Introduction

1. INTRODUCTION

1.1 CANCER

Cancer is defined as disease in which some abnormal cell divide uncontrolled. Cancer has ability to invade other tissue. Cancer cell can also spread to other body part by blood and lymphatic system. Cancer is not considered as one disease but much disease. Cancer is disease in which a cell group displays without any control growth (cell division out of the normal limit), invasion and sometime metastasis (cancer cell spread to other body part or other location in the body with medium of lymphatic system and blood circulation). Almost all cancer creates tumors but some cancer like leukemia (blood cancer) do not having tumor in any body part. Oncology is defined as branch or department of medicine related with the diagnosis, study, treatment and preventive action of cancer is formerly known as oncology.

Cancer affect to human of any ages or any fetuses. Risk factor for cancer for most varieties increase as age increase. Cancer cause around 13% of all death. As per research of Cancer Society of American, 8.2 million human being dies because of cancer in world during 2008. Cancer also affect to all animals.

1.1.1. History

Carcinoma is Greek medical term is for a malignant tumors derived from epithelial tissues. Carcinos is translated term into latin cancer which is also known as crab. Scientist Galen termed “oncos” to represent tumor, which is source for the latest word termed as oncology. Approximately 1600 B.C, Egypt is a country from where oldest cancer surgical treatment was discovered. After that, Second cancer surgical treatment discovered by Avicenna from Canon of Medicine. Root cause for breast cancer is due to milk clotting in mammary gland which was discovered by German Professor named Wilhem Fabry. In 1775, First root cause of cancer found by British surgeon Percivall Pott, that cancers of the scrotum was common disease throughout chimney sweep.

1.1.2. Types of Cancer

There are 100 types of cancer available. Types of cancer can be grouped into broad category based on type of cells that forms the tumor and so, tissue is the origin root of tumor.
Main category of cancers includes:

- **Carcinoma**: A tumor (usually malignant) produced from epithelial tissue. This cancer group describes the common cancer, in which include common form of breast cancer, prostate cancer, lung cancer and colon cancer.
- **Sarcoma**: A tumor (usually malignant) produced from connective cells, or mesenchymal cells.
- **Germ cell tumors**: Tumor produced from toti potent cell. In adult human, most commonly seen in testicle tissue and ovary; in fetuses, babies, and young children most commonly seen on the body mid line, exactly on the tip of the tail bone; in horse most commonly seen on the poll (skull base).
- **Blastoma or blastic tumors**: Maliganat tumor which found from immature embryonic tissue. Blastoma is mostly seen in children and infants.
- **Leukemia**: These cancer cause from blood generation tissue like bone marrow cells and generate huge numbers of abnormal blood cell and enter into blood.
- **Lymphoma and myeloma**: Lymphoma cancer is due to immunological system of body.
- **Central nervous system cancers**: Central nervous system cancer is due to brain cord tissues and spinal cord tissues.

### 1.1.3. Origin of Cancer

All type of cancer start with cells which is basic unit of body. It is important to know mechanism for conversions of normal cells into cancer cells for understanding of cancer root causes. Construction of body is of large number of different type of cells. As per normal mechanism of growth and division of cells, cells are in controlled pathway to create new more cells for keeping healthy body. Replacement of new cells with old cells occurs when cells become damaged or old. However, sometimes this ordered procedure goes in wrong pathway. The genetic materials like DNA, RNA of cells can becomes damaged or changed, produce mutation that effect to normal cell growth, cell division process. When this type of condition happened, new generated cells do not die even body does not need new cells. The excess new generated cells form a tissue mass known as tumor.
1.1.4. Signs and Symptoms

Roughly, Symptom of cancers can be divided in three major group:

- Local symptom: Unusual lump or swelling, hemorrhage (uncontrolled bleeding), pain and ulcer. Comparison of surround tissue may create various types of symptom like jaundice in which color of eyes and skin becomes yellowish.
- Metastatic Symptom (spreading): enlargement of lymphatic node, coughing and hemoptysis, hepatomegaly (enlargement of liver), pain in bone, easily cracking in bone and neuron related symptoms. Advanced cancers may painful, which is not first symptoms.
• Systemic symptom: Decrease in body weight, decrease appetite power, lethargic, excessive sweating in which mostly night sweats occurs, anemia and specified neoplastic phenomena occurs, i.e. Due to thrombosis changes or hormonal changes, specific conditions occurs due to an active cancer.

**Common sites and symptoms of Cancer metastasis**

- **Brain**
  - Headaches
  - Seizures
  - Vertigo

- **Respiratory**
  - Cough
  - Hemoptysis
  - Dyspnea

- **Lymph nodes**
  - Lymphadenopathy

- **Liver**
  - Hepatomegaly
  - Jaundice

- **Skeletal**
  - Pain
  - Fractures
  - Spinal cord compression

Figure 02: Common sites and symptoms of cancer metastasis

1.1.5. Diagnosis

Diagnosis of cancer generally required microbiological and histological investigation of a tissue sample or blood sample by pathologist, still starting direction of cancer can be symptom observed or radio graphical abnormality detected. Almost all types of cancers are initially diagnosed by either by sign/symptom appearance or with screen. This type of diagnosis should only be done by suggestion of a pathologist or doctor who are specialized in diagnosis of all type of cancer and other disease.
1.1.5.1. Investigation

Investigation of people who are suspected with any types of cancer are done with various type of medical test like blood test, X-ray, CT scan and endography.

![Chest X-rays investigated lung cancer in the left lung](image)

Figure 03: Chest X-rays investigated lung cancer in the left lung

1.1.6. Anticancer Agents

Table 01: Classifications of currently used anticancer agent in market

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylating agent</td>
<td></td>
</tr>
<tr>
<td>Triazine</td>
<td>Dacarbazine</td>
</tr>
<tr>
<td>Ethylenimine</td>
<td>Thiotepa</td>
</tr>
<tr>
<td>Alkyl sulphonate</td>
<td>Busulfan</td>
</tr>
<tr>
<td>Nitrosourea</td>
<td>Streptozocin, Carmustine</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>Cyclophosphamide, Chlorambucil</td>
</tr>
<tr>
<td>Antimetabolite</td>
<td>5-Flourouracils, Methotrexate</td>
</tr>
<tr>
<td>Antitumor antibiotic</td>
<td></td>
</tr>
<tr>
<td>Anthracycline</td>
<td>Doxorubicin, Daunorubicin</td>
</tr>
<tr>
<td>Others</td>
<td>Actinomycin,</td>
</tr>
</tbody>
</table>
### Introduction

<table>
<thead>
<tr>
<th>Topo isomerase inhibitor</th>
<th>Mitomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topo isomerase I inhibitor</td>
<td>Topotecan</td>
</tr>
<tr>
<td>Topo isomerase II inhibitor</td>
<td>Etoposide</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mitotic inhibitor</th>
<th>Vinca alkaloid</th>
<th>Vincristine, Vinblastin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epothilone</td>
<td>Ixabepilone</td>
<td></td>
</tr>
<tr>
<td>Taxane</td>
<td>Paclitaxel</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>Dexamethasone, Methyl Prednisolone</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Miscellaneous</th>
<th>Asparaginase</th>
</tr>
</thead>
</table>

### Alkylating agents

- **Carmustine (01)**

- **Cyclophosphamide (02)**

- **Thiotepa (03)**

- **Busulfan (04)**
Antimetabolites

5-Flourouracil (05)

Methotraxate (06)

Antitumor antibiotics

Actinomycin-D (07)

Doxorubicin (08)

Topoisomerase inhibitors

Etoposide (09)
1.2 TARGET FOR CANCER $^{11-14}$

Current approach to cancer chemotherapy embraces an eclectic mixture of drugs and techniques, all designed to target selectively cancer cells. Real therapeutic progress has been achieved, although 'cancer' as a disease has not been defeated and remains a massive challenge for future generations of researchers. In this therapeutic area, probably more than in any other, the debate about the risk-benefit of treatment and the patient quality of life issues has taken centre stage and remains a major area of concern. Many advanced cancers remain essentially incurable, and there is a common perception that chemotherapy, with its distressing unwanted effects and minor increases in longevity, is largely superfluous in such cases. This is not necessarily the case: chemotherapy, although often unpleasant for the patient, may be a superior alternative to conventional palliative care. In the case of breast cancer, even extremely modest increases in life expectancy are sufficient to persuade women to a course of chemotherapy (in addition to surgical
Introduction

(resection), although this is also influenced by other domestic factors. The quest for less toxic forms of therapy is, of course, central to anticancer initiatives, and there is a bewildering array of new (or usually modified) drugs or novel combination regimens in clinical trial or at earlier stages of development. What follows is a selection of new and different approaches to the treatment of cancer that may bear fruit over the next decade. There are many targets for cancer therapy which are described as under:

1.2.1 Tyrosine Kinase Inhibitors

Tyrosine kinases are a subclass of protein kinase. Tyrosine kinase is enzyme that having ability to transfer phosphate group from adenosine triphosphate to tyrosine residue present in protein. Tyrosine kinase are a subclass of huge class of protein kinase. Phosphorylation reaction of protein with kinase is main mechanism in transduction of signal for control of tyrosine kinase activity. Protein kinase are unique and important component of transduction of signal path that transfer information related to extracellular or cytoplasm condition to nucleus, thereby affecting genetical transcription process or synthesis of deoxyribonucleic acid. The human gene contain around 550 protein kinase and an additionally 130 protein phosphates that control the activities of the different type of protein kinase. Classification of Protein kinase can be into three various categories:

1) Tyrosine kinase, with activity for tyrosine residue only
2) Serine/threonine kinase, with specific activity for serine and threonine residues only
3) Kinases with specific activity for all three residues

Tyrosine kinase can be again subdivided in protein that have extracellular ligands binding domain (receptor tyrosine kinase) and enzyme that are confined to cytoplasmic material and/or nuclear cellular compartment (non-receptor tyrosine kinase). Abnormally activation or inactivation of protein tyrosine kinase has been seen in much human neoplasm, make them good molecular target for cancer treatment. There is currently three type of small molecular weight tyrosine kinase inhibitor that has approval of food and drug administration and many other target molecules are under clinical trial.

1.2.2 Angiogenesis and metalloproteinase inhibitors

Tumor cell produce metalloproteinase and angiogenic factor that facilitate tumor growth, invasion process of normal tissues and metastatic. Target the mechanism involved can provide us
with drug or molecules that blocking metastatic. Several inhibitors of angiogenesis or metalloproteinase are in clinical trial.

1.2.3 Cyclooxygenase Inhibitors\textsuperscript{16}
There is strong epidemiological evidence that chronic use of cyclo oxygenase (COX) inhibitors protects against cancer of the gastrointestinal tract and possibly other sites as well as the COX II is excess expressed in about 85\% of cancers and prostanoid starting from this sources may involve in activation of signal path that make able cell to escaping from apoptosis cell death. The COX II inhibitors like celecoxib, rafecoxib reduces mammary and gastrointestinal cancer incidence in animal models and causes regression of existing tumors, and it is in trial in humans as an inhibitor of a familial type of colon tumor. Overall, COX-2 is now considered to be a potentially important target for anticancer drug development. The recent literature is daunting and includes some debate about their precise mechanism of action.

1.2.4 p53 As Anticancer Agents\textsuperscript{17}
More than 50\% of human tumors carry p53 tumors suppress gene mutation, and there have been many attempts to capitalise on this. Virally mediated introduction of the wild-type (normal) p53 gene has not been very successful, but therapy with oncolytic virus ONYX 015, given into the tumor in conjunction with standard chemotherapy, has given good preliminary results.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{p53_figure.png}
\caption{Schematic illustration of the role of p53 protein\textsuperscript{17}}
\end{figure}
1.2.5 Antisense Oligonucleotides\textsuperscript{17}

Antisense oligonucleotide is synthetically sequence of one strand deoxy ribonucleic acid complement to specified coding region of messenger ribonucleic acid, which having ability to decrease gene expression. An antisense molecules or drugs, augmerosen, down regulate the anti apoptotic factor. In an early clinical trial, it sensitized malignant melanoma to standard anticancer drugs. These 'drugs' have to be delivered by viruses or other 'vectors'.

1.3 RAF INHIBITOR

1.3.1 Introduction to RAF-1\textsuperscript{8}

RAS-RAF-MEK-ERK pathway shows a downstream path for some important growth related tyrosine kinase receptor in which frequently mutation occurs or over expressed in cancer so these pathway is formerly known as a logical therapeutically target of cancer. Still that this pathway considered in growth promoting pathway in some contents, it is also same as apoptosis and suppressing. Some new drugs or molecules target these paths have been developed and currently are under clinical trials. From that, Best of one most important novel molecule is BAY 439006. In initial stage, it was developed as RAF inhibitor, but after that it was observed that it also target some useful tyrosine kinase like VEGFR-2, FLT-3. This tyrosine kinase having important contribution in anti-proliferative and anti-angiogenic property. Today, by use of BAY 439006, very good results have been seen especially in kidney cell cancer which is strongly related to vascular tumor. These reviews will give overall of ERK signal path in common and neoplastic cells, with especially target on new therapy by target the ERK path at the level of RAF kinase.

![Figure 05: Mechanism of Action of RAF Inhibitor\textsuperscript{18}](image-url)
### 1.3.2 RAF as a therapeutic target of cancer\textsuperscript{19-20}

Some important processes like Proliferations, differentiations, cells death are coordination process which all is proved helpful in maintenance of homeostasis amongst diverse cell type in upper level microorganism. Growth factor have important role in regulation of these all processes. Growth factor exhibits its role by targeting signaling transduction cascades with bind to its cognate membrane receptor. Some other component of this regulatory cascade from the growth factors to final effectors become out of regulation and contribution to transformation and tumor genesis process. So, RAF termed from V-RAF that is responsible for rapid growth fibro sarcomas in mice is a serine/threonine kinase which is a part of the mitogen activated protein kinase signaling transduction. There are mainly three type of RAF like RAF-1, A-RAF and B-RAF.

All type of RAF have difference in their tissue distribution like RAF-1 is unique distribution, which is totally opposed in the type of A-RAF, which is almost fully abundant in urological organ, like kidney, testis, ovarian, prostate gland, and B-RAF is seen in neural tissue cells. So, overall these all three type of RAF family member like RAF-1, A-RAF and B-RAF can be represented in some hematopoietic cell of both myeloid and lymphoid origin. Total Twenty two RAF kinase inhibitors take part in the RAS-RAF-MEK-ERK signaling transduction cascade that controls cell growths, differentiations, and proliferations process in order to external stimuli. By Activated RAF, Stimulation of cascade is started as well as MEK, ERK are sub sequentially phosphorylated and activation occurred. Then ERK controls functioning of cells by Phosphorylation of about more than fifty substrate in cytosols and nucleus membrane. Mutation form of RAF oncogene has been observ in diversed neoplasia, like hematopoietic, breast, cervical tissue, renal, laryngeal, hepatic cellular and lung carcinoma, and in lung biopsy recovered from cigarette smoker. Over expressed RAF observed in lots of malignant hematopoietic cell line. So, roles of all type of RAF in the starting transformation event is not clear still the line was create tumor.

Mechanism which is responsible for activation of RAF expressed including mutation, deletion, genetically rearrangement, and genetically amplification. In other addition, there is also mutation in that elimination of RAF kinase activity. Mutations that alter the position of lysine in ATP
binding site of the catalytic domain render the kinase into inactivation mode. B-RAF mutation observed in around 2% of human cancer, with specifically large number of frequency in melanoma (45-65%), ovarian tissue (30%), thyroid gland (25%) and colorectal (9%) cancers. Most frequently mutation (90%) observed is valine amino acid for glutamic acid substitution at position 600 termed V600E, B-RAF. V600E and B-RAF showing permanent 500 times fold elevation in kinase activity. Other additionally, Stimulation of V600E and B-RAF constitutes ERK signaling in human cell and it transforms establishment of new cells, permit them to grow as tumor in nude mice. In melanoma, Stimulation of V600E and B-RAF, proliferation and survival occurs. Decrease in B-RAF signaling with RNA inhibition in proliferation process and improve apoptosis. This data do validation of B-RAF as useful and exciting target in mammalian cancer.

1.4 RECENTLY APPROVED/ MARKETED RAF INHIBITORS

1.4.1 Trametinib

![Trametinib](image)

Trametinib is a cancer drug which having brand name Mekinist. Trametinib is a MEK inhibitor having potent anticancer activity. It having good result in metastatic melanoma cancer during Phase III Clinical trial. In B-RAF, Replacement of amino acid valine at position 600 leads to activation of B-RAF. Trametinib was approved in May-2013 by Food and Drug Administration as single drug and mostly used for the treatment of metastatic melanoma cancer in which mutation occurred by V600E. It was found during clinical trial that Trametinib got resistant to single drug so, Trametinib approved with combination with Dabrafenib.
1.4.2 BAY 439006

BAY 439006 is very important drug of class of RAF kinase inhibitors which is passed phase I of clinical trials and currently under phase II and Phase II of clinical trials. BAY 439006 is available in oral form like tablets and capsules in market. It is fall in novel series of compound of bi aryl urea compounds which having combination of two anticancer property which is useful to prevents tumor growth. One property of anticancer in these agent is inhibit proliferation with the means of targeting ERK pathways and second property of anticancer is that it inhibit angiogenesis by mean of targeting tyrosine kinase receptor like VFGFR and PDGFRH and related signal cascades. Initially, Development of BAY 439006 was as RAF kinase inhibitor, still it show their activity in many tyrosine kinase receptors like VEGFR-2, VEGFR-3, PDGFRH, FLT-3, which is associated with tumorgenesis and angiogenesis. During cell mechanical assay, In breast cancer cell line, melanoma cancer cell line, pancreatic gland cancer cell line and colon cancer cell line, these agent decrease basal Phosphorylation process in ERK pathway. During In-vivo antitumor screening of BAY 439006, it found that BAY 439006 is potent and dose dependent in some model. Example like HCT-116, DLD-1 is involved in colon cancer model, A548, NCIH-460 is involved in lung cancer model, and MIA, PACA-2 is involved in pancreatic gland cancer models, above all type of agents having mutation of K RAS. Wild type RAS with intact RAF involved in SKOV3 ovarian cancer cell line still over expressing epidermal factor and HER2 receptor result into constitutive activation of ERK signal pathway; COLO-205, HT-29 is of colon cancer cell lines having mutation of BRAF. MDAMB-231 is of breast cancer cell lines having mutation of both BRAF and KRAS. From above all it is concluded and suggested that BAY 439006 having unique importance in tumor dependence on signal concerned ERK pathways and useful in treatment of cancer. BAY 439006 also exhibit activity in larger colon cancer as well as ovarian cancer.

Clinical trial in phase I of BAY 439006, as a single drug and in combination with other anticancer drugs have been finished. Generally, toxicity and side effect with BAY 439006 was slightly to mild like reversibly rash produced on skin, diarrhea, fatigue. BAY 439006 also having dose limiting toxicity in which over dose may create some side effect like diarrhea, skin rash, hypertension and vomiting. Dose limit of BAY 439006 is 600 mg per day to adult human. BAY 439006 shown activities in various types of cancer tumor which is measured not only by disease regression but also by bio-marker study.
1.4.3 ISIS 5132\textsuperscript{21}

ISIS 5132 is very potent 20 nucleotide phosphorothioate 2V-deoxynucleotide. It is antisense inhibitor which targets the 3V-nontranslation region of RAF-1, messenger ribo nucleic acid, which inhibit cell growth of tumor both in vitro and in vivo. ISIS 5132 is in clinical trial of phase I and phase II completed. In phase I trial, total 87 patients involved and repeated three times and in phase II trial, total 31 patients involved and repeated two times. Clinical trial of phase I observed constantly and intermediately by dose schedule, and define modest transistance and non recurrent thrombocytopenia as main hematological toxicities. Dose limiting toxicity and side effect associated with ISIS 5132 is Hemolysis anemia and kidney failure is of this study. During phase II clinical trial of ISIS 5132 with patient, ISIS 5132 fails to exhibit clinical anticancer activity with hormone refractory prostate cancer, rectal cancer, and ovary cancer, but some cancer patient may give suggestion of cytostatic effect. Further molecular development of these agents stopped because of no clinical response of anticancer or antitumor achieved. Therefore, to disregard degradation and improvement in extracellular drug delivery, a liposome formulation and development of same formulation of ISIS 5132, and Lerafan, is currently under research.

1.4.4 Pyridoimidazolones\textsuperscript{22}

![Pyridoimidazolone](image)

Pyridoimidazolone is new potent inhibitor which target to V RAF murine sarcoma viral oncogen homologues B1 (B-RAF). There was synthesized a novel series of potential B-RAF inhibitors containing a new pyrido imidazolone hinge binding group. In the report five compounds with potency than the marketed agent sorafenib that was targeted as B-RAF inhibitor. These agents have low metabolic property and better pharmacokinetic profile that can be changed by structural modification, warranted screening of these agents in vivo to measure that this characteristic converted in anticancer activity.
1.4.5 pyrazolo[1,5-a]pyrimidines\textsuperscript{22}

\begin{align*}
\text{Ar} & = \begin{array}{c}
\text{Ar} \\
\text{Ar} \\
\text{Ar}
\end{array}
\end{align*}

Scientists have reported that the pyrazolo pyrimidine structure targeting to B-RAF from high throughout screening has been changed to incorporation of kinase hinge regions which interact with group. This molecule guide hit for optimization of lead to slightly improve in enzyme and intracellular activities while maintained better selectivity of kinase.

1.4.6 Scaffold hopping for B-RAF kinase inhibitors\textsuperscript{23}

\begin{align*}
\text{Ar} & = \begin{array}{c}
\text{Ar} \\
\text{Ar} \\
\text{Ar}
\end{array}
\end{align*}

Scientist has been employed a scaffold hopping strategy for B-RAF kinase projects and he has replaced original scaffold of pyrazolo[1,5-a]pyrimidine with thieno pyrimidine and thieno pyridine scaffold to achieve optimum pharmacophore moiety to generate new B-RAF inhibitor with better potency and pharmacokinetic property.
1.4.7 Amide and Urea analogues of benzothiazole

There was synthesized a sequence of amide analogues of benzothiazole and reported its initial screening of anti proliferative activity by use of various cancer cell line. Based on these results, he has found that the selected analogues inhibit RAF-1 kinases.

1.4.8 RAF 265

1.4.9 PLX4032
1.4.10 Sorafenib

![Sorafenib](image)

Erlotinib which is marketed potent RAF inhibitor was synthesized as 4-amino quinazoline group.

1.4.11 Erlotinib

![Erlotinib](image)

1.4.12 Gefitinib

![Gefitinib](image)

Gefitinib is also marketed potent RAF inhibitors.
1.5 RECENT DEVELOPMENT IN RAF INHIBITORS

1.5.1 Vemurafenib

![Vemurafenib](image1)

Vemurafenib got food and drug administration approval for the treatment of last stage melanoma cancer. This drug discovery done by using strategy of drug designing by fragmentation study based on lead discovery to receive regulatory approval. European commission give approval of vemurafenib as mono therapy for treatment of melanoma cancer of adult patients targeting mutation of B-RAF V600E positively unrespectable or metastatic melanoma cancer which is very high aggressively skin cancer.

1.5.2 Gefitinib

![Gefitinib](image2)

Gefitinib is potent, novel anticancer agents used for treatment of various cancer like breast cancer, lung cancer and other type of cancer. Gefitinib target to epidermal growth factor receptor inhibitor which produce interference in signaling by EGFR in cells. So, this drug only useful in mutated cancer and over active epidermal growth factor receptor cancer. However, Meta
analyses not showing any improve in all survival, that’s why, it is not extending life. Gefitinib is selective inhibitor of tyrosine kinase domain of EGFR. So, gefitinib is considered as EGFR inhibitor. The targeted protein in gefitinib is a family of various receptor which including HER-1, HER-2, HER-3. EGFR shows over expression in the various types of cells of human cancer. E.g. lung cancer cell line and breast cancer cell line. These lead to inappropriate activation of the anti apoptotic RAS signal pathway, eventual leads to uncontrol cells proliferation. Further development investigation on gefitinib sensitized non-small cells lung cancers proved that EGFR tyrosine kinase domain mutation have responsibility to activate anti apoptotic pathway. This mutation confirmed improved sensitivity to tyrosine kinase inhibitor like sorafenib and mefitinib of the various type of non-small cells lung cancer histology. Adrenocarcinoma is one type of cancer that mostly occurred due to this type of mutation. This mutation is widely seen in Asian, womens, nonsmokers. Mechanism of action of gefitinib drug is that inhibition of EGFR kinase by bonding to ATP binding sites of enzyme. So, Functions of EGFR tyrosine kinase involve in activation of anti apoptotic RAS signaling transduction cascade inhibition proceed and inhibition of malignant cancer cell proceed.

1.5.3 Dabrafenib

Dabrafenib is agent associated with mutation of genetic BRAF and used for treatment of cancer. Dabrafenib act as anticancer agent by mechanism of inhibition of related enzyme BRAF which play critical role in cell growth regulation. Dabrafenib has proved clinical trial of phase I and II in patient with melanoma cancer and have proved good safety criteria. Food and drug
administration approved dabrafenib is a single drug cancer treatment for cancer patient with B-RAF V600E mutation positive advance melanoma cancer. Clinical trial data of Dabrafenib exhibit that resistant to Dabrafenib and various B-RAF inhibitors achieved in 6 to 7 month. To disregard this resistant, Formulation should developed which having combination of Dabrafenib with trametinib which is MEK inhibitor. Developed formulation of combination of Dabrafenib and trametinib proved very efficient and resistance to both drug increased so food and drug administration give approval to these combination formulation for the treatment of cancer patient with mutation of metastatic and melanoma.

1.6 QUINAZOLINE NUCLEAS AS RAF INHIBITOR

1.6.1 Structure of Quinazoline

![Quinazoline (25)](image)

1.6.2 Chemistry of Quinazoline

Chemical name of quinazoline is Benzopyrimidine. Quinazolines are amongst most useful class of heterocyclic compound. This compound having different type of biological activites such as anticancer, antihistaminic, antitubercular, antibacterial, antifungal, antimalarial, anti-inflammatoy, anthelmentic and antihypertensive activities. Quinazolines is heterocyclic compounds made up by fusing of one benzene six membered heterocyclic ring and other pyrimidine six membered heterocyclic ring. The chemical formula of unsubstituted quinazoline ring is C₈H₆N₂. Color of unsubstituted quinazoline is yellow and amorphous. Derivatives of quinazoline termed as quinazoline analogues. The derivatives were found with other activities like insecticidal and analgesic. Quinazoline and condensed quinazolines are reported to show potent cytotoxic, antimicrobial activites.

Second, sixth and eighth position of this nucleus is very important for structural activity studies and disubstituted quinazolines are reported to possess antiviral, antihypertensive and antibacterial functions. Biological activity of quinazoline upon addition of different heterocyclic
rings and halogens at above mentioned positions results in derivatives with significant antibacterial and antifungal activities. Unsubstituted amine or substituted amine on fourth position of quinazoline ring and halogen atoms like chlorine, bromine or electron rich substituent on sixth position of quinazoline ring known to increase the activity against bacteria. Introduction of stryl moiety at 2\textsuperscript{nd} position in quinazoline imparts prominent chemotherapeutic action. Quinazolines when attached to thiazole ring possess antibacterial, antifungal activities. Attachment of Position 3\textsuperscript{rd} should be at different heterocyclic ring for improved chemotherapeutic actions. Nitro group at 6\textsuperscript{th} position and phenyl group at 8\textsuperscript{th} position of quinazoline moiety possess better anticancer activity. Electron withdrawing substituent in 3\textsuperscript{rd} position gives better hypotensive activity. Increment in lipophilicity at fourth position of quinazoline ring is preferred for better inhibitory activity. Substitution at 2\textsuperscript{nd} and 3\textsuperscript{rd} position n like Phenyl ring, bridge phenyl ring, heterocyclic rings are reported to possess antimicrobial activity. The substitution of aryl and heteroaryl groups at C-2 and N-3 respectively has shown enhanced anti-inflammatory and analgesics activity. Incorporation of different heterocyclic moiety like pyridine, triazole and phenothiazine at 2\textsuperscript{nd} position of quinazoline moiety elucidated for insecticidal, antibacterial and antifungal activity.

1.7 REPORTED METHODS FOR SYNTHESIS OF QUINAZOLINES

1.7.1 Niementowski Quinazoline Synthesis\textsuperscript{32}

The chemical reaction between of anthranilic acid with substituted amide gives 4-oxo-3,4-dihydroquinazolines (3H-quinazolin-4-ones). Reaction was discovered by scientist Niementowski so reaction is formerly known as Niementowski quinazoline synthesis. In 1984, Niementowski reported that 2-phenyl-4-hydroxyquinoline was formed when anthranilic acid and acetophenone were heated to 120-130 °C. On heats at 200 °C, anthranilic acid and heptaldehyde formed and minimal yields of 4-hydroxy-3-pentaquinoline. The temperature essential for this reaction is more important than its synthetic procedures. During depth study of reaction, both reaction path having possibility to occur and both path have slightly supportive to each other. The reaction is start with production of Schiff base, then followed via intramolecular reaction to yield product of imine intermediate. After that with removal of one water molecule and ring closing proceed with generation of quinazoline derivatives. All evidence support to above mechanism should be in temperature of 125-135 °C.
1.7.2 Camps Quinoline Synthesis\textsuperscript{33}

A chemical reaction in which conversion of o-acyl amino acetophenone into two separate hydroxyl quinazoline molecules with help of hydroxide ion is known as camps quinazoline reaction. Reaction was investigated by scientist named camps and in reaction two quinazoline ring was formed so reaction is known as camps quinazoline reaction. The product formed proportions of hydroxyl quinazoline (A and B) formed are depend on the reaction condition and molecular structure of precursor. Reaction product is predicted as quinazoline, It is expected that keto form stable in solid condition and in solution, formed the product quinoline.

1.7.3 Doebner Reaction\textsuperscript{34}

The chemical reaction between aniline and substituted aldehyde in presence of pyruvic acid produces quinolines-4-carboxylic acid is known as Doebner reaction.
1.8. MOLECULAR MODELING – STRUCTURE BASED STUDIES

1.8.1. Introduction to Docking Methodologies\textsuperscript{35-39}

The target with generated 3D structure is grows fast and crystalline structure formed by structural genomic initiation are broadly available. Reason for the increase in number of solve structural target is the available latest technique for crystalline structure measurement, like high throughput screening X-ray crystallography. Because of extension in structural measurement project performed by genomic help scientist to select large type of target protein or molecules selected for therapeutic use.

1.8.2. Molecular docking\textsuperscript{40-44}

Docking program is defined as best fit ligand that bind to specified protein for purpose of solve optimization problem. For this docking study, both ligand and protein should be flexible in nature. “hand-in-glove” is better suitable than a “lock-and-key”. During course of processes, ligands and proteins both adjust their confirmations to achieve best fit model and these type of conformation adjustment result into full binding is termed as “Induced fit”. The main purpose of docking study is to achieve optimized confirmation for both protein and ligand should be such that free energy of whole confirmation should be minimized.

Docking is generation of non covalent bonded protein with ligand complexes \textit{in silico}. The main task of docking is to assumption of complex by given structure of proteins and ligands. Conceptual, docking study is energy minimization process related with finding of low free energy bound mode of ligands with proteins binding sites. Docking is composed of two component. One component is pose searching and second component is scoring. Protein flexibility is computational expensive; so most of current docking program treat proteins either as rigid protein or permit flexibility only to side chain functional group. Handling of ligand can classified into two groups. (I) whole molecule approach and (II) fragment based approach. Best docking method estimation is the force involve in the proteins and ligands combination with electrostatic force, vanderwaals force and hydrogen bonding force and place the ligand in appropriate active site.
1.8.3. Mechanics of docking

For performing docking study, key requirement is the interested protein in which ligand should be docked. Generally, Structure elucidation by the bio physical techniques like X-ray crystallography, proton NMR spectroscopy, carbon NMR spectroscopy or Infrared spectroscopy. Input for docking study is protein structure and database of ligand. Successful docking study depend on two performance that is search algorithm and scoring function.

1.8.3.1. Search algorithm

Search space composed of all orientation and confirmation of proteins pair with ligand. However in current computational practice, it’s not possible to exhaust search space involving all possible distortion of every drugs and all possible rotation and translation orientation of ligand relation to the proteins at level of granularity. Most of all docking program in account for a flexible ligands, and some attempts to make a flexible protein receptor. Each "snapshot" of ligand protein pair is termed as pose.

Posing, Scoring and Ranking

Posing: Posing is process in which estimation can taken from snapshot that known conformation and orientation of ligand fitted in target molecule or not fitted. Posing method generally gives not perfect results.

Scoring: Posing and ranking both are involved into scoring. The pose scoring is a fine measurement of fit of ligands into the active site of target molecule. The rank scoring is generally complex and useful to predict the binding energy.

Ranking: Ranking is latest technique relative to pose score which take multiple result from an starting scoring phase to re-assessment them. Ranking is most precise method for estimation of free energy binding.

On other hand, In posing phase involve without docking energy calculation such as electrostatic force and vanderwaals force. Involvement of detail calculation like entropy is in ranking process.
1.8.4. Applications\textsuperscript{50-51}

The binding between molecular ligands and receptor protein results into activate or inhibit enzyme. If proteins are receptor, molecular ligand binding results into agonist or antagonist. Docking is very useful and applied in field of drug discovery and drug designing. Application of Docking to:

1. Hit identification: Docking combined with a scoring function can be used to quickly screen large databases of potential drugs \textit{in silico} to identify molecules that are likely to bind to protein target of interest.

2. Lead optimization: Docking is useful to expect to where ligand bind to protein. Lead optimization is important for designing of more potent molecules.

3. Bioremediation: This can useful to expect pollutant that can be degraded by enzyme.

1.8.5. Methodology\textsuperscript{52-57}

Docking algorithms used in study: Glide

Full form of GLIDE is Grid based ligand docking with energetic. Glide is very useful software which is used for docking study which is published by Schrodinger. In this software, grid creation step needs ligand file and site for docking in maestro format. There are some charged group in protein which are not in ligand pocket so these groups are not taking part in docking study so neutralization of these groups are essential which is done by Schrodinger protein script. The grid box center can defined as center of ligand which is taking part in docking. Estimation of dimension of grid box can done from size and fitting of ligand at active site. The grid box size for ligand can be set at 10 Å for purpose of continuation of next docking step, A scalling factor of 0.9 applied to vanderwaals force of ligands atom.

Scoring Functions of GLIDE

Glide score are of two type which employed by GLIDE 3.5:

(i) Glide Score 3.5 SP which is used by Standard Precision Glide;

(ii) GLIDE Score 3.5 XP, which is used by Extra Precision Glide.
This function utilizes same term in calculation but it is formulated with different purpose. Specifically, GLIDE score 3.5 SP is a “simpler”, have more latest function which is better for identification of ligand that have a good tendency for binding with target molecule. This is good for identification even it having pose limitation. These versions try to decrease wrong negatives and are perfect for screening of much database application. In opposite site, GLIDE score 3.5 XP is a strong function that imply some penalty for every poses that break down principle of some traditionally physical chemistry like charge and strong polar group be effective to exposure into solvents. GLIDE XP score is better efficient in minimization of wrong positive and important in lead optimization or other study where restricted numbers of compound will consider during experiment but the computationally formed compounds need to be as much in quality as possible.

Main step employed in docking study

a) Preparation of protein structure

Process of protein preparation involves.

(1) To obtain the three dimension structure of protein from Protein Data Bank (PDB).

(2) Add hydrogen atom to the protein which is downloaded from PDB, include the proton essential to define the right ionized form and tautomerization state of amino acid residue like Val, Thr, Asp, Vpr.

(3) To correct the missed side chain or atom of residues.

(4) To perform energy minimization step of protein structure with help of relative force to destroy steric clash that is already available in structure.

Lastly, Generated protein structure will be used in creation of grids for sequential docking calculation.

b) Preparation of ligand

For preparation of ligand involve three main parameter which must fulfill for successful docking study. (1) Atom position assigned should be with right atom type (2) Formation of all ligand should be in ionization mode with pH 7.0±2.0 with ionizer (3) Formation of automer and chirality for ionized form.
c) Docking methodology

Complete molecular docking is based on two parameters that are search algorithm and score functions.

(1) Search algorithm is a docking tool in which extensive search all possible conformation of the ligand in a three dimension space. Again, docking program will do calculation of energy for every rotation done for each and every possible rotatable bond it can search. Every binding energy value calculation will be published as a “binding pose”. There are also other different type of algorithm is possible in docking study which are as below.

- Molecule dynamic
- Monte Carlo method
- Fragmentation based method
- Point complementary method
- Distance geometry method

(2) Scoring function is defined as procedure in which acceptance of binding energy and give number or score to represent strangeness of binding force. Finally, this binding energy will be apply for selection of good conformation of the ligand by mean of minimum energy.

- Calculation formula for scoring function
  
  \[ \text{Fitness} = \text{vdW} + \text{Hbond} + \text{Elec} \]

- Calculation formula for calculate binding energy
  
  \[ \Delta G_{\text{bind}} = \Delta G_{\text{vdw}} + \Delta G_{\text{hbond}} + \Delta G_{\text{elect}} + \Delta G_{\text{conform}} + \Delta G_{\text{tor}} + \Delta G_{\text{s}} \]