Module -9

Lecture -5

Molecularly targeted therapies for cancer

Conventional therapies

Tumors are conventionally treated with surgery, chemotherapy and radiation
Side effects and lack of specificity are the major problems

Chemotherapy acts on dividing cancer cells and this inherently affects other rapidly dividing normal cells as well (hair, gastrointestinal epithelium and bone marrow) leading to the side effects that include

Nausea and vomiting , Suppression of the immune system, Hair loss,
Renal and hepatic toxicity

Relapse and drug resistance are also major problems

Resistance to treatment

Tumor cells may initially respond to chemotherapy but after some time develop resistance to the same drug through several mechanisms of drug resistance

Loss of importing of drugs is one of the mechanisms by which a drug may be excluded by the tumor cells

Overexpression of p-glycoprotein multidrug efflux pump can effectively decrease the intracellular drug concentration of several drugs

Loss of expression of drug metabolizing components such as cytochrome p-450 so that the prodrug will not be activated into an active drug

Inactivation of apoptotic proteins such as p53 by mutation, overexpression of antiapoptotic proteins such as Bcl-2

Increased inactivation of drugs or toxic intermediates by activating the relevant enzymes such as nucleoside deaminase

Alterations in the structure of drug targets such as bcr-abl
Blood-brain barriers or inaccessible tissues such as testis

Molecularly targeted therapy

We have learnt how oncogene and tumour suppressor gene networks regulate if cancer cells have to proliferate or die

These decisions are further influenced by the tumour microenvironment and stress signals, such as DNA damage

Recent work suggests that a subpopulation of cancer stem cells may be important for initiating tumour development.

The term ‘targeted therapy’ refers to a new generation of cancer drugs designed to interfere with a specific molecular target (typically a protein) that is believed to have an important role in tumour growth or progression.

A detailed understanding of the molecular changes underlying cancer is necessary for the identification of appropriate targets.

Molecular targets

A protein becomes a druggable target if it contains a binding site/catalytic cleft or enzymatic activity

Thus the kinases are very attractive targets
There are 518 kinases in the human genome and 90 are tyrosine kinases

Transcription factors are thought be not suitable as targets except for the nuclear hormone receptors such as estrogen receptors/progesterone receptors. Tamoxifen is a good example and it modulates ER-α activity and is useful in treating breast cancer

The enzymatic activity present in Ras inhibits Ras signaling. Thus this enzyme is not suitable as a target since we should not inhibit this inhibitory activity

Although protein-protein interaction sites are good targets it becomes inconvenient if there are too many points of contact between 2 proteins

Oncogene addiction

"oncogene addiction" refers to a condition in which the tumor cells are addicted to an oncogene. Tumor maintenance is dependent on a continued activity of this mutated or constitutively activated oncogene.

Specific oncogene-dependent tumor development in mouse models (*MYC, RAS, BCR-ABL*) and their reversal by removal or inhibition have been demonstrated.

Targeting of these oncogenes is generally done with specific antibodies or small molecule inhibitors

BCR-ABL

The t(9;22) reciprocal chromosome translocation generates the *BCR-ABL* oncogene

The fusion product (p210) is an aberrant tyrosine kinase that is abundant in CML

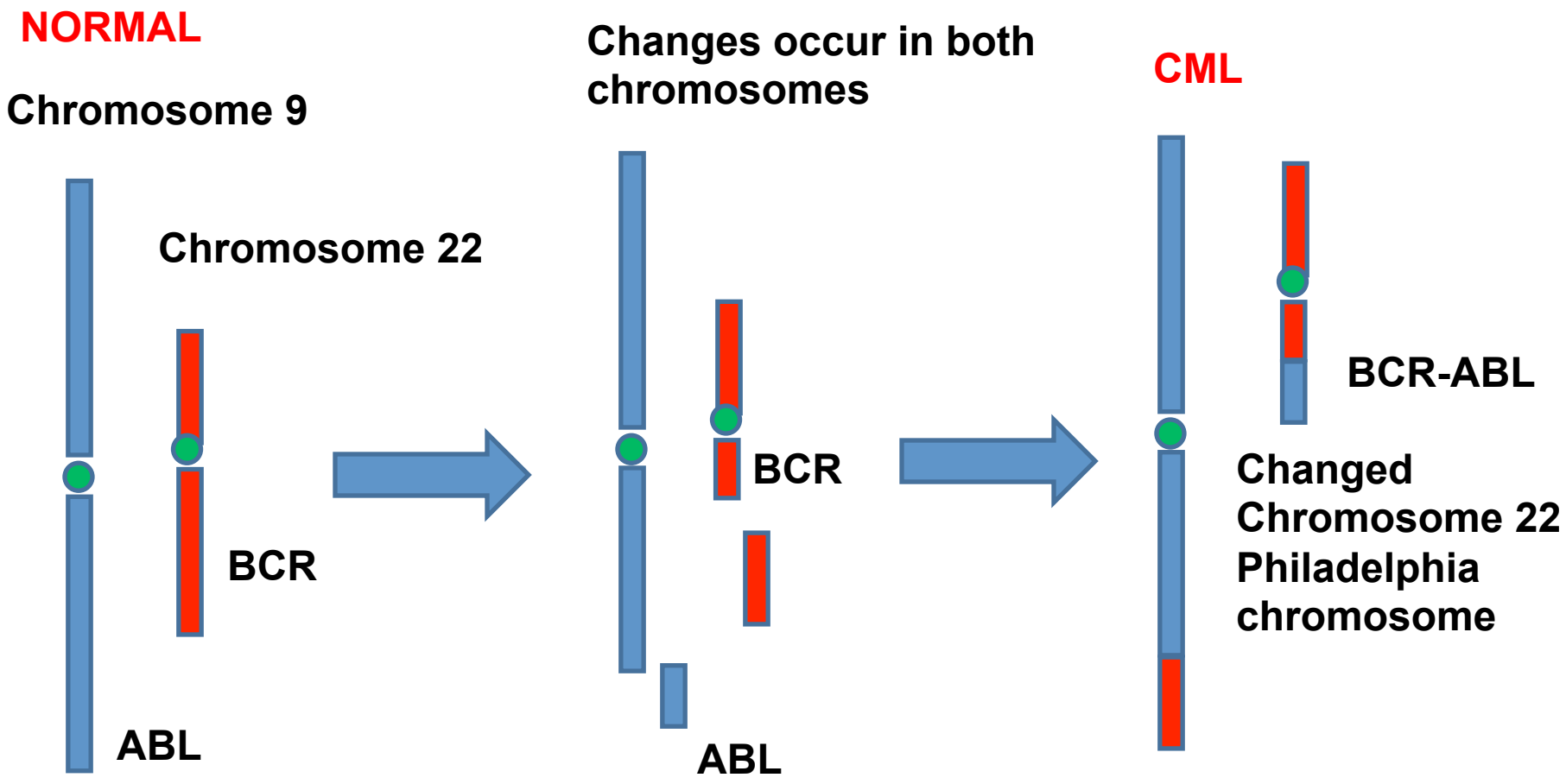
There are many different domains in the fusion protein and thus it is able to interact and activate many pathways

Ras signaling, PI3K/Akt and JAK/STAT

It can also activate Myc, NF-κB, Rac, Hck, Fes and Jun etc

These interactions enable it to control proliferation and survival of cancer cells

Formation of BCR-ABL



Imatinib

Imatinib mesylate /Gleevec was approved for the treatment of chronic myeloid leukemia (CML) on May 10, 2001.

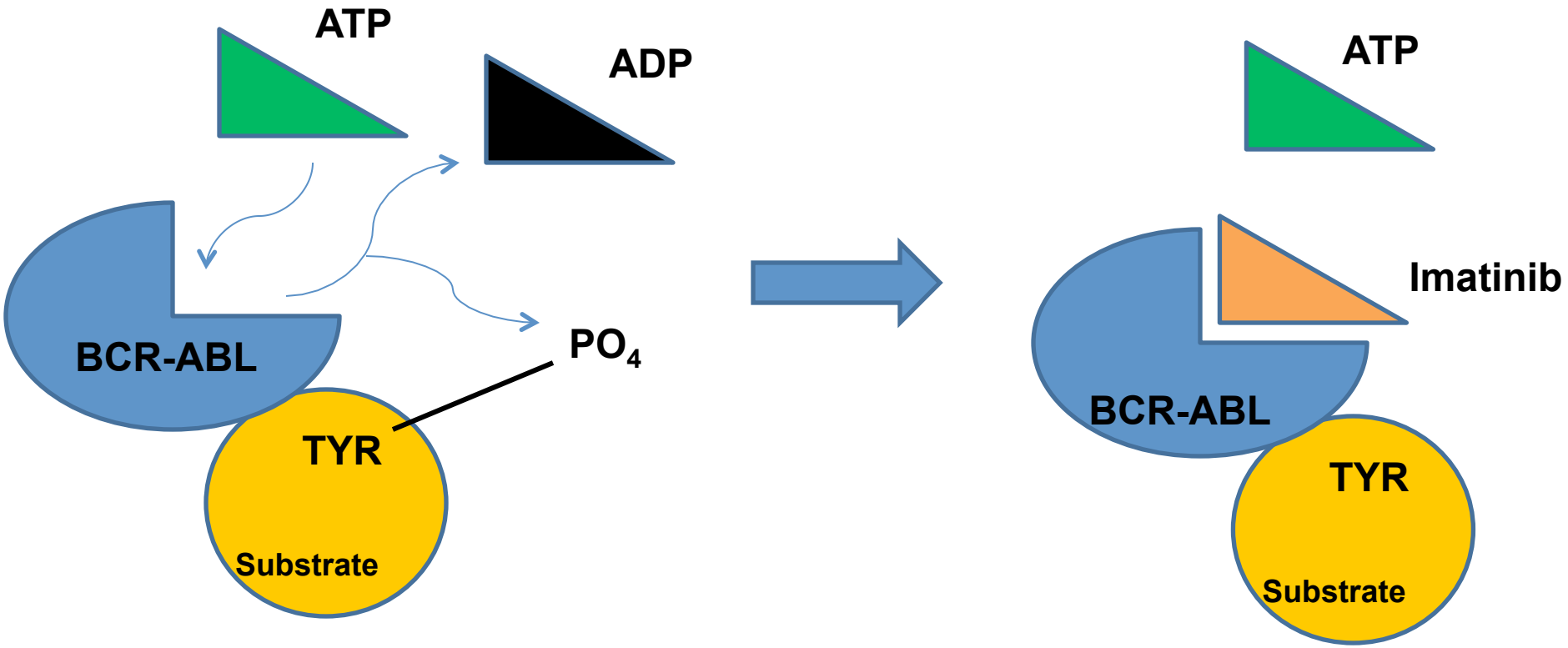
Imatinib not only inhibits BCR-ABL but is almost equally potent against platelet-derived growth factor receptor alfa (PDGFRA), and c-KIT receptor tyrosine kinases

Gastrointestinal stromal tumors (GISTs) are the most frequent mesenchymal tumors of the gastrointestinal (GI) tract

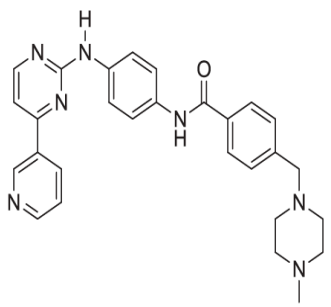
KIT mutations are detected in about 75–85% of GISTs, while PDGFRA mutations amount to 5–10%.

Imatinib is a competitive inhibitor of the BCR-ABLtyrosine kinase as well as other tyrosine kinases (e.g. KIT and PDGFR). It competes with ATP for binding to the kinases, preventing the transfer of the gamma-phosphate group to the suitable tyrosine residues, and is able to inhibit their downstream pathways.

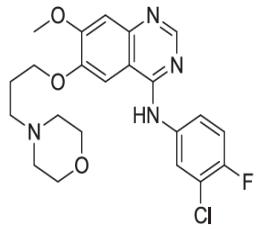
Imatinib competes for ATP binding site



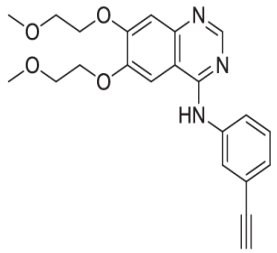
Approved molecularly targeted drugs



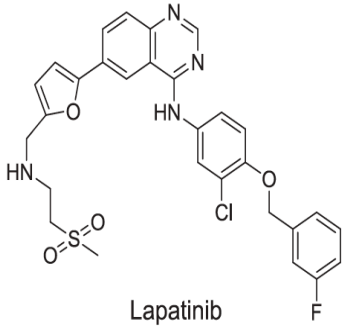
Imatinb



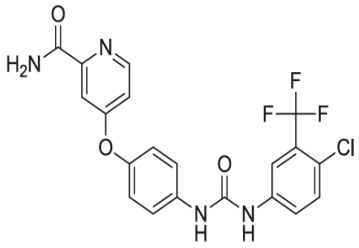
Gefitinib



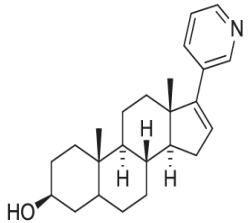
Erlotinib



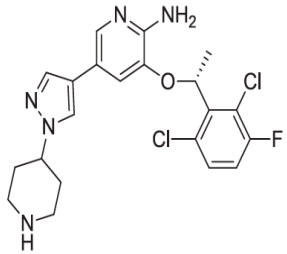
Lapatinib



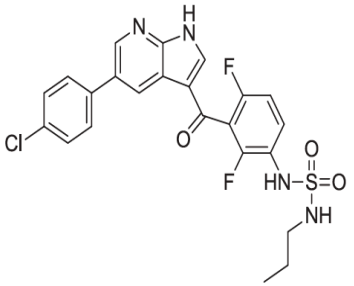
Sorafenib



Abiraterone



Critozinib



Vemurafenib

Resistance to Imatinib

Although Gleevec was successful initially, many problems were encountered subsequently

more than 40 mutant forms of *BCR-ABL* are known.

overexpression of *BCR-ABL* by amplification,

upregulation of drug efflux pumps,

downregulation of drug influx pumps,

alternative overexpression of nonreceptor TK such as Lyn,

enrichment of CML stem cell progenitors that are resistant to *BCR-ABL* -directed therapies

ErbB receptor tyrosine kinases

HER2/neu overexpression in breast and ovarian cancers correlates with poor prognosis

Trastuzumab (Herceptin) antibody binds extracellular domain IV of HER2

It is approved by the FDA for the treatment of HER2+ metastatic breast cancer and in the adjuvant setting in combination with chemotherapy

Trastuzumab downregulates HER2 signaling by masking a protease cleavage site on ECD IV and blocks shedding of the receptor. ECD shedding constitutively activates the TK domain, and blocking this correlates with positive responses to therapy. additional activity through antibody-dependent cell cytotoxicity.

Trastuzumab is also indicated for treatment of HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma

Cardiomyopathy is a side effect in some cases

ErbB receptor tyrosine kinases

FDA has approved several HER1-directed Mabs (Cetuximab, Panitumumab), which disrupt autocrine loops in human malignancies (colon, head and neck) and have higher responses when combined with chemotherapy

Several small molecule inhibitors have also received FDA approval: erlotinib and gefitinib are HER1-specific (lung and pancreas cancer), and lapatinib is a dual HER1/HER2 inhibitor (breast cancer resistant to trastuzumab)

PI3K/AKT

Class IA PI3Ks are implicated in cancer because of the presence of activating somatic mutations in the catalytic subunit p110a (*PIK3CA*) that are identified in approximately 30% of epithelial cancers (breast, colon, prostate, endometrial)

At least 10 PI3K p110 KD-targeted agents (BEZ235, BGT226, BKM120, XL765, XL147, GDC0941, SF1126, PX-866, CAL-101, GSK1059615) have entered early-phase clinical trials

RET

Gain-of-function mutations in the *RET* proto-oncogene leads to hereditary (98%) and sporadic (30%) medullary thyroid carcinoma (MTC), a rare calcitonin-producing tumor arising from parafollicular C cells

The advantage of TKIs currently in trials that target RET is that they also target other RTKs that are implicated in proliferation and angiogenesis.

B-RAF

BRAF is a cytoplasmic S/T kinase that lies immediately downstream of RAS and is a key player of the RAS/RAF/MEK/ERK pathway, with a major activating mutation (V600E) detected in melanoma (50-70%), PTC (36-69%), CRC (5-12%), and NSCLC (1-4%).

V600E BRAF mutation constitutively activates ERK signaling that induces proliferation and promotes transformation

Sorafenib (Nexavar) is a multitargeted KI that has activity against B-RAF, VEGFR-2/3, c-Kit, PDGFR, Flt-3, and FGFR1

FDA approved for advanced RCC and HCC

Enhancing tumor suppressor activity

Tumor cells have a program of gene silencing by epigenetic modification of the DNA and/or histones, especially of tumor suppressors.

Targets that activate tumor suppressors are currently under development (eg, P53-MDM2 inhibitors).

2 DNA hypomethylating agents, 5-azacytidine (Vidaza) and 5-aza-2'-deoxycytidine (Decitabine) are approved for the treatment of higher-risk myelodysplastic syndrome (MDS).

Vidaza is a nucleoside analog with a ribose structure that gets incorporated into RNA and DNA via ribonucleotide reductase (RNR).

HDAC inhibitors

In tumors, HDACs favor deacetylation and tightening of histones, leading to epigenetic silencing.

DNA methylation and histone deacetylation work in concert in gene silencing because of direct binding interactions between DNMTs and HDACs.

Vorinostat (Zolinza, suberoylanilide hydroxamine acid) is the first HDACI to be approved for the treatment of advanced cutaneous T-cell lymphoma. The side effects include nausea, diarrhea, fatigue, and anorexia.

Belinostat (PXD-101, Curagen) is a novel HDAC-I of class I and II histone deacetylases. This class of compounds has demonstrated anticancer activity in malignant mesothelioma.

P53-HDM2 interaction inhibitor

Human double minute 2 (HDM2) is a cancer target, as it inhibits p53 tumor suppressor activity.

HDM2 binds to a 15-residue α-helical segment of p53 (Kd = 600 nM).

High-throughput screening and medicinal chemistry identified tetra-substituted imadazoles called Nutlins (Hoffman-La Roche).

Nutlin-3 disrupts HDM2-p53 complexes with an IC_{50} of 90 nM, has good preclinical activity, and has entered phase I trials.

Bcl-2

The first agent to enter clinical trials targeting Bcl-2 was an antisense (G3139, Genasense) phosphothiolate oligodeoxynucleotide that targets Bcl-2 mRNA.

The agent was evaluated alone in CLL, with minimal response. Subsequently, combinations with other anticancer modalities (chemotherapy, immunotherapy) have been evaluated in CLL, AML, MM, SCLC, NHL, and melanoma.
A randomized phase III study with fludarabine plus cyclophosphamide, with or without Genasense, in relapsed or refractory CLL with long-term follow-up appears to show a survival advantage in a subset of patients

Several classes of small molecule inhibitors targeting the Bcl-2 anti-apoptosis members have entered early-phase clinical trials.

Levo-gossypol (AT-101, Ascenta) that inhibits Bcl-2, Bcl-Xl, and Mcl-1, is in phase II clinical trials for CLL (with rituximab) and docetaxel

Antisense therapy to IAPs

Inhibitors of apoptosis proteins (IAP) are potent inhibitors of caspases

XIAP is over expressed in many types of human tumors (GBM, AML, CRC, BC, pancreatic cancer, prostate cancer, gastric cancer) and overexpression correlates with poor prognosis.

AEG35156 is a second-generation antisense oligonucleotide [ASO] (19-mer that incorporates 2'-O-methyl with a phosphorothioate backbone), which targets XIAP mRNA

Phase I/II trials are ongoing with AEG35156 in combination with chemotherapy for pancreatic cancer, BC, NSCLC, AML, NHL, and other solid tumors and in combination with Sorafenib in patients with hepatocellular carcinoma (HCC).

Survivin inhibition is achieved by a second-generation ASO LY2181308 (Lilly/Isis) and is currently in phase I clinical trials.

Death receptors

Tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand or Apo2 ligand (TRAIL/Apo2L) members of the TNF superfamily induce apoptosis upon binding to cell surface death receptors TNFR1, TNFR2, CD95/FAS, and TRAIL receptors 1 and 2 (DR 4 and DR5), directly activating the caspase cascade via an initiator caspase (caspase-8) within the death-inducing signaling complex.

The preferential expression of DRs on malignant cells provides for a potential target for cancer therapy.

Several therapeutic agonist Mabs targeting DR4 and DR5 are in phase I/II clinical trials: LBY135 (Novartis), AMG 665 (Amgen), lexatumumab, HGS-TR2J (HGS), and Apomab (Genentech) are DR5 agonist Mabs, while CS-1008 (Sankyo) and mapatumumab (HGS) are DR4 agonist Mabs.

Antiangiogenesis

Bevacizumab (Avastin, Genentech/Roche) is a therapeutic monoclonal antibody that targets VEGF and has undergone clinical development as a single agent and in combination with chemotherapy and targeted therapies. It is the only approved antiangiogeneic therapy for 4 human malignancies (CRC, NSCLC, BC, GBM).

Several ATP-site small molecule inhibitors (SMIs) targeting the VEGFR (1, 2, and/or 3) TK domains are approved (sorafenib, sunitinib) or in early-phase clinical trials

FGFR1 antagonist FP-1039 (FivePrime) is a soluble fusion protein consisting of the extracellular domain (ECD) of human FGFR1 fused to the Fc portion of IgG1, with potential antineoplastic and anti-angiogenic activities

FP-1039 may also inhibit vascular VEGF-induced neo-angiogenesis.

CDK inhibitors

Discovery and development of ATP-site SMIs to CDKs as antiproliferative agents is based on the hypothesis that selective cell growth arrest and/or apoptosis could be induced due to impaired control of cell cycle progression in malignant cells.

Several CDK ATP-site SMIs, are currently in early-phase clinical trials.

Flavopiridol (Sanofi-Aventis), a natural product, is a potent pan-CDK SMI that blocks cell cycle progression at the G1/S and G2/M boundaries

UCN-01 (7-OH staurosporine), the second CDK SMI, has entered clinical trials. It inhibits protein kinase C (PKC) activity, promotes cell cycle arrest by accumulation in p21/p27, induces apoptosis in several preclinical models, and abrogates the G2 checkpoint by inhibition of CHK1. A novel strategy is to combine UCN-01 with DNA-damaging agents.

A synthetic lethal network

Gene X	Gene Y	Viability
++	++	viable
++	--	viable
--	++	viable
--	--	Not viable (death)

Two genes or proteins are synthetically lethal when inactivation of either gene/ protein is still compatible with cellular viability but inactivation of both leads to cell death

Two such genes are those encoding PARP-1 and BRCA-1

Synthetic lethal approach

Poly (ADP-ribose) polymerases (PARP) are a family of highly conserved enzymes that play a key role in signaling DNA single-strand breaks (SSB) through the Base excision repair (BER) pathway and double-strand breaks (DSB) through the DSBR pathway

The net negative charge on the poly ADP-ribose polymer opens up the damaged DNA to allow access to the other components of the repair process.

Inhibition of PARP-1 would lead to unrepaired SSBs and DSBs and this is a potent signal to initiate apoptosis

However, the DNA damage response pathway will activate many kinases and also BRCA1, and BRCA2, leading to cell cycle arrest and DNA repair

We can enhance apoptosis by PARP-1 inhibition in cancer cells that have an inherited DNA repair deficiency (eg, BRCA1, BRCA2)

Several PARP-1 inhibitors are now in early-phase clinical trials

Induction of differentiation

One idea is to induce differentiation as most of the cancer cells are poorly differentiated.
All trans retinoic acid is used to differentiate acute promyelocytic leukemia cells.
ATRA can induce the blast cells from this cancer into polymorphonuclear neutrophils.
Along with regular chemotherapy ATRA is given to these patients and this approach improves their survival and leads to complete remission.

Chromosomal translocation (15; 17) results in the fusion product of PML-RAR (retinoic acid receptor). This blocks the differentiation ability of RAR.
ATRA induces ubiquitination and proteosomal degradation of PML-RAR fusion protein and thus is able to restore the differentiation capabilities of RAR.

Immunotherapy was discussed already

Under development

HSP90 inhibitors
Hedgehog signaling inhibitors
mTOR inhibitors are under development

In this lecture we learnt about some of the problems encountered with conventional therapy
We also learnt as to how modern research on the molecular aspects of cancer provided us with some tools that can be used for designing novel approaches to treat cancer
We discussed the concept of oncogene addiction and this helps in molecularly targeted therapy
Successful implication of this approach in CML was discussed
We also noted that many antibodies such as herceptin are already in the clinics and you may also recollect our lessons on tumor immunotherapy
Targeting of kinases, protein-protein interactions, ways of enhancing apoptosis, HDAC inhibition, antiangiogenisis approach were also discussed in this lecture
We also discussed some of the novel approaches including synthetic lethal approaches to treat cancer
Finally I have also mentioned that this is an area that continues to grow with more and more discoveries and novel approaches to tackle this disease.

END