viability, cytokinesis.
❖ **Second messenger assa**y: It is used to evaluate the second Messenger mobilization in various cellular pathways.
❖ **Reporter gene assay** :It is used to evaluation of transcriptional and gene expression activity. Biochemical assays use biochemical aspiration secluded from the cell on behalf of using the whole cell. While cell based assays are more biologically relevant, biochemical assays are easier to perform[20-24].

Biochemical assays are also known as mechanism based assays . In vitro methods are less expensive and less time consuming process. It allows or to concede screening of a larger number of test compounds or amalgamation with in short time .

ENZYMATIC ASSAYS :

1.1:Tetrazolium salt assays : Presence of mitochondrial enzymes, those salts are under go reduction. Mitochondrial cells are present in cytosol of the cell and it is leads to formation of coloured compound. Those coloured compound are called as formazan .viable cell have the metabolic activity, so the presence of mitochondrial enzymes indicates the viable cell.
Non viable cells are not capable of reducing the salts, in this colour change indicates the cell viability and used for cell viability assays .Various tetra zolium salts are available those are MTT, XTT, WSTS. These three types of salts are salt based viability assays. These salts sensitive to light and this assay are performed in dark conditions [18,25,26].

MTT assay:MTT (3-(4,5-di methylthiazol -2-yl) -2,5-diphenyltetrazolium bromide) is a tetrazole which is yellow in colour .After it reacts with the mitochondrial enzymes, it is converted into a purple colour formazan compound[18,25,26].

XTT assay:XTT (2,3 -bis-(2-methoxy -4-nitro -5-sulfophenyl) -2H-tetrazolium -5-carboxyanilide) thisis also one of the tetrazolium salt, it is used in cell viability assay based on the principle of MTT assay. This assay is used to reduce the limitations of MTT assay and slowly replaced the MTT assay. This assay is more accurate and sensitive. This assay used to solubiling solvents is reduced [18,27,28] .

MTS assay :(3-(4,5-dimethylthaizol-2-yl) -5-(3-carboxymethoxyphenyl) -2-(4sulfophenyl) -2H-tetrazolium) this is the another tetrazolium salt ,those are under the presence of **phenazine methosulfate** .It produces the coloured formazan product. In the presence of phosphate buffered saline this formazan is qualified at the 490 nm. In this assay, there is no need to do the intermediate steps we can directly added reagents to the cell culture, this is major advantage. MTS assay is also known as one step MTT. This assay is used as a confirmatory assay after MTT, XTT or any other type of assays. [18,29,30].

1.2. Sulforhodamine B Assay(SRB): Itis a rapid, sensitive, inexpensive method. This method is suitable for an ordinary laboratory as well as for a very large scale anti -tumor screening[31]. Sulforhodamine B assay is in the form of pink coloured

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aminoxanthene dye, it is anionic in nature .under the acidic conditions it have the capability of binding to the basic amino acid group of residues Cell culture takes much dye as required for the reaction, placed the cell culture in incubator for 30 -35 min ,the remaining indicator is removed or washed by using 1% (vol/vol) of acetic acid. Measure the absorbance from 560 to 580 nm.
The staining of Sulforhodamine B dye is stoichiometric in nature, after incubation the amount of dye that is obtained from cell culture is directly proportional to cell mass. Tetrazolium salts assays, although are adequate for reliable quantification of viable cells. Many variablesare occur when the duration of incubation with dyes and cell culture is not proper then it leads to formation of formazan the dyes or reagents are toxic to some extent and can result in cytotoxicity. To subdue these limitations, majorly sulforhodamine B assay is used.
The end point of SRB is not destructive, not time critical and comparable with other flouroscence
Assay [18,31-33].

Propidium iodine assay : In this two cationic dyes are present that is, Ethidium bromide and PI, these are pass through the membrane of dead or drying cells and intractable with DNA.
These dyes are bind with DNA then increase their flouroscence. Cells are incubated with PI and the number of non viable cells are assessed by the subsequent flouroscence detection.
At -20, the second measurement will be taken after freezing the cells for 24 hours.PI will be intercalated into the DNA of all cells and the difference into the measurements it gives number of viable cells.
Coming to asset, it is simple, rapid, with out washing step.
Coming to detriment, PI also binds with double stranded RNA which present in cytoplasm. But this can be reduced to using the RNAase enzyme during the experiment[31].

Membrane Integrity Assay:

2.1.Tryptan blue exclusion dye test : This assay is a very honest and primordial assay For the determination of cell viability and cytotoxicity in cell culture .In this assay Tryptan Blue is used as a dye This Tryptan blue dye have the capability to interact into non intact cell membranes and also bind to the intracellular proteins then cell colour turns to blue colour . This assay is based on the assumption that every viable cell that is intact in nature will have a well maintained cell membrane and which will cure the inner cellular structures and cytoplasm from dyeing where as, dead cell do not have intact cell membranes, the inner cellular structures are exposed to dye like Tryptan blue, eosin.
When a cell culture is combined with indicators like Tryptanblue and going to incubation for 30 min then, live cells are involved or interact with indicators After some time ,only cytoplasm and death cells will get dyed or coloured. These wills indicate the count for viable cells. Cell viability can be determined prior to incubation with the test drug and after to determine the efficacy of the drug[34].

2.2:H-Thymadine incorporation assay :3H-Thyamidine is a nucleoside, it is radioactive in nature. This assay is used to appraise the evaluate cell viability, cell proliferation, cytotoxicity. 3H -thymadine is amalgamation into the chromosomal DNA strands of cell culture , then it undergoes proliferation, new cells are formed with the characters of same modified DNA which will contain the 3 H thymadine, By using a scintillation beta-counter ,easily identified the 3H -thymadine contains modified DNA .By using this counter technique it gives a data about the direct quantification of the extent of cell division is takes place. [18,37,38].

Non-conventional newer models:

1. Anti-angiogenic models :

Angiogenesis is a physiological multi step process by which the formation of blood vessels occurs from a new site or from pre existing vasculature. Angiogenisis a normal physiological process that is required for the formation of new blood vessels especially during embryonic stage, wound healing, inflammation. However, tumour development and metastasis during cancer are known to depend on Angiogenisis which serves as a feeding or nurturing mechanism for the tumour cells [39,40] Thus, targeting Angiogenisis could prove a very efficient strategy to control tumour progression and metastasis[41].

1.1. Zebrafish: Caudal fin regeneration model: Zebrafish is a fish found in fresh water belonging to the family **minnow** .It is a tropical fish [42,43] it is an important and relatively newer vertebrate model organism in drug development. It has regenerative abilities, the embryo is transparent and develops out side their mother, therefore it is easy to study Angiogenisis in real time during the embryonic development phase, the genome is already completely sequenced. The DNA make up and general anatomy of zebra fish's organ system is very similar to that of humans, easier drug administration[44-46] Apart from clinical advantages, zebrafish's availability is very low and economical. The maintainance cost of zebrafish is just 1% of the total maintainance that of mice[46].
This model is depends on the regeneration property of Zebrafish. Zebrafish can undergo Angiogenisis like apoptosis ,cytokine and vasculature very quickly or rapidly or swiftly, which is also a crucial element in metastasis and

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tumour development. Angiogenisis growth factors released by cells like VEGF and FDF are responsible for tumour development and the absence of these factors will let the tumour in a state of dormancy.
Adult zebrafish is kept for an administration period for about 15 days in an laboratory conditions and are divided into experimental groups that is, vehicle control group, standard group, test groups. Caudal finof Zebrafish are amputated using a razorblade. After which the reclamation of the caudal fin is photographically recorded or documented. Prior to the step of amputation of fins and during the observation, Zebrafish are insentience using a drug that is tricaine .Images are take prisoner frequently to advertence the growth of the caudal fin at every stage. Following vascular parameters are evaluated [45-48] .

1. Total Regerated Area(TRA)
2. Vascular Projection Area (VPA)

CAM MODEL : The chick chorioallantoic membrane(**CAM)** is an embryonic membrane which comprises of dense blood vessels and lymphatic vessels. CAM has network of capillaries and is highly approachable as it is originally proximately to the outer shell of the egg. The CAM model is an efficient tool to observe. Mainly there are 2 types of CAM assay [49] .

- **In-ovo method :**It is the primeval method to be used which had the advantage of excessive feasibility of the embryo but limited attainability to the chick chorioallantoic membrane. .
- **In- ovo method:** This is the method introduced in which the cultures of chick embryo were shell less .

CAM model basically includes grafting of tumour including agents and test compounds onto the developing CAM as per the respective experimental groups The egg used are of fertilized white leghorn chicken eggs, which are 3 days old. The CAM is then prepared for the grafting by removing 3 ml of albumin out using a syringe to create an air sacs. Air sacs allows for intact CAM development. On the 8 or 9, a square window cut out is made on the shell of the egg. After the disk application (disk made up of what an paper, nitro cellulose membranes, gelatin sponges, inert synthetic polymers that are soaked in the test compounds window is sealed to prevent decontamination. Visual advertency and measure or quantify of the Angiogenisis are accomplishing four days after the disk application. Following vascular parameters are evaluated[50] .

1. Total Regerated Area **(TRA)**
2. Vascular projection Area (**VPA)**

CONCLUSION :

Preclinical experimental pharmacology which involves the identification and optimization of chemical lead structures. Conventional pre clinical screening models for anti cancer drugs, nevertheless are powerful tools in drug amelioration but are totally deceptive and thus the situation of 95% friction or corrosion rates in clinical trials .Drug development for cancer involves a slight number of screening models with respect to the pre clinical stage. Xenograft model, hallow fiber assay are enormously used in vivo models but are slight in delivering high association because of the lack of immune system participation in the assessment process.
The assessment of outcomes in in-vivo techniques in general for anti cancer drug screening is subjective and relies on personal interpretation, which can bring upon discrepancies. In-vitro techniques, however make use of standardized evaluation of the outcome like spectrophotometric techniques, micro titer plate reader in HTS.
Non conventional newer screening models like Zebrafish: caudal fin regeneration assay and Chorioallantoic membrane assay have been flourish Those models are being continuously explored and modified to increase their authenticity and dexterously in screening anti cancer drugs.

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