CHAPTER I

1. Introduction

1.1. Inflammation

Inflammation is derived from the Latin word inflammare which means "to set on fire with passion" **[1]**. Inflammation is a part of body's defence mechanism through which the immune system recognizes and removes harmful foreign particle and begins the healing process. Sometimes, the immune system triggers an inflammatory response inappropriately, which causes acute autoimmune diseases **[2]**. Key symptoms of inflammation are redness (rubor), swelling (tumor), heat (calor), pain (dolar) and loss of tissue function, which result from inflammatory cell responses to infection or injury **[3]**.

Classically, inflammation is responsible for variety of changes in the body which are sometime visually seen. Thus, the sensation of heat is caused by the increased movement of blood through dilated vessels into the environmentally cooled extremities, also leads to the increased redness. The swelling is the result of increased passage of fluid from dilated blood vessels into the surrounding tissues and infiltration of cells into the damaged area. Pain is due to the direct effects of mediators and the stretching of sensory nerves due to oedema. The loss of function refers to either simple loss of mobility in a joint, due to the oedema and pain, or to the replacement of functional cells with scar tissue **[4]**.

1.1.1. Pathophysiology of inflammation

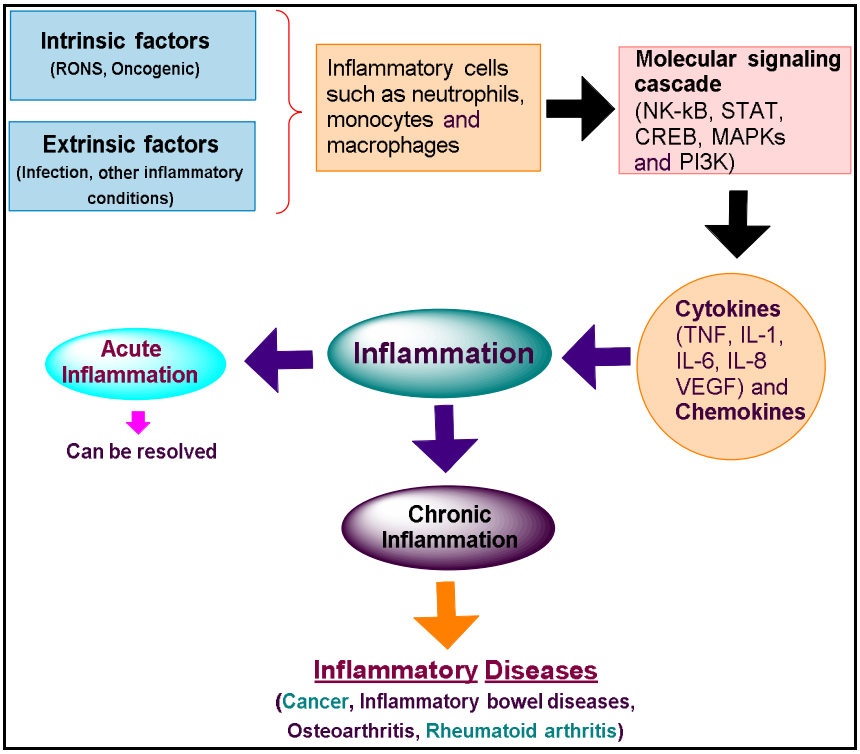


Figure 1: Pathophysiology of inflammation

Inflammation is a protective mechanism initiated in response to tissue damage caused by infection, trauma and chemical exposure, **Figure 1**. Generally inflammation is triggered by intrinsic factors such as reactive oxygen and nitrogen species, oncogenic or some extrinsic factors (infection or other inflammatory conditions). At the beginning of inflammation, neutrophils are the first cells to infiltrate under the direction of inflammatory mediators released at the site of inflammation. As the process of inflammation proceeds, various other players of inflammation such as lymphocytes and macrophages gets stimulated and recruited at the site of inflammation. This immune infiltration is facilitated by the increased expression of chemokines, growth factors and cytokines released products. The major aim of these cells is to boost the body's defence

mechanism with the addition of neutralizing agents. However, when the body is unable to resolve the acute inflammatory response, it leads to chronic and persistent inflammation. When these inflammatory responses become chronic, cell mutation and proliferation may occur, which often create an environment that could be conducive towards development of cancer. Chronic inflammation also triggers the development of various types of inflammatory diseases such as, Inflammatory Bowel Diseases (IBD), Osteoarthritis (OA) and Rheumatoid Arthritis (RA) **[5]**. In next section we have described the various types of inflammatory diseases and cancer.

1.1.2. Inflammatory diseases

Inflammatory diseases records major cause of death worldwide. Three out of five people are dying due to inflammatory diseases like stroke, chronic respiratory diseases, heart disorders, cancer, obesity and diabetes. According to World Health Organization (WHO), diseases associated with inflammation are greatest threat to human being. Number of patient suffering from chronic inflammatory diseases is going to increase exponentially in upcoming years. As per Rand corporation estimate, in USA alone nearly 60% of Americans suffers from chronic inflammatory conditions. Wellness 365, India has estimated around 1.5 million patients are suffering from Inflammatory Bowel Disease (IBD). In the next section, various types of inflammatory diseases are described in detail.

1.1.2.1. Cardio vascular diseases (CVD)

Pericarditis and endocarditic are two major cardio vascular diseases caused due to inflammation. Pericarditis is inflammation (swelling or irritation) of fluid filled sac surrounding heart (pericardium), which lubricates the heart and reduces friction during pumping. Pericarditis is generally caused

due to infection, metabolic disorder, cancer, thyroid, kidney diseases, trauma, surgery, RA and allergic reaction of medicines. The main symptoms are heavy chest pain, shoulder pain, back and neck pain **[6]**.

Endocarditis is an inflammation of the endocardium which is inner lining, chamber and valve of the heart. The disease pathogens from other part of body come in contact with heart through blood stream and causes problem in the heart. Treatment is necessary as early as possible otherwise it may cause partial or complete damage to the heart valve and my lead to heart failure. Normal healthy people are not affected by this disease but people with pre-existing heart defects develop many problems. Disease symptoms are developed over a period of time and it depends on the heart condition. Symptoms of endocarditis are sudden weight loss, pain in joint and muscles pain, oedema, fever, coughing, night sweats and shortness of breath or difficulty in breathing **[6]**.

1.1.2.2. Neurological disease

In inflammatory neurological disease, the inflammation is termed as neuroinflammation. Prolonged neurological diseases involving sustained activation of glia cells and recruitment of immune elements. In Multiple Sclerosis (MS) there is exclusive attack of leukocyte in CNS, but in Parkinson and Alzheimer there is no significant inflammatory penetration **[7, 8]**. Though neuroinflammation cause many problems but their some benefits which includes neuroprotection, assembling of neuron for repairing, remyelination (remyelination is the natural repair mechanism of demyelination), which protects against progressive axonal injury and consequently also diminishes long-term disability in MS patients.

1.1.2.3. Autoimmune disease (AD)

An autoimmune disease is a condition in which our immune system is triggered and attacks on self-molecule due to immunologic response to auto-reactive immune cells **[9]**. Immune system guards us from germs like bacteria and viruses. When immune system senses these foreign invaders, it sends out an army of fighter cells to attack them. Normally, the immune system can differentiate between foreign cells and our own cells. In an autoimmune disease, immune systems make mistakes by considering body cell as foreign bodies and it releases proteins called autoantibodies that attack healthy body cells. Some autoimmune diseases target whole body and some are organ specific. The autoimmune disease which affects whole body is known as systemic autoimmune disease/disorder whereas other is organ specific autoimmune disease/disorder.

i) Systemic autoimmune disease/disorder

Systemic autoimmune diseases are a wide range of related diseases characterized by dysregulation of an immune system which take place by activation of immune cells to attack autoantigens and resulted in inappropriate inflammation damages. Examples of systemic autoimmune diseases include systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia gravis, psoriasis, rheumatoid arthritis (RA) and inflammatory bowel diseases (IBD) **[10]**.

ii) Organ specific autoimmune disease/disorder

When immune system targets specific organs or tissues it is known as organ specific autoimmune diseases. The best example of organ-specific

autoimmune diseases includes Celiac disease, Graves's disease, Hashimoto's Thyroiditis, Type I diabetes mellitus and Addison's disease **[11]**.

1.1.2.4. Rheumatoid Arthritis (RA)

Rheumatoid arthritis is one of the most prevalent chronic systemic inflammatory diseases of the synovial membrane and joint. Untreated RA can lead to progressive joint destruction, resulting in disability, poor quality of life and increased mortality. About 1% of the population is affected with RA and the disease onset generally occurs between 30 to 50 years of age, with a higher incidence in women **[12]**. Signs and symptoms of RA are pain or aching, stiffness, tenderness and swelling in more than one joint. Other symptom includes weight loss, fever, fatigue or tiredness and weakness **[13]**. RA can also affect other tissues throughout the body and cause problems in organs such as the lungs, heart, and eyes. The presence of auto-antibodies (seropositivity) is associated with more severe symptoms such as joint damage which may lead to mortality.

1.1.2.5. Cancer

The heart of cancer is through cell progression and cell division in a cell cycle. Cell cycle is a sequence of ordered events that take place in a cell leading to division and duplication. The main phases of the cell cycle are interphase (G1, Synthesis of DNA, G2, and Mitosis), nuclear division and cytokinesis. Cell growth, replication of DNA and cell division is controlled by a complex series of signalling process which also includes checkpoint to confirm and correct mistakes. If mistake are not corrected, the cells commit suicide (apoptosis). In cancer, due to genetic mutations, above regulatory process malfunctions, resulting in uncontrolled cell growth and proliferation.

In general, cancer is divided on the basis of origin. There are mainly four types of cancer: Leukaemia, Carcinomas, Lymphomas, and Sarcomas.

- Leukaemia is a cancer of the blood, it begins when healthy blood cells change and grow uncontrollably. Leukaemia is of four types:

  a) Acute lymphocytic leukaemia (ALL)

  b) Chronic lymphocytic leukaemia (CLL)

  c) Acute myeloid leukaemia (AML)

  d) Chronic myeloid leukaemia (CML)

- The most common types of cancer are carcinoma, cancer of the skin or the tissue that covers the surface organs. Examples prostate cancer, breast cancer, lung cancer and colorectal cancer.

- Lymphoma is a cancer of lymphatic system. Hodgkin lymphoma and non-Hodgkin lymphoma are two main types of lymphoma.

- Sarcoma in the cancer of tissues that support and connect the body. Generally, sarcoma is developed in fat, muscles, nerves, tendons, joints, blood vessels, lymph vessels, cartilage or bone.

Worldwide, every year around 19.3 million new cancer cases are reported and 10 million cancer deaths were reported, **Figure 2 (i)**. Female breast cancer was most diagnosed cancer (11.7%), followed by lung (11.4%), colorectal (10.0 %), prostate (7.3%), and stomach (5.6%). Leading cause for cancer associated death were lung cancer estimated 1.8 million deaths (18%),

followed by colorectal (9.4%); liver (8.3%), stomach (7.7%), and female breast (6.9%) cancers **[14].**

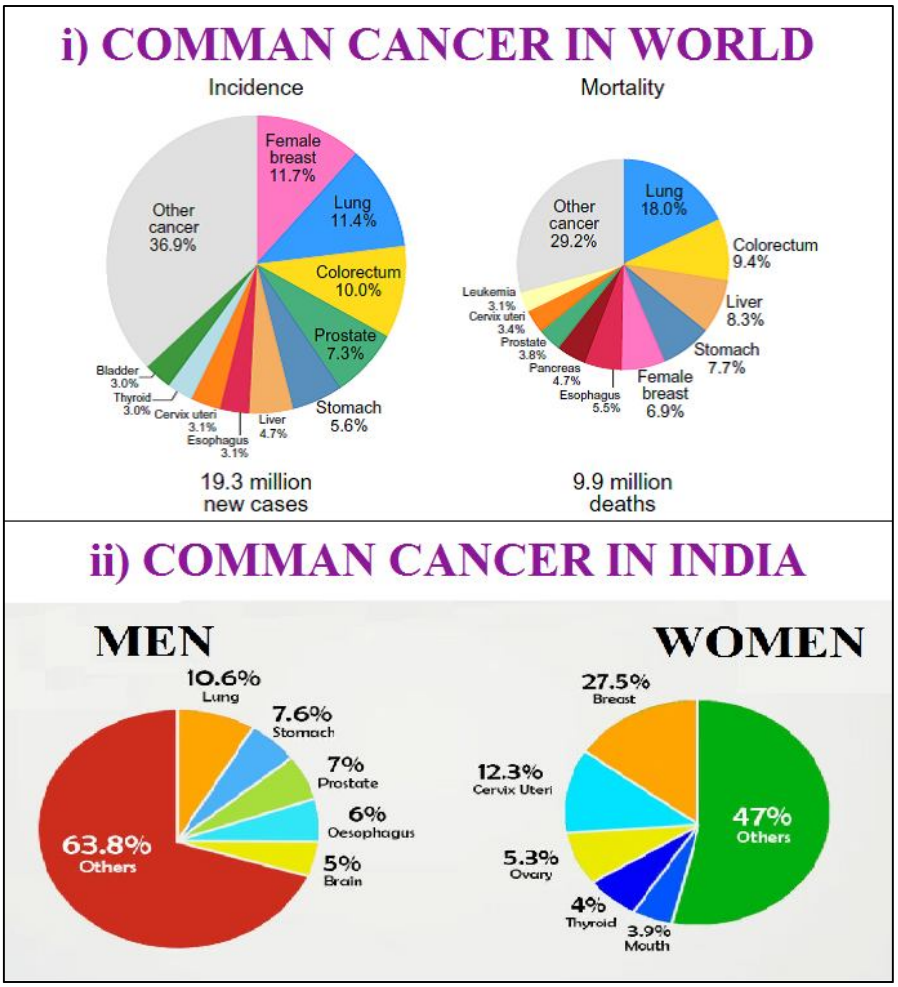


Figure 2: Reported cancer cases i) Worldwide, ii) India (men and women)

Annually 1.16 million cancer cases are reported in India, which contributed to ~0.78 million cancer associated death in India every year. The most common types of cancer in India for male are Lung (10.6%) and Stomach (7.6%). Most common type of cancer in female Breast cancer (27.5%), Cervical cancer (12.3%) and Ovarian cancer (5.3 %), **Figure 2 (ii)**. India had ranked 4th in the world with highest rates of Oral cancer (11.8%; Male). Thus it is clear

from above number that cancer is dangerous diseases with highest mortality rate. At present there is no exact treatment available for complete cure and care of cancer. In next section we have described available therapies for RA and Cancer treatment **[15]**.

1.1.3. Current pharmacologic therapies for the treatment of RA

Medications for RA mainly divided into two categories; Traditional therapies includes Non-steroidal anti-inflammatory drugs (NSAIDs) like Ibuprofen and Diclofenac and Corticosteroids (Steroids) like Prednisone, Dexamethasone and Triamcinolone, Diseases modifying anti-rheumatic drugs (DMARDs) like Methotrexate, Leflunomide, Hydroxychloroquine and Sulfasalazine. Other category is targeted therapies such as Biologic agents (proteins) and Kinase inhibitors (small molecules). Biological agents include Etanercept, Abatacept, Adalimumab, Anakinra, Certolizumab, Tocilizumab, Rituximab, Infliximab, Golimumab etc. There are many kinase inhibitors approved for RA such as Tofacitinib, Baricitinib (JAK inhibitors), Fostamatinib (SYK inhibitors) and some are under late stage of clinical development such as Leniolisib, Nemiralisib and Seletalisib (PI3Kδ inhibitors). Other operative therapies are Surgery, Arthroscopy (remove inflamed joint tissue) and Joint replacement **[16].** Available current therapies for the treatment for RA, along with their side effects are listed below, **Table 1 [17].**

Table 1: Current therapies for the treatment of RA

Sr. No	Classes	Drug names	Side effects
		Traditional therapies for RA	
1	Non-steroidal anti-inflammatory drugs (NSAIDs)	Ibuprofen, Diclofenac	Heartburn, Abdominal pain, gas, Vomiting, Constipation
2	Steroids	Prednisone, Dexamethasone, Triamcinolone	Weight gain, Osteoporosis Muscles weakness, Thinning of skin
3	Disease-modifying anti-rheumatic drugs (DMARDs)	Methotrexate, Leflunomide, Hydroxychloroquine, Sulfasalazine	Sickness, Loss of appetite, Sore throat, Diarrhoea, Headaches, Hair loss
		Targeted therapies for RA	
4	Biological treatments	Etanercept , Infliximab	Reactions at the site of the injections, Infections, Sickness, High temperature, Headaches
5	JAK inhibitors	Tofacitinib, Baricitinib	Nasopharyngitis, Diarrhea, Infection, Lymphoma, Risk of blood clots
6	SYK inhibitors	Fostamatinib	Diarrhea, nausea, dizziness, rash, Abdominal pain, Fatigue.
7	**PI3K inhibitors**	**Leniolisib, Nemiralisib, Seletalisib for RA**	**Hyperglycaemia, Fatigue, Dry skin, Weight loss**

All traditional therapeutics were developed using a practical approach without detailed understanding of their mode of action and therapeutic target. Thus, traditional treatments are associated with several side effects. In last few decades, more efforts were focus on understanding of inflammatory process and related pathways involved in the RA. Infliximab was the first TNF-antagonists approved for inflammatory diseases (Crohn's disease and RA). Biologic agents used are mainly injectable; hence these biological drugs increase the risk of infections. The high cost, cold storage condition, stability, logistic and immunological adverse effects are associated with biological agent, that have triggered discovery of small molecules based targeted therapies for safe and effective treatment for RA.

We are just in the beginning of small molecule era because biological agent has already proven successful therapeutic for RA, but treatment using small molecule will be more cost effective, logistic friendly and most important it will be orally available (tablet or capsule). First small molecule approved is Tofacitinib as JAK inhibitors (jakinibs) for the treatment of RA and inflammatory bowel disease. Similarly, Fostamatinib is approved as SYK inhibitors. PI3Kδ inhibitors such as Leniolisib, Nemiralisib and Seletalisib are in late stage of clinical development for treatment of inflammatory diseases.

1.1.4. Current pharmacologic therapies for the treatment of cancer

There are many therapies available for the treatment of cancer. Appointment of therapies depends upon many factors such as, the type and stage of cancer, patient general health, and preferences. Cancer therapies are divided in to two main types traditional and targeted. Traditional therapy includes Surgery, Chemotherapy, Radiation and Bone marrow transplant,

whereas target therapy includes immunotherapy (biological therapy) and small molecule inhibitors.

a. Traditional therapies for cancer

- **Surgery:** It is an operative in nature where surgeon removes as much as cancerous cell/tissues from the patient body.

- **Chemotherapy (Chemo):** DNA alkylating agents are administered as chemotherapeutic agents. Example of chemotherapeutic agents include Cisplatin, Oxaliplatin **[18]**, some mustard gas derivative is also given such as Melphalan and Ifosfamide **[19]**. Plant alkaloids (inhibits specific cell cycle phase in cancerous cell) as Paclitaxel, Topotecan **[20]** etc. In combination with chemotherapeutic agents, antitumor antibiotic are also used such as Doxorubicin, Mitomycin and Dactinomycin **[21]**. Chemotherapeutic agents causes many side effects in the body such as immune suppression (decreased red blood cell, white blood cell and platelets), anaemia, nausea, vomiting, anorexia, diarrhoea, hair loss (alopecia), infertility, organ damage etc.

- **Radiation therapy:** Radiation therapy involves usage of non-ionizing radiation to controls or kill cancerous cell by damaging its DNA in selected body parts **[22]**. It is used in synergistic with chemotherapy, before or after as suggested by radiation oncologist. The most common side effects for radiation therapy are fatigue and mild to moderate skin irritation. Other side effects are nausea, vomiting, damage epithelial surfaces of cell, intestinal discomfort, sore mouth, infertility etc.

- **Bone marrow transplant:** Bone marrow transplant is also known as stem cell transplant. It is a treatment in which bone marrow cells are

replaced with healthy cells from donor **[23]**. It is a dangerous procedure with much complication at early stage or late stage of treatment. The common side effect is infection due to low blood count. Other side effect are nausea, sores mouth, fatigue, low levels of platelets and red blood cells, anaemia, diarrhoea, infertility, thyroid problems etc.

b. Targeted therapies for cancer

- **(Immunotherapy) Biological therapy:** Immunotherapy is also coined as biotherapy (biological therapy), which is based on the activation of immune system to fight against cancer. It is highly selective and less toxic compared to traditional therapies. In immunotherapy, host immune system is activated passively or actively to recognize and destroy abnormal (cancerous) cells. Some of the approved biological anti-cancer agents are listed below, with common side effects, **Table 2 [24-26]**.

Table 2: Immunotherapies for the treatment of cancer

Sr. No.	Drug Name	Target	Indications	Common Side effect
1	Rituximab (Rituxa®), Ibritumomab (Zevalin®), Ofatumumab (Arzerra®), Obinutuzumab, (Gazyva®)	CD20	Non-Hodgkin's lymphoma, B-cell CLL, CLL	Infection, swelling at the injection site, fever, chills, nausea, vomiting, loss of appetite, fatigue
2	Isatuximab (Sarclisa®)	CD30	Multiple myeloma	
3	Necitumumab (Portrazza®), Panitumumab, Bevasizumab	EGFR, VEGF	Head and neck carcinoma, Colorectal cancer, Lung cancer, Renal cancer, Ovarian cancer	
4	Pertuzumab, Trastuzumab	HER2	Breast cancer	

All the above biological agents are monoclonal antibodies (mAbs) design to interact with a specific part of an antigen, which is highly specific for cancerous cell. Most common side effect seen is swelling or infection at the site of injection (on body), fever, chills, nausea, vomiting with these therapies. The major drawback is the production cost of monoclonal antibodies, logistic and storage conditions (require low temperature).

- **Small molecule based targeted therapy:** Small molecule based inhibitors have high potency and low toxicity because it selectively and specifically target cancer cells leaving normal cells untouched. Some of them are listed below on the basis of approval year, **Table 3 [27-31]**.

Table 3: Small molecule inhibitors for the treatment of cancer

Sr. No.	Drug Names (Approved year)	Targets	Indications	Originator
1	Imatinib (Gleevec) (2001)	Bcr-Abl/ PDGFR	Chronic myeloid leukaemia, Acute lymphoblastic leukaemia	Novartis
2	Dasatinib (Spraysel) (2006)	Bcr-Abl/ PDGFR/ Src	Chronic myeloid leukaemia, Acute lymphoblastic leukaemia	Bristol-Myers Squibb
3	Sunitinib (Sutent) (2006)	PDGFR/ VEGFR	RCC, Pancreas neuroendocrine tumor	Pfizer
4	Lapatinib (Tykerb) (2007)	EGFR/HER2	Breast cancer	Novartis
5	Icotinib (Conmana) (2011)	EGFR	Lung cancer	Betta
6	Ruxolitinib (Jakafi) (2011)	JAK1/2	Myelofibrosis	Incyte/Novartis

7	Axitinib (Inlyta) (2012)	VEGFR-1/2/3	Renal cell carcinoma	Pfizer
8	Afatinib (Gilotrif) (2013)	EGFR/HER2/HER4	Lung cancer	Boehringer Ingelheim
9	Ibrutinib (Imbruvica) (2013)	BTK	Mantle cell lymphoma, chronic lymphocytic leukemia	AbbVie/ Johnson & Johnson
10	**Idelalisib (Zydelig) (2014)**	**PI3Kδ**	**Chronic lymphatic leukemia, Follicular lymphoma**	**Gilead**
11	Acalabrutinib (Calquence) (2017)	BTK	Mantle cell lymphoma	AstraZeneca
12	Copanlisib (Aliqopa) (2017)	Pan-PI3K	Follicular lymphoma	Bayer
13	Duvelisib (Copiktra) (2018)	PI3Kγ/δ	Chronic lymphatic leukemia, Follicular lymphoma	Verastem
14	Anlotinib (Focus V) (2018)	VEGFR/ FGFRs	Lung cancer	Chia Tai Tianqing
15	Alpelisib (Piqray) (2019)	PI3Kα	Breast cancer	Novartis

Several small molecule based anti-cancer inhibitors are approved as drug and some are under clinical development. The common side effects associated with these agents are sore mouth, hair loss, nail colour changing, skin rashes, blood clotting, liver problem, diarrhoea, problems in wound healing, high blood pressure and skin problems might include rash or dry skin, stomach upset etc. The major limitation for targeted therapies is off target interactions. Also less selectivity and genetic mutation of target leads to resistance.

Thus, there is much opportunity in targeted therapy, due to known pathways involve in the cancerous cell division, growth and cell proliferation. In this regards, as a part of our ongoing research, we found development of PI3Kδ inhibitor could be an attractive targeted based therapy for safe and effective treatment of RA and cancer. We decided to develop potent, selective and orally bioavailable PI3Kδ inhibitor. In the next section, we explained relevance of PI3K enzyme in human body considering pathophysiology of RA and cancer.

1.2. Kinase

More than 500 kinase, have been discovered through *human genome* project. Kinases are enzyme which catalyzed the phosphorylation of substrate (Protein, Carbohydrate and Lipid) via transfer of phosphate from high energy ATP molecule to give phosphoanhydride and ADP, **Figure 3**. This phosphorylated substrate act as a biochemical messenger in signal transduction pathway to activate various cellular function such as cell growth, cell division, metabolism, protein regulation etc. which are important for normal cell physiology.

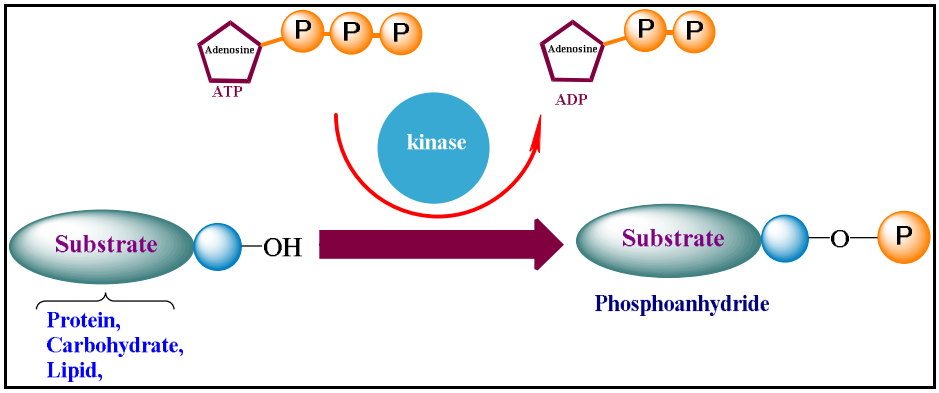


Figure 3: Kinases; catalyzed the phosphorylation of substrate

Depending upon substrate type, kinases are classified in the three main types:

- **Protein kinase:** Substrate is protein containing amino acid. The best example is CDKs (Cyclin dependent kinases) which are involved in initial cell cycle regulatory process and MAPs (mitogen-activated protein kinases). Above mention both kinase uses serine/threonine amino acid residues for phosphorylation, whereas TK (Tyrosine kinase) and RTK (Receptor tyrosine kinase) uses tyrosine amino acid residue **[32]**.

- **Carbohydrate kinase:** Depending upon types of sugar bound substrate, they are further divided in four classes; hexokinases, ROK (**R**epressor, **O**pen reading frame **k**inase) kinases, ribokinases and GHMP (**G**alactokinase, **H**omoserine kinase, **M**evalonate, and **P**hosphomevalonate kinase) kinases **[33]**.

- **Lipid kinase:** Substrate used in lipid kinase is lipids of plasma and cell membrane. On the basis of lipid structure, lipid kinase is divided into two types; SK (**S**phingosine **k**inases) where sphingosine (2-amino-4-trans-octadecene-1,3-diol) is substrate and PI3K (**P**hosphat**i**dylinositol-**3**-**k**inase) where phosphatidylinositol is used as substrate **[34-36]**.

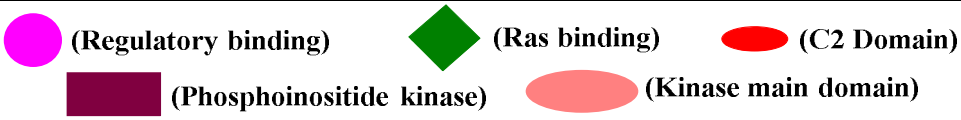
In next section, we will discuss the classification, biochemistry, signal transduction pathway and significance of PI3K enzyme inhibition.

1.2.1. Classification of PI3K

PI3Ks has been classified into three classes (I, II and III) based on substrate specificity, sequencing homology and types of regulatory subunits, **Table 4.** All three classes have phosphoinositide kinase and kinase binding domain in common. The class I PI3Ks consists of four kinases (PI3K-α, β, δ and

γ) and further grouped into two sub-classes: class IA and class IB. The PI3Kα and β are expressed in a wide variety of tissues and organs. PI3Kγ is found mainly in leukocytes, while expression pattern of PI3Kδ is restricted to spleen, thymus, hematopoietic cells and peripheral blood leukocytes. PI3Kγ and PI3Kδ are mainly expressed in rheumatoid arthritis (RA) synovium and regulate innate and adaptive immune responses **[37]**.

Table 4: PI3K enzyme classification

Class	Structural features of catalytic subunits	Subunits	
		Catalytic	Regulatory (Adaptor)
IA		p110α,β and δ	p85α, p85β and p55γ
IB		p110γ	p101
II		PI3K-C2α,β and δ	C2 domain at C-terminal
III		Vps34p analogues	p150
 (Regulatory binding) (Ras binding) (C2 Domain) (Phosphoinositide kinase) (Kinase main domain)			

i) Class I PI3Ks

Class I PI3Ks are heterodimers composed of catalytic subunit (p110) and a regulatory (adaptor) subunit (p85). Class I PI3Ks is most studied and explored class as compared to other classes because of its connection with many pathways and diseases such as inflammation, cardiovascular disease, metabolic diseases and tumorigenesis. It further subdivided into class IA and IB on the basis of regulatory sub units. The regulatory subunit controls receptor binding, activation and localization of the enzyme. In mammals Class IA PI3K

has three isoforms namely PI3Kα, PI3Kβ and PI3Kδ with the respective p110 catalytic subunit bound to the p85 regulatory subunit. PI3Kα and PI3Kβ are widely distributed in the body whereas p110δ which shows a more restricted distribution and is mainly found in leukocytes. Class IB is mainly expressed in leukocytes but is also found in the heart, pancreas, liver and skeletal muscles **[38]**.

ii) Class II PI3Ks

Class II PI3K has a single catalytic subunit (adaptor subunit is absent) with C2 domain at C-terminal and has three PI3K isoforms, PI3KC2α, PI3KC2β and PI3KC2γ, which are generally activated by Receptor tyrosine kinases (RTKs), cytokine receptors and integrins. PI3K-C2α and PI3K-C2β have a broad but rare tissue distribution, whereas the expression pattern of PI3K-C2γ seems to be more restricted. The exact cellular functions of this family remain unclear. Clinical importance of class II PI3Ks is still under exploration. Pharmacological inhibitors with selectivity for class II PI3Ks have not been reported **[39]**.

iii) Class III PI3Ks

Class III PI3K consists of a single catalytic Vps34 subunit and regulatory subunit p150. Vps34 only produces PI (3) P, an important regulator for membrane, proteins and vesicle trafficking. It has been also connected to vital functions such as a nutrient-regulated lipid kinase via mTOR (Mammalian target of rapamycin) **[40]**.

1.2.2. Biochemistry of PI3K

Phosphoinositide-3-kinases (PI3Ks) belongs to lipid kinase family, which constitute central signalling hub that mediates diverse and crucial cell

functions, including cell growth, proliferation, differentiation, motility and survival. Lipids Inositol ring (head) is a class of phospholipids, which contains phosphatidic acid to which this inositol ring is attached through its 19-OH group, **Figure 4.** When there are no phosphates group attached to inositol ring, it is known as phosphatidylinositol (PIP_2) and its phosphorylated derivative is known as phosphoinositide (PIP_3). In the cell, the main biochemical role of PI3Ks is to phosphorylate 3'-OH position of the inositol ring of PIP_2 to give PIP_3 which work as secondary messenger to activate other pathways. It is negatively regulated through dephosphorylating by phosphatase and tensin homolog (PTEN) gene **[41]**.

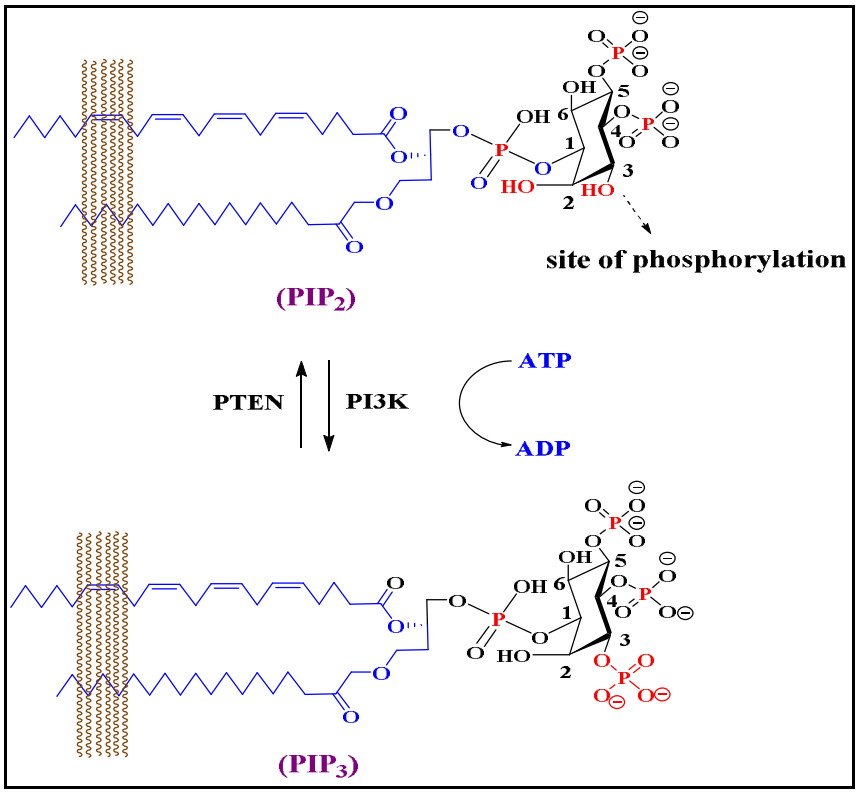


Figure 4: Phosphorylation with PI3K enzyme in cell

1.2.3. PI3K signal transduction pathways

As discuss in the earlier section, PI3Ks regulates intracellular lipids which affect a range of biological phenomena through phosphorylation of 3-OH group of inositol lipid ring to access the control of intracellular protein. Initially RTK (Receptor tyrosine kinase) is normally activated by antigen (extracellular stimuli, growth factors, cytokines, hormones) leading to dimerization to release specific protein to activate PI3K, **Figure 5 [42]**. Upon activation, PI3K catalyzes the phosphorylation of PIP_2 to produce PIP_3, which act as second messenger and activates many other signaling pathways such as PDK_1 (3-phosphoinositide-dependent protein kinase-1), which regulates downstream ATK or PKB (Protein kinase B) and m-TOR (Mammalian target of rapamycin) pathway. Above signal transduction is important to control many cellular functions such as cell growth, cell survival, cell proliferation, apoptosis etc. Uncontrolled activation of any enzyme (PI3K, RTK, PDK_1, ATK and m-TOR) in this pathway leads to many inflammatory diseases, ultimately ending with cancer. PTEN prevents activation of above mention downstream kinases **[43]**.

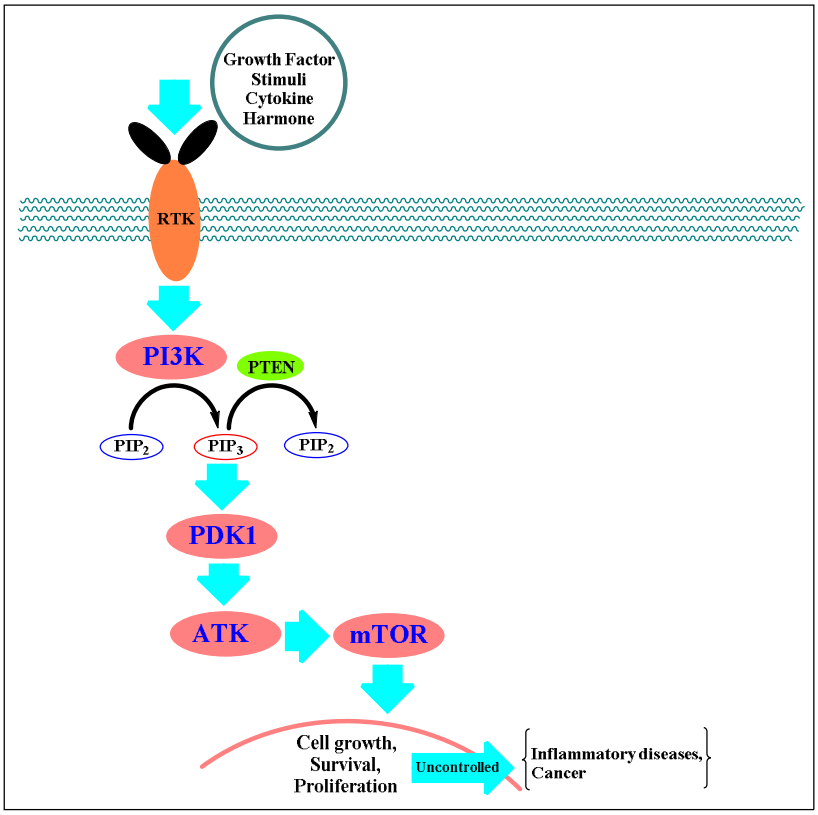


Figure 5: PI3K activation pathway

1.2.4. Clinical significance for targeting PI3Kδ isoform

PI3K is believed to be one of the key therapeutic targets for inflammatory diseases and cancer treatment because it is observed that hyperactivity of PI3K signaling is significantly correlated in autoimmune disorder and tumor progression. The p110δ isoforms of PI3K have gained more attraction as pharmacological targets for the treatment of RA and hematological malignancies because of its known effects on a variety of immune cells **[44]**. Selective inhibitions of PI3Kδ isoform with suitable inhibitors are already documented in the literature. It is also supported through *in vivo* studies (PI3K gene knock out/deletion) where in PI3Kδ gene deleted mice showed depressed immunological response.

Earlier efforts were mainly dedicated on developing the broad-spectrum (pan) inhibitors of the PI3K (α, β, γ and δ) isoforms, as potential therapeutics. However, knowing the side effects associated with PI3Kα and β isoforms inhibition (due to universal expression), recently, more efforts are directed towards the development of isoform selective inhibitors, particularly PI3Kδ selective inhibitors, for the safe and effective treatment of hematological malignancy and inflammatory disorders. There are many clinically approved PI3Kδ inhibitors, some are under clinical development. In the next section, we have listed some of the PI3K inhibitors.

1.2.5. PI3K inhibitors

PI3K inhibitors are listed in below table, some of them are launched and some are in clinical trials. CAL-101 (GS-1101/Idelalisib/ZydeligTM) is the only PI3Kδ selective inhibitor launched till now (July 2014). Other are either dual or pan PI3K inhibitors. Leniolisib and Parsaclisib are other PI3Kδ selective inhibitors which are in late phase of clinical development, **Figure 6**.

Table 5: PI3K Inhibitors in clinical development

Sr. No.	Selectivity	Generic Name	Development Phase	Indication	Originator
1	PI3K (δ)	Idelalisib Zydelig™	**Approved (July 2014)**	Chronic lymphocytic leukemia, Small lymphocytic lymphoma	Gilead Science

2	PI3K (δ)	Leniolisib	Late III	Activated PI3Kδ syndrome COPD, Asthma	Novartis
3	PI3K (δ)/ CK1ε	Umbralisib Ukoniq	**Approved (February 2021)**	Marginal zone lymphoma, Follicular lymphoma	TG Therapeutic
4	PI3K (δ)	-	II	Rheumatoid arthritis	Amgen
5	PI3K (δ)	Parsaclisib	II	Hematological malignancies, Solid tumors	Incyte Corporation
6	PI3K (α/δ)	Copanlisib Aliqopa™	**Approved (September 2017)**	Hematological and solid malignancies	Bayer
7	PI3K(δ/γ)	Duvelisib Copiktra™	**Approved (September 2018)**	Chronic lymphocytic leukemia, inflammatory Diseases	Infinity/ Verastem
8	PI3K (δ/γ)	Tenalisib	II	Relapsed/Refractory Indolent Non-Hodgkin's Lymphoma	Rhizen
9	PI3K α	Alpelisib Piqray™	**Approved (May 2019)**	Advanced or Metastatic breast cancer	Novartis

10	PI3K α	Taselisib	Late III	Metastatic breast cancer	Roche
11	PI3K α	Serabelisib	II	Breast cancer, Endometrial cancer Renal cell carcinoma	Takeda
12	PI3K/mTOR	Gedatolisib	III	Solid Tumor, Brest Cancer	Pfizer
13	PI3K/mTOR	Dactolisib	III	Advance solid tumor	Novartis
14	PI3K/mTOR	Samotolisib	II	Neoplasm	Eli Lilly and Co.
15	PI3K/mTOR	Bimiralisib	II	Solid Tumor, Brest Cancer	Piqur Therapeutic
16	PI3K/mTOR	Omipalisib	II	Advance Solid Tumor	GSK
17	PI3K/mTOR	Apitolisib	II	Breast cancer, Endometrial cancer	Genentech, Piramed
18	Pan PI3K	Pictilisib	Terminated	Solid Tumor, Brest Cancer	Roche
19	Pan PI3K	Buparlisib	III	Breast Neoplasm	Novartis
20	Pan PI3K	Pilaralisib	II	Endometrial Neoplasm	Sanofi

CAL-101 (GS-1101/Idelalisib/**Zydelig™**) is an approved (July 2014), oral PI3Kδ selective inhibitor, **Figure 6 [45, 46].** It has been shown that Idelalisib has therapeutic effects without inhibiting PI3K signaling crucial for normal function of healthy cells. Idelalisib is the first FDA-approved PI3K inhibitor which is used in combination with rituximab for the treatment of relapsed or refractory chronic lymphocytic leukemia and follicular lymphoma. The most common side effects of Idelalisib are decreased in neutrophil count, hypertriglyceridemia, hyperglycemia, hepatotoxicity, ALT (alanine aminotransferase is a liver enzyme) elevation and fever. Drug induced pneumonitis is also observed with Idelalisib. Due to above toxicity, Idelalisib cannot be use for RA and other inflammatory conditions such as asthma, COPD (chronic inflammatory lung disease) etc.

CDZ173 (Leniolisib) is an oral and selective inhibitor of the PI3Kδ isoform developed by Novartis. It is used for activated PI3Kδ syndrome (APDS). Leniolisib showed excellent potency against PI3Kδ with the IC_{50} of 0.011 nM and is under late stage of clinical development, **Figure 6 [47]**.

Umbralisib (TGR-1202/ **Ukoniq**) is an oral, selective PI3Kδ/ CK-1ε inhibitor approved (February 2021) for marginal zone lymphoma and follicular lymphoma. Common side effects are low blood cell counts, decreased blood platelets, abdominal pain, pneumonia, urinary tract infection (UTI) and fever. Due to acute toxicity, Umbralisib is not tested for inflammatory disease **[48]**.

Duvelisib (IPI-145/**Copiktra™**) is an approved (September 2018), oral dual inhibitor of PI3K-δ/γ. Preclinical studies revealed that Duvelisib causes direct killing in primary chronic lymphocytic leukemia cells in a dose- and time-dependent manner, **Figure 6 [49]**. The most common side effects of

Duvelisib are diarrhea, nausea, low blood cell counts, bone pain, muscle pain, fever, cough, tiredness or cold symptoms such as stuffy nose, sneezing, sore throat.

Copanlisib (BAY 80–6946/**Aliqopa^{TM}**) is approved in September 2017. It is administered intravenously. It is potent, highly selective and reversible pan-class I PI3K inhibitor with predominant activity against the p110α and p110δ isoforms, **Figure 6 [50]**. Copanlisib has many side effects such as burning, crawling, itching, numbness, prickling, cracked lips, diarrhea, difficulty in swallowing, lack or loss of strength swelling or inflammation of the mouth etc.

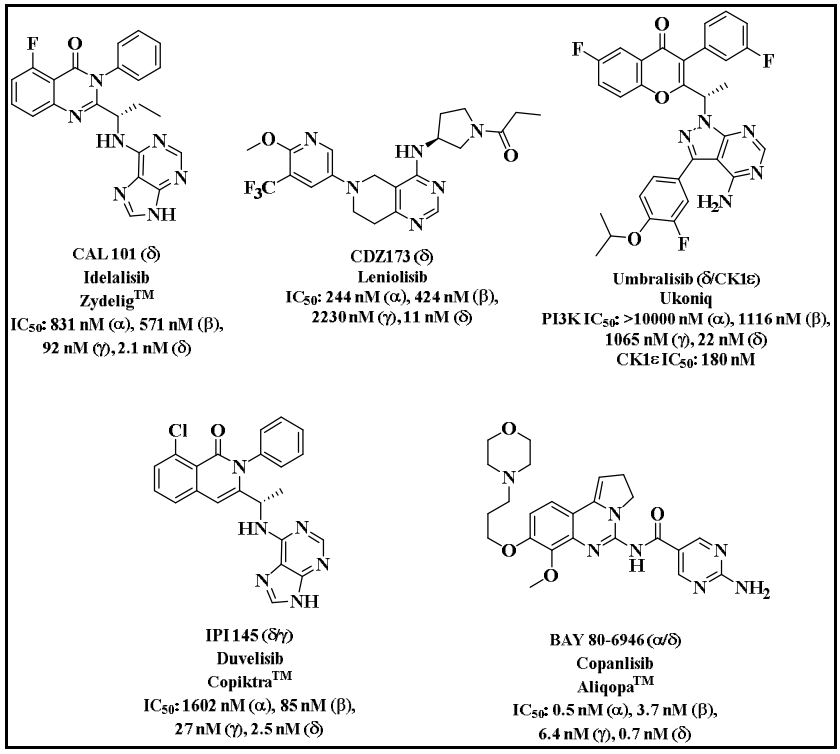


Figure 6: Isoform-specific inhibitors

Dactolisib (NVP BEZ235) is an oral dual PI3K/mTOR inhibitor; it is in phase II clinical trials. Dactolisib has severe side effects such as elevated

blood glucose level, ALT elevation, diarrhoea, hair loss, mucosal inflammation and skin rash **[51]**. Apitolisib (GDC-0980/RG7422) and Gedatolisib (PF-05212384/PKI-587) are orally bioavailable dual inhibitor of PI3K and mTOR kinase, currently in phase II and phase III clinical development, respectively **Figure 7 [52, 53].** Due to non-selective in nature Apitolisib and Gedatolisib has many side effects such as hyperglycemia, mucosal inflammation, liver dysfunction (increase in aspartate aminotransferase, alanine aminotransferase) etc.

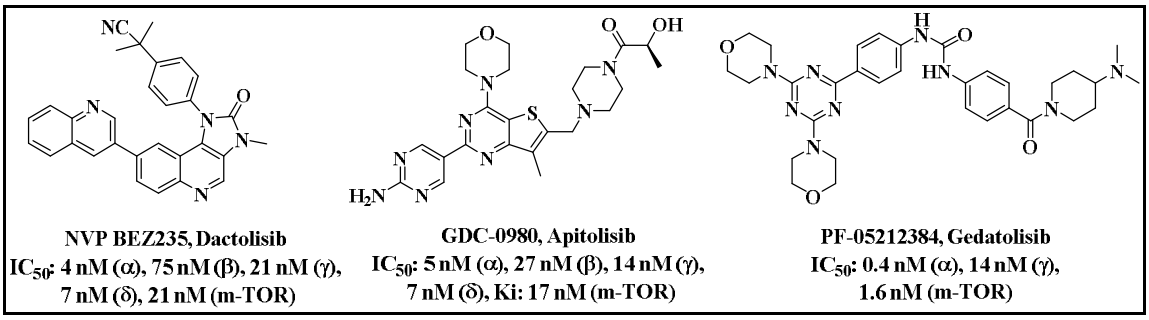


Figure 7: Dual PI3K/mTOR inhibitors

Buparlisib (NVP-BKM120), Pilaralisib (XL-147) and Pictilisib (GDC-0941) are orally pan-class I, reversible inhibitors of PI3K, **Figure 8 [54, 55]**. Buparlisib and Pilaralisib are in phase III and II clinical development, whereas Pictilisib was discontinued due to toxicity. There are many side effects associated with pan PI3K inhibitors such as anxiety, depression, hyperglycemia, fatigue, rashes on skin etc.

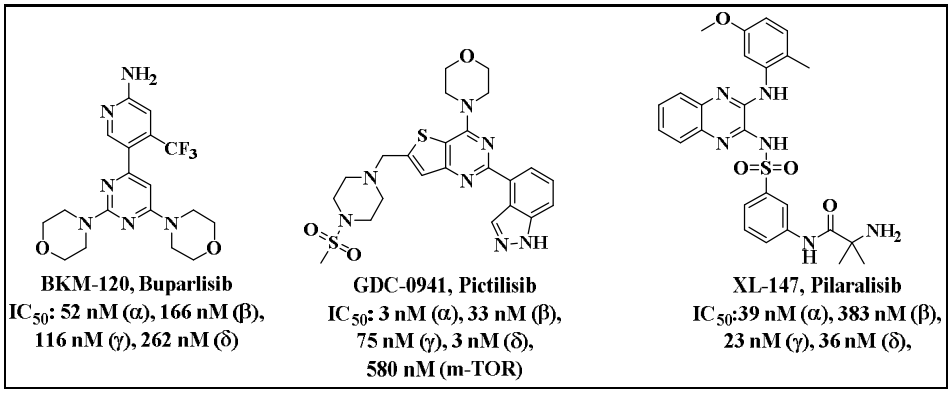


Figure 8: Pan-PI3K inhibitors

Currently available PI3K inhibitors, especially PI3Kδ inhibitors have significantly improved the clinical situation, but exhibits adverse effects such as neutropenia, hypertriglyceridemia, hyperglycemia, diarrhea/colitis, skin rashes, pyrexia, or liver enzyme elevation with severe organ toxicity. Therefore due to undesired toxicity most of the PI3Kδ inhibitors are not targeted for RA treatment. So there is need to explore a new pharmacophore and strategies to design potent, selective and safe PI3Kδ inhibitor to overcome the side effects associated with existing inhibitors for the safe and effective treatment of RA and chronic lymphocytic leukemia (CLL).

1.3. Objectives

Our objective is to design a novel, orally active, PI3Kδ selective inhibitor as an anti-inflammatory agent for the treatment of RA and anti-cancer agent to treat B-cell CLL. Literature survey reveals that, PI3K isoform selectivity (particularly PI3Kδ) can be achieved by modulating pharmacophore core structure or substituent on it. As a part of ongoing research activity, in the current investigation, we decided to optimize two standards compounds (Dactolisib and INK-654/666), wherein we mainly adopted bioisosteric

replacement strategy to develop novel PI3Kδ inhibitors. In next section, we have described:

a) Design of imidazo-quinoline derivative, benzofuran based pyrazolo-pyrimidine derivatives and 2,4-disubsitued quinoline pyrazolo-pyrimidine derivatives as PI3Kδ selective inhibitors

b) Synthesis and Characterization of designed PI3Kδ inhibitor

c) *In vitro* PI3Kδ inhibitory activity study

d) Isoform selectivity (α/β/γ/δ) study

e) Pharmacokinetic study

f) *In vivo* study

g) *In vitro* CYP and hERG inhibition study

h) Safety pharmacology study

i) Molecular modelling study

1.4. Design strategy

As discuss in the earlier section, knowing the potential side effects associated with the PI3K isoforms inhibitors, recently, more efforts are directed towards the development of isoform selective inhibitors, particularly PI3Kδ selective inhibitors, for the effective treatment of autoimmune, inflammatory diseases, such as RA and CLL.

Berndt et al. reported PI3Kδ crystal structure in complex with IC-87114 (PDB ID: **2WXK**). PI3Kδ enzyme structure shows four regions in the binding site; a) hinge region (ATP-adenine binging site), b) specificity pocket,

c) affinity pocket and d) hydrophobic region, **Figure 9 (a) [56]**. Thus for potent PI3Kδ selective inhibition, newly design compound must interact with the key amino acid residues in hinge region which is conserved for all PI3K isoforms, specificity pocket and hydrophobic region. Interactions in the affinity pocket helps to increase potency of inhibitors. Thus, to achieve all the key interactions, molecule adopts "propeller shaped" orientation. Most of the PI3Kδ selective inhibitors either approved or in clinical development possess "propeller shaped" orientation. The best example of such structural orientation is Idelalisib, **Figure 9 (b)**.

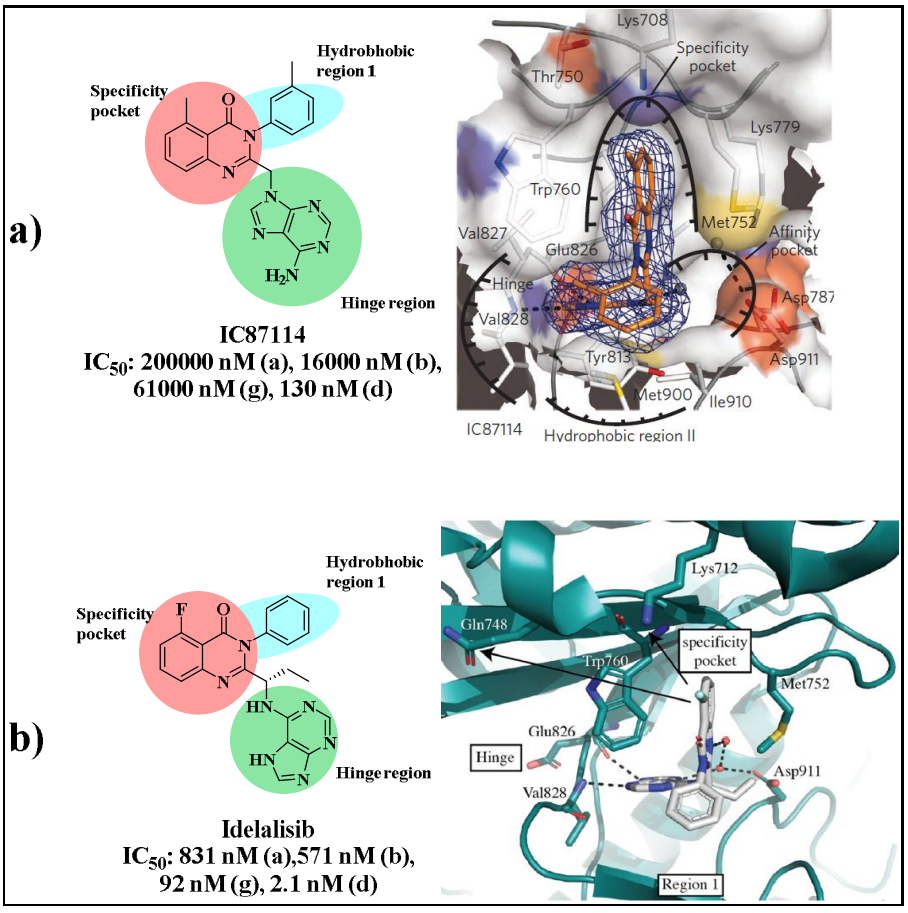


Figure 9: PI3Kδ crystal structure complexed with a) IC-87114 (PDB ID: **2WXK**) and b) Idelalisib (PDB ID: **4EX0**)

In Idelalisib, purine ring binds in the hinge region through *N3* and *N9* via hydrogen bond to nitrogen of δGlu_{826} and δVal_{828}, respectively, **Figure 9**. Quinazolinone interacts with Met_{752} and Trp_{760} in the specificity pocket. Phenyl ring extend towards hydrophobic region 1 and orients perpendicular to the quinazolinone (PDB ID: **4EX0**). Thus, overall orientation of Idelalisib is propeller shaped. SAR (Structure activity relationship) data published for Idelalisib by Gilead scientists clearly indicates that for potency, interaction in hinge region and specificity pocket is most important **[57]**. Whereas substitution on phenyl ring (hydrophobic region) help to increase selectivity as well as potency.

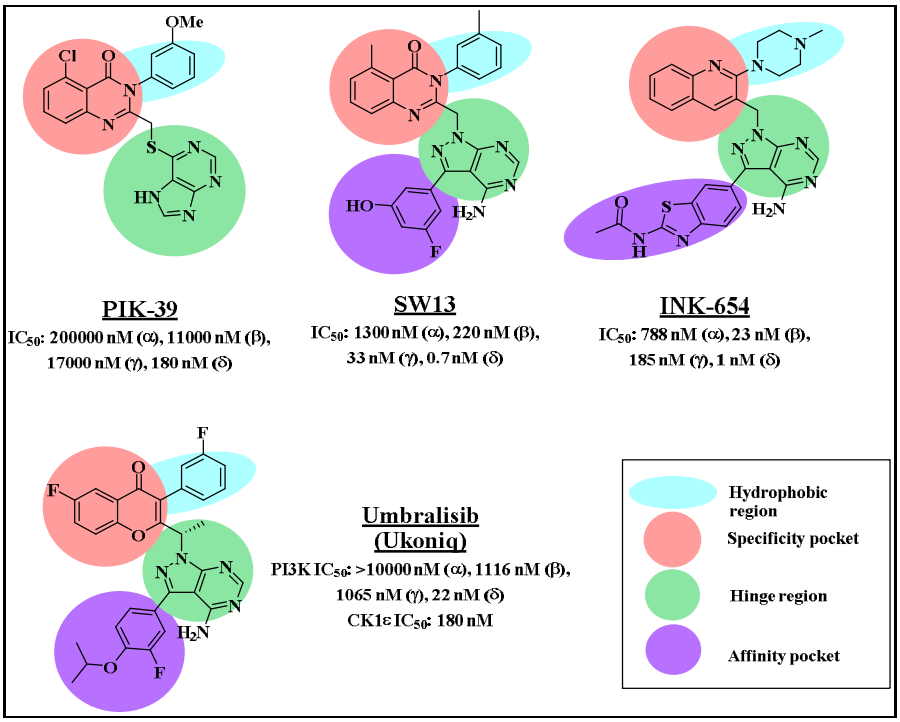


Figure 10: Potent PI3Kδ inhibitors with similar structural features

PIK-39, SW-13, INK-654 and Umbralisib are few more examples having propeller shape orientation, **Figure 10.** SW13, INK-654 and Umbralisib

are more potent PI3Kδ inhibitors as compared to IC87114 and PIK-39 due to strong interactions in affinity region with Asp_{787} or Asp_{911} **[56]**.

Considering an unmet need of developing PI3Kδ selective inhibitors for the safe, effective treatment of RA and CLL, we aim to design, novel, potent and orally bioavailable PI3Kδ selective inhibitor mainly by favoring the suitable interactions of the designed molecule with PI3Kδ ATP binding pockets targeting hinge region, specificity pocket, affinity region and hydrophobic region.

In Chapter II, we synthesized imidazo-quinoline based PI3Kδ inhibitor by introducing spacer in Dactolisib, so that newly designed compounds can fit in the ATP binding site of PI3Kδ protein, similar to Idelalisib, **Figure 11.** Introduction of spacer would make interaction in hydrophobic region accessible by adopting propeller shaped orientation. Initially, *in silico* modelling study of Dactolisib and new design compounds were done in PI3Kδ enzyme, which showed promising result. It was observed that imidazo-quinoline of Dactolisib has hydrogen bond interaction in hinge region with Val_{828}. New designed compounds also showed favorable interaction (hydrogen bonds) with Val_{828} in hinge region, specificity pocket, hydrophobic region and affinity pocket. Hence, we expect that these newly designed, compounds with spacer would be novel, potent and orally bioavailable PI3Kδ selective inhibitors by retaining the key interactions of inhibitors, in the PI3Kδ ATP binding pocket.

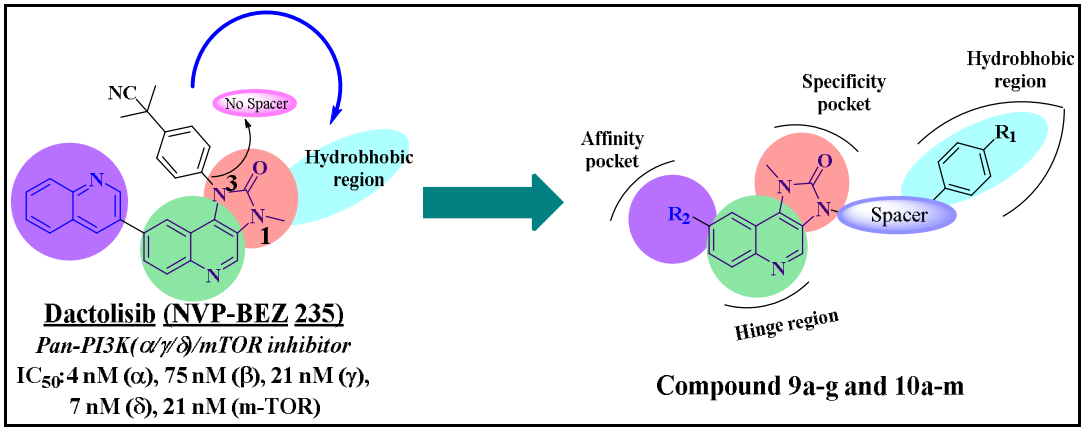


Figure 11: Imidazo-quinoline derivatives

There are many PI3Kδ inhibitors reported in literature, among them we further selected Intellikine compound (INK-666 and INK-654) as reference compound due their selectivity and potency for PI3Kδ inhibition. As discuss earlier, Alex Bernd et al. has reported the crystal structure of PI3Kδ protein with INK654 and INK666 showing INK-666 and INK-654 as PI3Kδ selective inhibitors, **Figure 12 [56]**. In crystal structure of PI3Kδ enzyme, interactions of INK-654 and INK-666 at ATP binding site showed that *N*-methyl piperazine ring is projected towards hydrophobic region, pyrazolo pyrimidine ring interacts with Val_{828} through hydrogen bond in hinge region, Core quinoline ring is sandwiched between Trp_{760} and Met_{752} in specificity pocket and benzthiazole ring is projected deeper in affinity pocket which leads to selective PI3Kδ inhibition. The SAR study of INK compounds showed potent PI3Kδ inhibition with excellent selectivity.

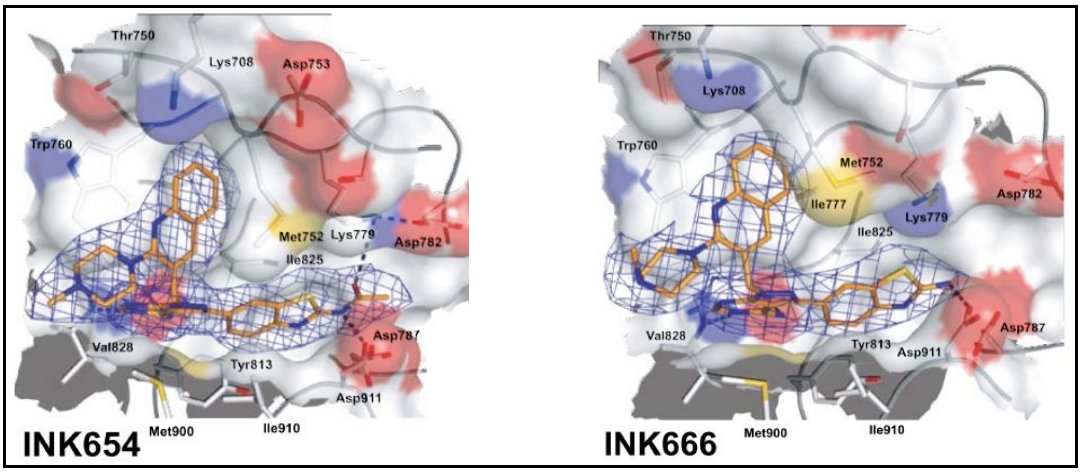


Figure 12: INK-654 and INK-666 co-crystal with PI3Kδ enzyme (PDB ID: **2WXK**)

We designed to two new scaffolds as PI3Kδ inhibitor by bioisosteric replacement of quinoline ring in INK-666 with benzofuran and 2,4-disubstituted quinoline ring.

Chapter III contains new scaffold, where 2,3-disubstituted quinoline ring of INK-666/INK-654 was bioisosterically replaced with benzofuran ring to get novel, potent and selective PI3Kδ inhibitors with benzofuran analogue, **Figure 13**. Substituted benzofuran has been widely used as pharmacophore and exhibits variety of therapeutic applications, such as antitumor, analgesic, anti-inflammatory, antihyperglycemic antimicrobial, antifungal and antiparasitic activities.

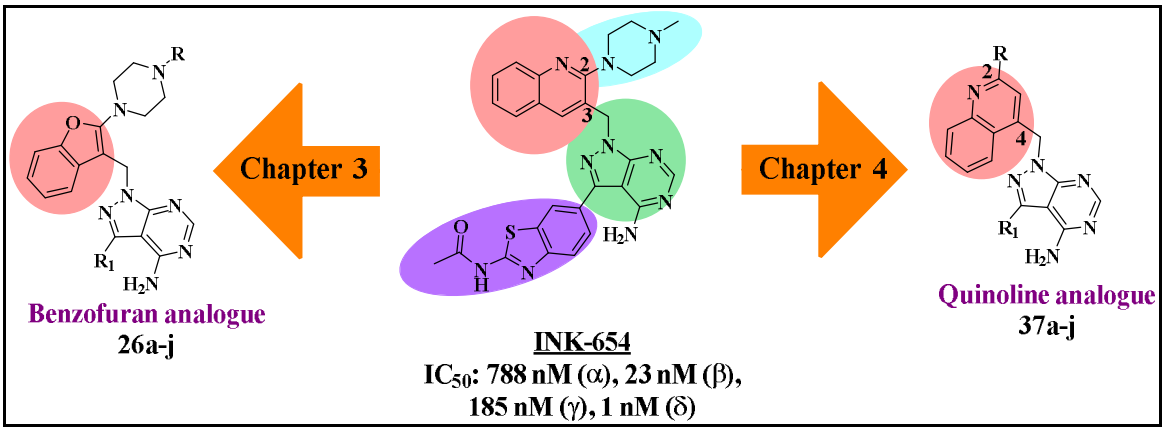


Figure 13: Benzofuran and 2,4-disustituted quinoline derivatives as PI3Kδ inhibitor

Literature around Intellikine scaffold (INK-666/INK-654) showed that the SAR is well established for 2,3-disubstitued quinoline ring where as its 2,4-disubstituted quinoline derivative are not discovered. Hence, in Chapter IV we synthesised 2,4-disubstituted quinoline based PI3Kδ inhibitor to understand SAR, **Figure 13 [58]**.