IJBPAS, January, 2016, 5(1): 08-30

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

AN UPDATED REVIEW OFCANCER AND ANALYTICAL STUDY OF ANTI-CANCER DRUG GEFITINIB-THE FIRST SELECTIVE INHIBITOR OF EPIDERMAL GROWTHFACTOR RECEPTOR'S (EGFR)

HAFIZ A. MAKEEN[1], AL BRATTY MOHAMMED[2], MD SHAMSHER ALAM[2*]

[1]Department of Clinical Pharmacy, College of pharmacy, Jazan University, P.O. Box 114, Jazan 45142, Saudi Arabia

[2]Department of Pharmaceutical Chemistry, College of pharmacy, Jazan University, P.O.Box 114, Jazan 45142, Saudi Arabia

***Corresponding Author:E Mail: mosalam@jazanu.edu.sa**

ABSTRACT

Cancer is the group of diseases. The present study is emphasizing on the emerging hallmarks and enabling characteristics of cancer. It covers the molecular basis and with very clearlytheincidence and mortality figures. Treatment modalities and its limitations are nicely highlighted. It also includes the new generation of targeted therapeutic drugs.

Gefitinib is an orally active, selective EGFR-TK inhibitor (EGFR-TKI) that causes complete inhibition of EGF-stimulated EGFR auto phosphorylation in cell lines.In preclinical studies, gefitinib has demonstrated antitumor activity against a variety of human cancer cell linesexpressing EGFR, including lung, ovarian, breast, and colon.TKIs are ideal substances for LC-MS/MS measurements with positive ionization due to their chemical structures combined with their proton affinity. Therefore several LC-MS/MS tests were developed recently. Because of its clinical advantages, there is an increase in the number of Gefitinib formulations in the market for a variety of indications. Therefore, there is a need for a sensitive and reliable analytical method for the estimation of Gefitinib in pharmaceutical formulations.

Keywords: Gefitinib, Liquid Chromatography, Plasma, LC-MS/MS,validation,metastasis,Pharmacokinetics

INTRODUCTION

General introduction to the cancer

Cancer is a cellular disease characterized byalteration in control mechanisms that govern the balance between cell proliferation, cell death and differentiation. In this disease, cancer cells grow abnormally and form a mass or tumor **[1].**Tumors can be benign or malignant. Benign tumors may grow but they do not invade surrounding normal tissue, spread to other tissues and are usually not life threatening. On the other hand, malignant tumors are life threatening. They grow, invade and spread (metastasis) to other tissues in the body.

Cancer cells acquire six essential hallmarks or traits in comparison to normal cells, **(Figure-1).** The first hallmark is self-sufficiency in growth signals, the second is insensitivity to growth-inhibitory (antigrowth) signals, the third is evasion of programmed cell death (apoptosis), the fourth hallmark is limitless replicative potential, the fifth is sustained angiogenesis and finally the sixth hallmark is tissue invasion and metastasis **[2].**

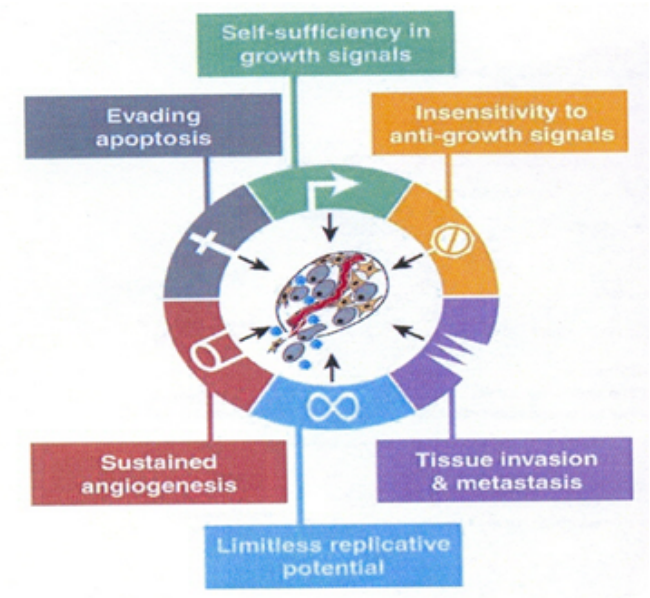

Figure-1: Crucial Hallmarks of Cancer Cells

These hallmarks were updated in 2011. Two emerging hallmarks and two enabling characteristics were added to the current hallmarks **(Figure-2).** The emerging hallmarks are the ability of cancer cells to avoid immune destruction and their ability to deregulate cellular energetics. The first of the enabling characteristics is the development of genomic instability in cancer cells which generates random mutations and the second is tumor-promoting inflammation. Tumor microenvironment plays a major roleenabling cancer cells to acquire these hallmarks**[3].**

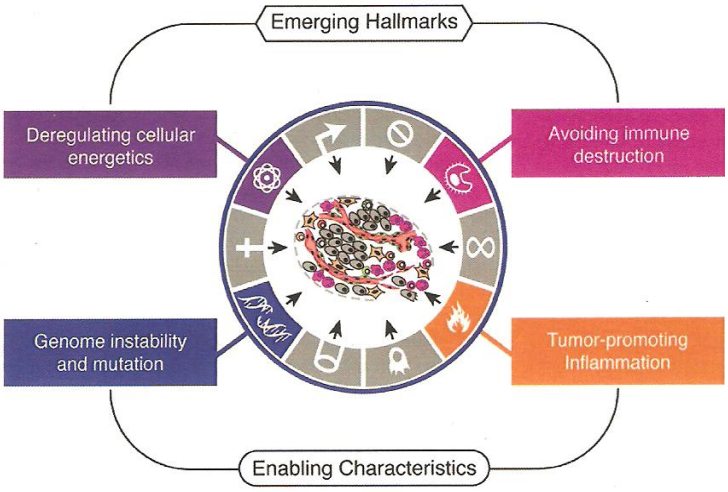

Figure -2: Emerging hallmarks and enabling characteristics of cancer

Cancer is governing by several factors. Environmental factors**[4],** heredity, immune deficiency, some viral infections and carcinogenic agents are all linked to various cancers**[5].** Doll and Peto have listed various factors that may lead to cancer. These factors included tobacco, alcohol, diet, food additives, reproductive and sexual behavior, occupation, pollution, industrial products, medicines and medical procedures and infections **[6].** Knowledge of and a close understanding of how exposure to these risk factors can be reduced is an important weapon in the war on cancer.

Overview of the molecular basis of cancer

Cancer can be measured a genetic disease. Oncogenes and tumor suppressor genes play a major role in the pathogenesis of cancer. DNA damage can lead to the activation of oncogenes and loss of tumor suppressor genes. Oncogenes can be considered tumor inducing factors and they develop from normal genes called proto-oncogenes. They have important roles in all phases of carcinogenesis. Activated oncogenes can produce excessive amounts of the normal gene product or an abnormal gene product. This can result in an abnormal cell growth and proliferation. The platelet derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), tyrosine kinases and apoptosis regulator BCL2 are examples of oncogenic proteins. These oncogenes can be targeted by cancer therapies**[7].**Imatinib is a prime example of a cancer therapy that targets a cancer causing oncogene in order to treat chronic myelogenousleukaemia. Imatinib is a small molecule that targets the tyrosine kinases ABL and C-KIT and the PDGFR. It is used in the treatment of chronic myelogenousleukaemia**[8]** and gastrointestinal stromal tumors (GIST)**[9].**Cetuximab is a monoclonal

antibody which targets EGFR and is used in the treatment of colorectal cancer**[10].** In contrast, tumor suppressor genes regulate and inhibit inappropriate cellular growth and proliferation. Mutation or loss of this gene can result in loss of normal cell growth control. P53 is an example of a tumor suppressor gene. Mutation of P53 is one of the most common genetic changes associated with cancer. In addition, alteration in DNA repair genes can lead to cancer. DNA repair genes encode for protein that corrects errors that may occur during DNA duplication **[11].** Cancers evolve as a result of a series of mutations in both oncogenes and tumor suppressor genes, the classic example of this being the Fearon-Vogelstein model of colorectal cancer **[12].**

Incidence and Mortality Figures of cancer

According to GLOBOCAN 2008 about 12.7 million cancer cases and 7.6 million cancer death are estimated to have occurred in 2008. GLOBOCAN is an estimate of cancer incidence and mortality worldwide produced by the International Agency for Research on Cancer (IARC), which is a part of the World Health Organization (WHO). According to the same agency, the most frequently diagnosed and the leading cause of cancer death in female is the breast cancer which accounts for 23% of the total cancer cases and 14% of cancer death. However, in males, lung cancer was the leading cancer site accounting for 17% of total new cancer cases and 23% of the total cancer death**[13].**

In the European Union (EU), 1,281,436 is the predicted total number of cancer deaths in 2011. As the total number of cancer deaths in the (EU) was 1,256,071 in 2007, there is an increase in the number of cases by 25,365. In 2011, the estimated standardized cancer death rates in (EU) also are expected to be 142.8/100,000 men and 85.3/100,000 women compared with 153.8/100,000 men and 90.7/100,000 women in 2007 **(Figure -3) [14].**

In the United States, cancer is considered the second leading cause of death after cardiovascular disease. In 2010, more than 1.5 million new cancer cases were predicted to be diagnosed and about 569,490 cancer deaths are expected to be recorded in the United States. The overall survival rate from 1999 to 2005 for all types of cancer was 68% and the five most common causes of death from cancer in men in the United States in 2007 were lung, prostate, colorectal, pancreas and leukaemia and in women they were lung, breast, colorectal, pancreas and ovarian cancer. **Figure-4** represents the ten leading cancer types for the estimated new cancer

cases and deaths in male and female in 2010 in the United States**[15].**

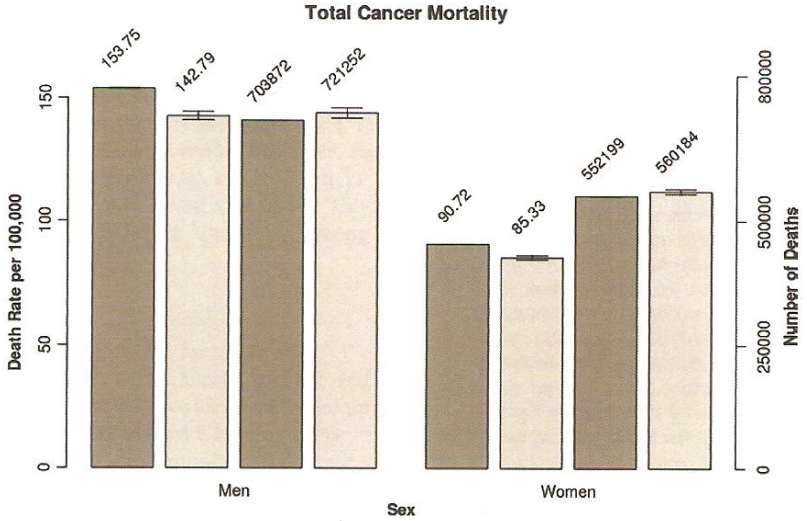

Figure-3: Cancer statistics in Europe: Bar plots of standardized death rates per 100 000 population and certified deaths for the year 2007 (dark grey) and the predicted death rates and number of deaths for 2011 (light grey) for total cancer mortality in the (EU) in men and women.

Estimated New Cases*

	Males			Females	
Prostate	217,730	28%		Breast	207,090	28%
Lung & bronchus	116,750	15%		Lung & bronchus	105,770	14%
Colon & rectum	72,090	9%		Colon & rectum	70,480	10%
Urinary bladder	52,760	7%		Uterine corpus	43,470	6%
Melanoma of the skin	38,870	5%		Thyroid	33,930	5%
Non-Hodgkin lymphoma	35,380	4%		Non-Hodgkin lymphoma	30,160	4%
Kidney & renal pelvis	35,370	4%		Melanoma of the skin	29,260	4%
Oral cavity & pharynx	25,420	3%		Kidney & renal pelvis	22,870	3%
Leukemia	24,690	3%		Ovary	21,880	3%
Pancreas	21,370	3%		Pancreas	21,770	3%
All Sites	**789,620**	**100%**		**All Sites**	**739,940**	**100%**

Estimated Deaths

	Males			Females	
Lung & bronchus	86,220	29%		Lung & bronchus	71,080	26%
Prostate	32,050	11%		Breast	39,840	15%
Colon & rectum	26,580	9%		Colon & rectum	24,790	9%
Pancreas	18,770	6%		Pancreas	18,030	7%
Liver & intrahepatic bile duct	12,720	4%		Ovary	13,850	5%
Leukemia	12,660	4%		Non-Hodgkin lymphoma	9,500	4%
Esophagus	11,650	4%		Leukemia	9,180	3%
Non-Hodgkin lymphoma	10,710	4%		Uterine Corpus	7,950	3%
Urinary bladder	10,410	3%		Liver & intrahepatic bile duct	6,190	2%
Kidney & renal pelvis	8,210	3%		Brain & other nervous system	5,720	2%
All Sites	**299,200**	**100%**		**All Sites**	**270,290**	**100%**

Figure-4: Ten leading cancer types for the estimated new cancer cases and deaths in men and women in 2010 in the United States

These statistics are essential for evaluating the current cancer prevention and management strategies. It is clear from the current statistics that there is a real need for new therapies for treating cancer and/or new strategies to make the current available treatment modalities work better.

Treatment modalities of cancer

Surgery, radiotherapy and systemic therapy are the main cancer treatment modalities.

Surgery: Surgery is the oldest modality available to treat patients with cancer. It is highly effective at treating cancer that have not metastasized and in these cases, treatment can be curative. Catching the disease early therefore is important but for many cancers, the disease has already disseminated at the time of diagnosis. Patients with limited metastatic disease may still benefit from surgical resection of the metastasis. Furthermore, in patients with advanced metastasis, surgery can be used to relieve pain, improve functional abnormalities (e.g. gastrointestinal obstruction) and may improve the patient's quality of life **[16].**

Radiation therapy

Radiation therapy can be used to eradicate localized tumor masses or disseminated disease (leukaemia) with bone marrow or stem cell transplants. Radiation can also be used for tumors located at non-operable areas of the body. The usefulness of radiation can be limited by its toxic effects on normal tissues that are located beside the tumor **[17].**

Systemic therapy

Not all cancers can be cured by surgery or radiation therapy, especially metastatic cancers. In these cases, systemic treatment is essential. These include chemotherapy (both traditional cytotoxic and molecularly targeted therapies), hormonal therapy and biological therapy. Chemotherapy is the mainstay of systemic therapies and its origins go back to observations made in the middle 1940s, when mustard gas was shown to produce bone marrow suppression and lymphoid hypoplasia in service men exposed to the gas. This incident led to the use of mustard gas derivatives (nitrogen mustard alkylating agents) in the treatment of Hodgkin's disease and other lymphomas**[18].** Since the use of nitrogen mustards emerged, a series of cytotoxic agents were developed, the vast majority of which kill cancer cells mainly by damaging DNA, interfering with DNA synthesis or inhibiting cell division. Cytotoxic chemotherapy agents have been classified by their effect on the cell cycle or their mechanism of action. Agents that affect the cell only during a specific phase of the cell cycle often are referred to as phase specific agents or schedule dependent agents. On the other hand,

agents that affect the cell during any phase of the cell cycle are often referred to as phase non-specific agents or dose dependent agents. **Figure- 5**,shows the mechanism of action and the effects of the cytotoxic drugs on phases of cell cycle. It does also show examples of phase specific and phase non-specific cytotoxic agents**[19].**

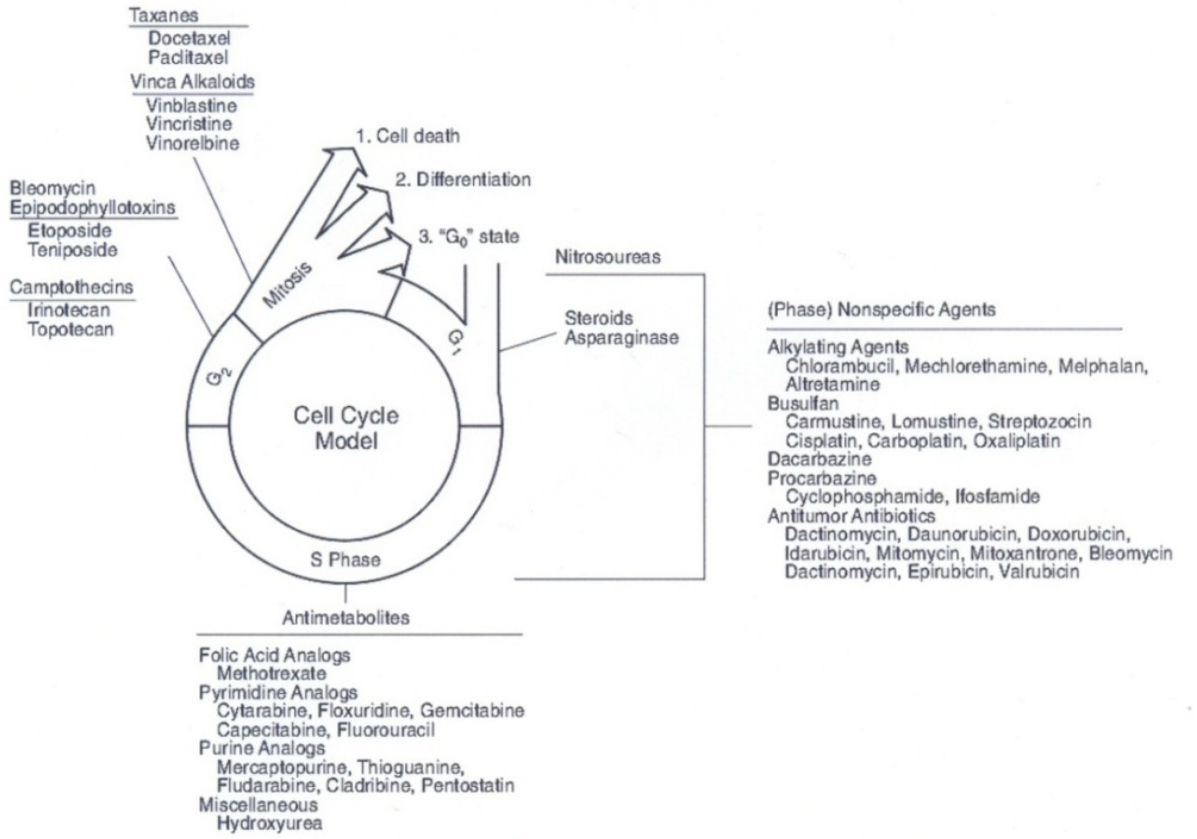

Figure -5: Effects of cytotoxic drugs on phases of the cell cycle

Cancer chemotherapy may be used as a primary, palliative, adjuvant or neoadjuvant treatment modality. It is considered a primary or a curative modality in some diseases like leukaemias, lymphomas and testicular cancer. Chemotherapy is initiated for palliative purposes in most of solid tumors to relieve symptoms either because of the nature of the tumor or because of advanced disease at time of treatment**[20].** Adjuvant therapy is defined as the use of systemic chemotherapy to eradicate micro-metastatic disease following surgery or radiotherapy or both of them. The main purpose of chemotherapy in this situation is to reduce tumor recurrence rates and prolong patient's long term survival**[21].** Chemotherapy may also be given in neoadjuvant or preoperative setting. The purpose in this case is to make other treatment modalities like surgery more effective by reducing tumor burden and to destroy micro-metastases.

With increased understanding of cancer cell biology, other systemic therapeutic agents have been introduced. The following section describes the key mechanisms of action and provides a few examples of hormonal, biological and molecular targeted agents used for the treatment of cancer.

Hormonal therapy is type of anti-neoplastic therapy currently in the clinic. The drugs related to this group oppose or alter the effect of male or female hormones in hormone dependent tumors. This group of drugs inhibits tumor growth by blocking the receptors or by eliminating the endogenous hormone which feeds the tumor. The problem with this group is resistance where not all the tumors arising from hormone sensitive tissue respond to hormonal therapy. Examples of hormones used as anti-neoplastic drugs include the progestin hydroxylprogesterone, the anti-estrogen tamoxifen and the anti-androgen flutamide**[22].**

Biological therapy is another form of systemic cancer therapy. In this group a protein, antibody or gene can be administered to the cancer patient in order to kill cancer cells or to support the patient immune system**[23].** The immune system plays an important role in determining cancer development. Immune-suppressed patients like AIDS patients face an increased risk of developing cancer compared to immune-competent individuals**[24].** Supporting the immune system can help prevent or treat cancer. Biological therapies aim to support individual immune response as well as providing selective anti-tumor effects. Immunotherapy is an example on biological systemic therapy and it usually involves stimulating the host's immune system to fight the cancer cells. The agents used in immunotherapy are naturally occurring cytokines which have been produced by the aid of the recombinant DNA technology. Examples of agents used in immunotherapy include interferons and interleukins (ILs). The proposed mechanism of action of Interferon-α for example is direct anti-proliferative efficacy, an immunomodulatory effect on natural killer cells, T cells, B cells and macrophages, anti-angiogenic efficacy and a differentiating effect on tumour cells**[25].** Interleukin-2 (IL-2) is a recombinantly produced lymphokine with an immunoregulatory action and its use in patients with advanced renal cell carcinoma has induced tumor regression and increased patient survival with a favorable toxicity profile when used in low doses**[26].**

The new generation of targeted therapeutic drugs

By understanding the mechanisms by which cancer cells exhibit abnormal growth, angiogenesis, apoptosis and metastasis, it has been possible to design drugs that inhibit these processes**[27].** Molecular targeted therapeutic agents were introduced to kill tumor cells without disrupting the physiological functions of normal cells**[28].**Bevacizumab, cetuximab, trastuzumab, erlotinib, gefitinib and dasatinib are examples of clinically used molecular targeted agents**[29].** A list of them and other molecular targeted agents, their targets and clinical uses is available in **table-1.** Bevacizumab is a monoclonal antibody with antiangiogenic activity. It targets the vascular endothelial growth factor (VEGF)**[30].** Bevacizumab is available in the clinic for the treatment of non-small-cell lung cancer **[31]**and colorectal cancer. The other monoclonal antibodies cetuximab and trastuzumab are also used clinically **[32]**for the treatment of solid tumors including colorectal cancer and breast cancer. They inhibit growth factor receptors leading to decreasing cell proliferation and increasing death of tumour cells. Cetuximab targets the epidermal growth factor receptor (EGFR). It was reported as active and save option for the treatment of colorectal cancer. Trastuzumab is now a standard drug for Her-2 positive breast cancer patients. Other examples of targeted drugs are erlotinib and gefitinib which inhibit the epidermal growth factor receptor tyrosine kinase (EGFR). They are used for the treatment of different types of solid tumors including non-small cell lung cancer **[33].** Dasatinib also is another example of molecular targeted agents. It has the advantage of being used for the treatment of leukemias. It targets and inhibits the BCR-ABL tyrosine kinase. Dasatinib can be used for the treatment of patients with chronic myelogenousleukaemia (CML) or Philadelphia positive acute lymphoblastic leukaemia (ALL). It has shown efficacy in patients with CML or Philadelphia-positive (ALL) patients who are resistant to imatinib as it inhibits imatinib BCR-ABL mutations**[34].**

Table 1: List of some molecular targeted agents currently in the clinic

Generic name	Trade name	Molecular Target	Clinical use
Bevacizumab	Avastin	VEGF	CRC, NSCLC, mBC, RCC
Cetuximab	Erbitux	EGFR	CRC
Panitumumab	Vectibix	EGFR	CRC
Trastuzumab	Herciptin	HER-2	BC
Imatinib	Glivec	PDGFR	ALL, CML, GIST
Gefitinib	Iressa	EGFR	NSCLC
Erlotinib	Tarceva	EGFR	NSCLC
Dasatinib	Sprycel	BCR-ABL	ALL, CML
Sorafenib	Nexavar	EGFR	HCC, RCC
Sunitinib	Sutent	PDGFR, VEGF	GIST
Lapatinib	Tyverb	HER-2	NSCLC

VEGF= Vascular endothelial growth factor, EGFR= Epidermal growth factor receptor, PDGFR= Platelet derived growth factor receptor, ALL= Acute lymphoblastic leukaemia, BC= Breast cancer, mBC= Metastatic breast cancer, HER-2= Human epidermal growth factor 2, CML= Chronic myelogenousleukaemia, CRC= Colorectal cancer, HCC= Hepatocellular carcinoma, RCC= Renal cell carcinoma, GIST= Gastro intestinal stromal tumour, NSCLC= Non-small-cell lung carcinoma.

Current cancer treatment modalities can produce good cure and survival rates in some types of cancers such as certain types of leukaemia and testicular cancer**[35].** But unfortunately, limitations that reduce the effectiveness of systemic therapy exist and these are discussed in the following section.

Limitations of chemotherapy

Cancer chemotherapy can be limited by several factors. These factors include administration technique problems, adverse effects, patient performance statusand resistance, details of which are summarized below.

Administration techniques

Administration techniques including dose intensity and the schedule of chemotherapy are considered among the limitations of the efficacy of cancer therapy. Dose intensity is defined as the amount of drug administered per unit of time **[36].** Lengthening the interval between successive courses of chemotherapy or decreasing the dose can negatively affect treatment outcomes. The dose intensity for most chemotherapy regimens is limited by major dose-related toxicities such as bone marrow suppression. The schedule of chemotherapy administration is also an important determinant of response. The optimal schedule is influenced by the pharmacokinetic properties of the anticancer agent. For example, phase-specific agents have cytotoxic effects only when the cell is in a particular phase of the cell cycle. If a phase-specific agent with a short half-life is administered by a bolus injection, it may not affect all the cells. On the other hand, if it was given by continuous infusion, more cancer cells may be exposed to its cytotoxic effect. Increasing the duration of exposure is of course limited by toxicity to normal tissues.

Adverse effects

Adverse effects of systemic therapy are among the most important treatment

limitation factors. Several adverse effects are common to most conventional chemotherapeutic agents. These adverse effects include nausea, vomiting, myelosuppression (including anaemia, neutropenia and thrombocytopenia), mucositis, alopecia, infertility and secondary malignancies. Some of these adverse effects like meylosuppression for example and some drug specific cytotoxicities like cardiotoxicity induced by doxorubicinare dose limiting side effects**[37].** Compared to conventional therapy, molecular targeted agents on the other hand, offer potential for improved efficacy and less toxicity as they act more selectively against cancer cells than normal cells.

Patient performance status

Patient performance status, tolerance and specific factors also can play a major role in the usefulness of a chemotherapeutic course. Patient specific factors (e.g. performance status, co-morbidities, renal and hepatic function, and pharmacogenomics), tumor-specific factors (e.g. pathology, stage, and molecular profile), and treatment goal (e.g. palliation or cure) in addition to the cost of the treatment are all considered when determining the treatment option**[38].**

Resistance

Cancer cell resistance to systemic therapy is one of the most important limitation factors of cancer chemotherapy. Resistance can be classified into primary, acquired or multi drug resistance (MDR) and apparent resistance caused by limited drug delivery to the solid tumor. Drug resistance may be either inherent in cancer cells (primary) or acquired i.e. develop over time during chemotherapy even after dramatic initial response. Some cell lines that became resistant to single chemotherapy agents may also be resistant to other agents that can be structurally non-related. This phenomenon is called multidrug resistance (MDR). The primary mechanism believed to be responsible for MDR is an increase in P-glycoprotein in the cell membrane. This protein mediates efflux of the chemotherapy agent causing a decreased accumulation of drug within the cells**[39].**

ANALYTICAL STUDY OF ANTI-CANCER DRUG GEFITINIB- THE FIRST SELECTIVE INHIBITOR OF EPIDERMAL GROWTH FACTOR RECEPTOR'S (EGFR)

General introduction to Gefitinib

Gefitinib is an orally active inhibitor of the epidermal growth factor receptor (EGFR; erbB1) tyrosine kinase, involved in signal transduction processes and implicated in the proliferation and maintenance of cancer cells. Mutation or overexpression of EGFR, as well as erbB2, can lead to cellular transformation andhas been linked to poor prognosis in cancer. Gefitinib, a novel low molecular weight anilino-quinazoline, produces potent inhibition of EGFR/tyrosine kinase activity (IC50, 0.027 μmol/L) and inhibits EGF stimulated tumor cell growth with an IC50 of 0.054 μmol/L**[40].** Tyrosine kinase triggers chemical processes that cause the cells, including cancer cells, to grow, multiply and spread. Gefitinib attaches to EGFRs and thereby blocks the attachment of EGF and the activation of tyrosine kinase**[41].** This mechanism for stopping cancer cells from growing and multiplying is very different from the mechanisms of chemotherapy and hormonal therapy. TKIs of the 4-anilinoquinazoline class competitively inhibit adenosine triphosphate (ATP) binding at the intracellular ATP-binding cleft of the catalytic tyrosine kinase domain and thereby block the HER- signalling pathway, resulting in decrease in tumour cell proliferation and growth, induction of apoptosis, and inhibition of angiogenesis and metastasis**[42].** The structure of Gefitinib is shown in **Figure 6.**

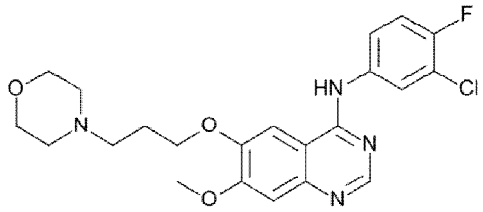

Figure- 6: Gefitinib

The potential benefit of gefitinib treatment for non-small cell lung cancer patients with brain metastasis has been discussed recently, while whether the drug can pass the blood–brain barrier remains unknown. However, the pharmacological effects of gefitinib could be expected to be more closely related to its concentrations at the site of action, i.e., in the central nervous system (CNS), than in serum. Such data would be important in light of recent findings on the potential importance of active transport proteins such as P-glycoprotein for penetration of drugs into the CNS. In the last years several conventional HPLC-methods with optical detection were developed for the quantification of TKIs**[43].** A disadvantage of classic HPLC-methods is their restricted

ability to distinguish between multiple components which cannot be separated chromatographically. Otherwise TKIs are ideal substances for LC-MS/MS measurements with positive ionization due to their chemical structures combined with their proton affinity. Therefore several LC-MS/MS tests were developed recently. Because of its clinical advantages, there is an increase in the number of Gefitinib formulations in the market for a variety of indications. Therefore, there is a need for a sensitive and reliable analytical method for the estimation of Gefitinib in pharmaceutical formulations.

LITERATURE REVIEW ON GEFITINIB

Bouchet et al. 2011., described a rapid and specific method for quantification of nine TKIs in plasma samples**[44].** Chromatography was performed on a Waters Acquity-UPLC® system with BEH C18-50*2.1 mm column, under a gradient of ammonium formate–acetonitrile. An Acquity-TQD® with electrospray ionization was used for detection. Samples were prepared by solid phase extraction (Oasis® MCX μElution) and eluate was injected in the system. Calibration curves ranged from 10 to 5000 ng/mL for imatinib, its metabolite, nilotinib, lapatinib, erlotinib and sorafenib and from 0.1 to 200 ng/mL for dasatinib, axitinib, gefitinibandsunitinib. Peaks of each compound (retention time from 0.76 to 2.51 min) were adequately separated. The mean relative extraction recovery was in the range of 90.3–106.5% thanks to the use of stable isotopes as internal standard. There was no significant ion suppression observed at the respective TKI retention times. This rapid, sensitive and specific UPLC/MS-MS method is able to perform simultaneous quantification of nine TKIs in human plasma and usable for routine therapeutic drug monitoring.

Chandrashekara et al. 2013.,developed a simple and rapid reverse-phase high-performance liquid chromatographic (RP-HPLC) method and validated for the simultaneous separation and estimation of gefitinib (an anti-cancer drug) and its process-related impurities**[45].** Five process-related impurities of gefitinib were separated on an Inertsil ODS-3V column (250 3 4.6 mm i.d.; particle size 5 mm) using 130 mM ammonium acetate and acetonitrile (63:37, v/v) as mobile phase (pH 5.0) with a photodiode array (PDA) detector. The correlation coefficients for gefitinib and its process- related impurities were in the range of 0.9991–0.9994 with limit of detection and limit of quantification in the range of 0.012–0.033 and 0.04–0.10 mg/mL, respectively. The recovery values were in the range of 98.26–99.90% for gefitinib and 95.99–100.55% for process-

related impurities, while precision values were less than 3%. The method was found to be specific, precise and reliable for the determination of gefitinib and its impurities in gefitinib bulk drug.

Deng et al. 2013.,developed a sensitive and simple liquid chromatography/electrospray mass spectrometry (LC-MS/MS) method for determination of gefitinib in rat plasma using one-step protein precipitation**[46].** After addition of estazolam as internal standard (IS), protein precipitation by acetonitrile was used as sample preparation. Chromatographic separation was achieved on an SB-C18 (2.1 mm × 50 mm, 3.5 μm) column with methanol-0.1 % formic acid as mobile phase with gradient elution. Electrospray ionization (ESI) source was applied and operated in positive ion mode; multiple reaction monitoring (MRM) mode was used to quantification using target fragment ions m/z 447.0→127.7 for gefitinib and m/z 294.7→266.8 for the IS. Calibration plots were linear over the range of 5-2000 ng/mL for gefitinib in rat plasma. Lower limit of quantification (LLOQ) for gefitinib was 5 ng/mL. Mean recovery of gefitinib from plasma was in the range 78.6-93.0 %. RSD of intra-day and inter-day precision were both less than 15 %. This developed method is successfully used in pharmacokinetic study of gefitinib in rats.

Gotze et al. 2012., developed and validated a specific, simple and rapid quantification method for various TKIs in human plasma**[47].** A simultaneous test for six TKIs (erlotinib, imatinib, lapatinib, nilotinib, sorafenib, sunitinib) was developed using liquid chromatography tandem mass spectrometry in a multiple reaction monitoring mode. After protein precipitation the specimenswere applied to the HPLC system and separated using a gradient of acetonitrile containing 1% formic acid with 10 mM ammonium formiate on an analytic RP-C18 column. The calibration range was 10–1000 ng/mL for sunitinib and 50–5000 ng/mL for the other TKIs with coefficients of determination ≥0.99 for all analytes. The intra- and inter day coefficients of variation were ≤15%. and the chromatographic run time was 12min. Plasma specimens were stable for measurement for at least 1 week at 4 °C. Clinical applications of the assay are exemplarily discussed.

Honeywell et al. 2010., developed fast, sensitive, universal and accurate method for the determination of four different tyrosine kinase inhibitors from biological material using LC–MS/MS techniques**[48].** Utilizing a simple protein precipitation with acetonitrile a 20 µl sample volume of biological matrixes can be extracted at 4 ◦C with minimal effort. After centrifugation

the sample extract is introduced directly onto the LC–MS/MS system without further clean-up and assayed across a linear range of 1–4000 ng/ml. Detection was performed using a turbo-spray ionization source and mass spectrometric positive multi-reaction-monitoring-mode (+MRM) for Gefitinib (447.1m/z; 127.9m/z), Erlotinib (393.9m/z; 278.2m/z), Sunitinib (399.1m/z; 283.1m/z) and Sorafenib (465.0m/z; 251.9m/z) at an ion voltage of +3500V. The accuracy, precision and limit-of-quantification (LOQ) from cell culture medium were as follows: Gefitinib: 100.2±3.8%, 11.2 nM; Erlotinib: 101.6±3.7%, 12.7 nM; Sunitinib: 100.8±4.3%, 12.6 nM; Sorafenib: 93.9±3.0%, 10.8 nM, respectively. This was reproducible for plasma, whole blood, and serum. Themethodwas observed to be linear between the LOQ and 4000 ng/ml for each analyte. Effectiveness of the method is illustrated with the analysis of samples from a cellular accumulation investigation and from determination of steady state concentrations in clinically treated patients.

Lankheet et al. 2012., developed a fast and accurate method for simultaneous determination of anticancer tyrosine kinase inhibitors (TKIs) dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib in human plasma to support pharmacokinetic-guided dosing in individual patients using high-performance liquid chromatography and detection with tandem mass spectrometry (HPLC-MS/MS)**[49].** Stable isotopically labeled compounds of the eight different TKIs were used as internal standards. Plasma proteins were precipitated and an aliquot of supernatant was directly injected onto a reversed phase chromatography system consisting of a Gemini C18 column (502.0mm i.d., 5.0mm particle size) and then compounds were eluted with a gradient. This method was validated over a linear range from 20.0 to 10,000 ng/mL for erlotinib, gefitinib, imatinib, lapatinib, nilotinib and sorafenib, and from 5.00 to 2500 ng/mL for dasatinib and sunitinib. Results from the validation study demonstrated good intra- and inter-assay accuracy (<13.1%) and precision (10.0%) for all analytes. This method was successfully applied for routine therapeutic drug monitoring purposes in patients treated with the investigated TKIs.

Roche et al. 2009., developed a highly sensitive and selective liquid chromatography tandemmass spectrometry (LC–MS/MS)method to quantify cellular levels of the tyrosine kinase inhibitors (TKIs) dasatinib and lapatinib**[50].** Cellular samples were extracted with a tert-butylmethyl ether: acetonitrile (3:1, v/v):1M ammonium formate pH 3.5 (8:1,

v/v) mixture. The TKIs were quantified using a triple quadrupole mass spectrometer which was operated in multi reaction-monitoring mode employing positive electrospray ionisation. The limit of detection and limit of quantification for lapatinib was determined to be 15 and 31 pg on column, respectively. The limit of detection and quantification for dasatinib was 3 and 15 pg on column, respectively. The method allowed for sensitive and accurate determination of cellular levels of dasatinib and lapatinib. In addition, we examined the potential for this method to be utilised to quantitate other TKIs, using gefitinib, erlotinib, imatinib and sorafenib as examples. In principle, these agents were also quantifiable by this method, however, no drug specific validation studies were undertaken with these TKIs. The data indicates that in the cancer cell-linemodel, DLKP, significantlymorelapatinib accumulates in cells in comparison to dasatinib. Additionally, over-expression of the membrane protein drug transporter, P-glycoprotein (P-gp) a common cancer drug resistance mechanism, greatly reduces the cellular accumulation of dasatinib but not of lapatinib.

Venkataramanna et al. 2011., established degradation pathway for gefitinib is as per ICH recommendations by validated and stability indicating reverse phase liquid chromatographic method**[51].** Gefitinib is subjected to stress conditions of acid, base, oxidation, thermal and photolysis. Significant degradation is observed in acid and base stress conditions. Two impurities are studied among which one impurity is found prominent degradant. The stress samples are assayed against a qualified reference standard and the mass balance is found close to 99.5%. Efficient chromatographic separation is achieved on a Agilent make XDB-C18, 50 × 4.6 mm with 1.8 µm particles stationary phase with simple mobile phase combination delivered in gradient mode and quantification is carried at 250 nm at a flow rate of 0.5 $mL \cdot min^{-1}$. In the developed RPLC method the resolution between gefitinib and the potential impurities is found to be greater than 5.0. Regression analysis shows an r^2 value (correlation coefficient) of greater than 0.998 for gefitinib and the two potential impurities. This method is capable to detect the impurities of gefitinib at a level of 0.01% with respect to test concentration of 0.5 $mg \cdot mL^{-1}$ for a 4-µL injection volume. The developed RRLC method is validated with respect to specificity, linearity & range, accuracy, precision and robustness for impurities determination and assay determination.

Zheng et al.2011., developed a specific, sensitive, and rapid method based on high-

performance liquid chromatography coupled to tandem mass spectrometry (LC–MS–MS) was for determination of gefitinib in human serum and cerebrospinal fluid (CSF)**[52].** The analyte was detected by tandem mass spectrometry operating in positive electrospray ionization mode with multiple reactions monitoring (MRM). Gefitinib was extracted from serum or CSF samples with ethyl acetate using icotinib as internal standard. The method was validated over the concentration range of 1.00–1,000 ng mL^{-1} in human serum and 0.05–50.0 ng mL^{-1} in CSF. For both matrices, inter- and intraday precision (CV%) were less than 15% and accuracy was within 85–115%. Average extraction recoveries were 78.9 and 61.8% in human serum and CSF, respectively. Linearity, recovery, matrix effects, and stability were validated in the two matrices. The method was successfully used for analysis of clinical samples from lung cancer patients with brain metastases treated with gefitinib in the dosage range of 250–500 mg day^{-1}.

Wang et al. 2011.,developed a novel, rapid and specific liquid chromatography-tandem mass spectrometric (LC-MS/MS) method and validated for the simultaneous quantification of gefitinib and its predominant metabolite, O-desmethylgefitinib in human plasma**[53].** Chromatographic separation of analytes was achieved on an Alltima C18 analytical HPLC column (150 mm × 2.1 mm, 5 μm) using an isocratic elution mode with a mobile phase comprised acetonitrile and 0.1% formic acid in water (30:70, v/v). The flow rate was 300 μL/min. The chromatographic run time was 3 min. The column effluents were detected by API 4000 triple quadrupole mass spectrometer using electrospray ionization (ESI) in positive mode. Linearity was demonstrated in the range of 5-1000 ng/mL for gefitinib and 5-500 ng/mL for O-desmethylgefitinib. The intra- and inter-day precisions for gefitinib and O-desmethylgefitinib were ≤10.8% and the accuracies ranged from 89.7 to 104.7% for gefitinib and 100.4 to 106.0% for O-desmethylgefitinib. This method was used as a bioanalytical tool in a phase I clinical trial to investigate the possible effect of hydroxychloroquine on the pharmacokinetics of gefitinib. The results of this study enabled clinicians to ascertain the safety of the combination therapy of hydroxychloroquine and gefitinib in patients with advanced (Stage IIIB-IV) non-small cell lung cancer (NSCLC).

Zhao et al. 2005., developed and validated a rapid, sensitive and specific method using liquid chromatography–tandem mass spectrometry (LC/MS/MS) for determination of gefitinib in human plasma

and mouse plasma and tissue**[54].** Sample preparation involved a single protein precipitation step by the addition of 0.1 mL of plasma or a 200 mg/mL tissue homogenate diluted 1/10 in human plasma with 0.3 mL acetonitrile. Separation of the compounds of interest, including the internal standard (d8)-gefitinib, was achieved on a Waters X-Terra™ C18 (50 mm × 2.1 mm i.d., 3.5 μm) analytical column using a mobile phase consisting of acetonitrile–water (70:30, v/v) containing 0.1% formic acid and isocratic flow at 0.15 mL/min for 3 min. The analytes were monitored by tandem mass spectrometry with electrospray positive ionization. Linear calibration curves were generated over the range of 1–1000 ng/mL for the human plasma samples and 5–1000 ng/mL for mouse plasma and tissue samples with values for the coefficient of determination of >0.99. The values for both within- and between-day precision and accuracy were well within the generally accepted criteria for analytical methods (<15%). This method was subsequently used to measure concentrations of gefitinib in mice following administration of a single dose of 150 mg/kg intraperitoneally and in cancer patients receiving an oral daily dose of 250 mg.

Guetens et al. 2005., described the development of an on-column focusing gradient capillary LC method coupled to tandem mass spectrometry (quadrupole-linear ion trap) for the quantitative determination of the anticancer agent ZD1839 (Gefitinib, Iressa) in blood plasma**[55].** Plasma samples (0.2 ml) were extracted with methyl tert-butyl ether. The analytes of interest, ZD1839 and the internal standard [2H8] ZD1839 (ZD1839-d8) were eluted on a 50 mm × 1 mm, 5 μm particle size, capillary ODS Hypersil column using an aqueous ammonium acetate gradient at 40 μl/min. Mass spectrometric detection was performed by a Q-Trap tandem mass spectrometer with electrospray positive ionisation, and monitored in the multiple reaction monitoring transitions 447 > 128 and 455 > 136, respectively. The limit of quantification of ZD18395 was 0.1 ng/ml.

CONCLUSION

The present study covers all the emerging hallmarks and enabling characteristics of cancer. It represents the molecular basis and the incidence and mortality figures. Treatment modalities, its limitations, adverse effects and its resistance are nicely focused.

A sensitive method for the analysis of gefitinib (a new generation of targeted therapeutic drug) in plasma is necessary in conducting therapeutic drug monitoring and pharmacokinetic studies. The developed

liquid chromatography tandem mass spectrometry (LC–MS/MS) method proved to be robust, allowing quantification with sufficient precision, accuracy and sensitivity and reached the level of reproducibility demanded by clinical patient sample analysis. Hence, the method can be readily incorporated into the routine testing of gefitinib.

ACKNOWLEDGMENTS

Hafiz A. Makeenis highly thankful to Institute of Cancer Therapeutics (ICT),University of Bradford, U.K. for throughout support. All of the authors are thankful to College of Pharmacy, Jazan University, KSA for providing necessary facilities. We hereby declare that there is no any financial/commercial conflict of interest involved.

REFERENCES

[1] Weinberg RA, How cancer arises, Sci Am, *275*, 1996, 62-70.

[2] Hanahan D and Weinberg RA, The hallmarks of cancer, Cell,100, 2000, 57-70.

[3] Hanahan D and Weinberg RA, Hallmarks of cancer: the next generation, Cell,144, 2011, 646-674.

[4] Montesano R and Hall J, Environmental causes of human cancers, Eur J Cancer,37 2001, 67-87.

[5] Fagan-Dubin L. Causes of cancer, Cancer Nurs, *2*, 1979, 435-41.

[6] Doll R and Peto R, The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today, J Natl Cancer Inst, 66, 1981, 1191-1308.

[7] Croce CM, Oncogenes and cancer, N Engl J Med, 358, 2008, 502-511.

[8] Goldman JM and Melo JV, Targeting the BCR-ABL tyrosine kinase in chronic myeloid leukemia, N Engl J Med, 344,2001, 1084-86.

[9] Cohen MH, Cortazar P, Justice R and Pazdur R, (). Approval summary: imatinib mesylate in the adjuvant treatment of malignant gastrointestinal stromal tumors, Oncologist, 15,2010, 300-07.

[10] Scartozzi M, Bearzi I, Mandolesi A, Giampieri R, Faloppi L, Galizia E, Loupakis F, Zaniboni A, Zorzi F, Biscotti T, Epidermal growth factor receptor (EGFR) gene promoter methylation and cetuximab treatment in colorectal cancer patients, Br J. Cancer, 104, 2011, 1786-90.

[11] Weinberg RA, How cancer arises, Sci Am, 275,1996, 62-70.

[12] Fearon ER and Vogelstein B, A genetic model for colorectal tumorigenesis. Cell, 61, 1990, 759-67.

[13] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D, Global

cancer statistics, CA Cancer J Clin, 61,2011, 69-90.

[14] Malvezzi M, Arfe A, Bertuccio P, Levi F, La Vecchia C and Negri E, European cancer mortality predictions, Ann Oncol, 22, 2011, 947-56.

[15] Jemal A, Siegel R, Xu J and Ward E, Cancer statistics, CA Cancer J Clin, 60,2010, 277-300.

[16] Nagula S, Ishill N, Nash C, Markowitz AJ, Schattner MA, Temple L, Weiser MR, Thaler HT, Zauber A and Gerdes H, Quality of life and symptom control after stent placement or surgical palliation of malignant colorectal obstruction, J Am Coll Surg, 210, 2010, 45-53.

[17] Camma C, Giunta M, Fiorica F, Pagliaro L, Craxi A and Cottone M, Preoperative radiotherapy for resectable rectal cancer: A meta-analysis, JAMA, *284*, 2000, 1008- 15.

[18] Joensuu H,. Systemic chemotherapy for cancer: from weapon to treatment, Lancet Oncol, 9,2008, 304.

[19] Kimble MAK, Applied therapeutics, the clinical use of drugs, 8 edn, Kirkwood, J. Cancer immunotherapy: the interferon-alpha experience. Semin Oncol *29*, 2002 18-26.

[20] Sepulveda C, Marlin A, Yoshida T and Ullrich A, Palliative Care: the World Health Organization's global perspective, J Pain Symptom Manage, 24, 2002, 91-96.

[21] Mazzeo F, Berliere M, Kerger J, Squifflet J, Duck L, D'Hondt V, Humblet Y, Donnez J and Machiels JP, Neoadjuvant chemotherapy followed by surgery and adjuvant chemotherapy in patients with primarily unresectable, advanced-stage ovarian cancer, Gynecol Oncol, 90, 2003, 163-69.

[22] Ali S and Coombes RC, Endocrine-responsive breast cancer and strategies for combating resistance, Nat Rev Cancer, *2*, 2002, 101-12.

[23] Mihich E, Historical overview of biologic response modifiers, Cancer Invest, 18, 2000, 456-66.

[24] Franceschi S, Dal Maso L, Arniani S, Crosignani P, Vercelli M, Simonato L, Falcini F, Zanetti R, Barchielli A, Serraino D, Risk of cancer other than Kaposi's sarcoma and non-Hodgkin's lymphoma in persons with AIDS in Italy, Cancer and AIDS Registry Linkage Study, Br J Cancer, 78,1998, 966-70.

[25] Kirkwood J, Cancer immunotherapy: the interferon-alpha experience. Semin Oncol, *29*, 2002, 18-26.

[26] Bleumer I, Oosterwijk E, Oosterwijk-Wakka JC, Voller MC, Melchior S, Warnaar SO, Mala C, Beck J and Mulders PF, A clinical trial

with chimeric monoclonal antibody X-G250 and low dose interleukin-2 pulsing scheme for advanced renal cell carcinoma, J Urol, 175,2006, 57-62.

[27] Hanahan D and Weinberg RA, Hallmarks of cancer: the next generation, Cell, 144, 2011, 646-74.

[28] Eckhardt S, Molecular targeted therapy: a strategy of disillusions or optimism? J Lab Clin Med, 147,2006, 108-13.

[29] Soria JC, Blay JY, Spano JP, Pivot X, Coscas Y and Khayat D, Added value of molecular targeted agents in oncology, Ann Oncol, *22*, 2011, 1703-16.

[30] Zondor SD and Medina PJ, Bevacizumab: an angiogenesisinhibitor with efficacy in colorectal and other malignancies, Ann Pharmacother, 38,2004, 1258-64.

[31] Herbst RS, Ansari R, Bustin F, Flynn P, Hart L, Otterson GA, Vlahovic G, Soh CH, O'Connor P and Hainsworth J, Efficacy of bevacizumab plus erlotinib versus erlotinib alone in advanced non-small-cell lung cancer after failure of standard first-line chemotherapy (BeTa): a double- blind, placebo-controlled, phase 3 trial, Lancet,377,2011, 1846-54.

[32] Syed S, Takimoto C, Hidalgo M, Rizzo J, Kuhn JG, Hammond LA, Schwartz G, Tolcher A, Patnaik A, Eckhardt SG, A phase I and pharmacokinetic study of Col-3 (Metastat), an oral tetracycline derivative with potent matrix metalloproteinase and antitumor properties, Clin Cancer Res, 10, 2004, 6512-21.

[33] Herbst RS, O'Neill VJ, Fehrenbacher L, Belani CP, Bonomi, PD, Hart L, Melnyk O, Ramies D, Lin M and Sandler A, Phase II study of efficacy and safety of bevacizumab in combination with chemotherapy or erlotinib compared with chemotherapy alone for treatment of recurrent or refractory non small-cell lung cancer, J Clin Oncol, 25,2007, 4743-50.

[34] Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, Moiraghi B, Shen Z, Mayer J, Pasquini R,.Dasatinib versus imatinib in newly diagnosed chronic- phase chronic myeloid leukemia, N Engl J Med *362*, 2010, 2260-70.

[35] Brenner H, Long-term survival rates of cancer patients achieved by the end of the 20th century: a period analysis, Lancet, *360*, 2002, 1131-35.

[36] Gianni AM and Piccart MJ, Optimising chemotherapy dose density and dose intensity new strategies to improve outcomes in adjuvant therapy for breast cancer, Eur J Cancer, 36 (1),2000, 1-3.

[37] Gharib MI and Burnett AK, Chemotherapy-induced cardiotoxicity: current practice and prospects of prophylaxis, Eur J Heart Fail, 4,2002, 235-42.

[38] Wedding U, Honecker F, Bokemeyer C, Pientka L and Hoffken K, Tolerance to chemotherapy in elderly patients with cancer, Cancer Control, 14,2007, 44-56.

[39] Gottesman, M.M. (1993). How cancer cells evade chemotherapy: sixteenth Richard and Hinda Rosenthal Foundation Award Lecture. Cancer research 53, 747-754.

[40] Rajani B, Mukkanti K, Method Development and Validation for the Estimation of Gefitinib in Tablet Dosage Form by RP-HPLC, Int J.PharmaSci, 4(3), 2014, 518-21.

[41] Sordella R, Bell DW, Haber DA and Settleman J, Gefitinib-sensitizing EGFR mutations in lung cancer activates anti apoptotic pathways, Science, 305, 2004, 1163-67.

[42] Moyer JD, Barbacci EG, Iwata KK, Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase, Cancer Res, 57 (21), 1997, 4838-48.

[43] Widmer N, Béguin A, Rochat B, Determination of imatinib (Gleevec® in human plasma by solid-phase extraction–liquid chromatography–ultraviolet absorbance detection, J ChromatograBAnalytTechnol Biomed Life Sci, 803, 2004,285–92.

[44] Bouchet S, Chauzit E, Ducint D, Castaing N, Canal-Raffin M, Moore N, Titier K, Molimard M, Simultaneous determination of nine tyrosine kinase inhibitors by 96- well solid-phase extraction and ultra-performance LC/MS-MS, ClinicaChimica Acta,412, 2011, 1060–67.

[45] Chandrashekara KA, Udupi A, Reddy CG, Separation and Estimation of Process- Related Impurities of Gefitinib by Reverse-Phase High-Performance Liquid Chromatography, J Chromatogra. Sci, 2013, 1–7.

[46] Deng X, Zhang P, Zhang Q, Nian S, JiansheMA, Gradient Elution LC-MS/MS Determination of Gefitinib in Rat Plasma and its Pharmacokinetic Study, Lat Am J Pharm, 32 (2), 2013, 292-96.

[47] Gotze L, Hegele A, Metzelder SK, Renz H, Nockher WA, Development and clinical application of a LC-MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma, ClinChimActa, 413, 2012, 143–49.

[48] Honeywell R , Yarzadah K, Giovannetti E, Losekoot N, Smit EF,

Walraven M, . Simple and selective method for the determination of various tyrosine kinase inhibitors used in the clinical setting by liquid chromatography tandem mass spectrometry, J Chromatogra, B 878, 2010, 1059–68.

[49] Lankheet NAG, Hillebrand MJXH, Rosinga JHM, Schellens JH, Beijnen ADR. Huitema, Method development and validation for the quantification of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib in human plasma by liquid chromatography coupled with tandem mass spectrometry, Biomed. Chromatogra, 27, 2013, 466–76.

[50] Rochea S, McMahonb G, Clynesa M, Connor RO, Development of a high-performance liquid chromatographic–mass spectrometric method for the determination of cellular levels of the tyrosine kinase inhibitors lapatinib and dasatinib, J Chromatogra B, 877, 2009, 3982–90.

[51] Venkataramanna M, Somaraju IV, Babu KS, Identification of Degradant Impurity in Gefitinib by Using Validated RRLC Method, Amer J Anal Chem, 2, 2011 75-83.

[52] Zheng X, Hu P, Liu J, Zhao Q, Wang M, Jiang J, Zhong W. LC– MS– MS Quantitative Determination of Gefitinib in Human Serum and Cerebrospinal Fluid. Chromatogra, 74, 2011, 41–49.

[53] Wang LZ, Lim MY, Chin TM, Thuya WL, Nye PL, Wong A, Chan SY, Goh BC, Ho PC, Rapid determination of gefitinib and its main metabolite, O-desmethylgefitinib in human plasma using liquid chromatography-tandem mass spectrometry, J ChromatogrBAnalytTechnol Biomed Life Sci, 15 (22), 2011, 2155-61.

[54] Zhao M, Hartke C, Jimeno A, Li J, He P, Zabelina Y, Specific method fordetermination of gefitinib in human plasma, mouse plasma and tissues using high performance liquid chromatography coupled to tandem mass spectrometry, J Chromatography B, 819(1), 2005, 73–80.

[55] Guetens G, Prenena H, Boeck GD, Dongen WV, Esmans E, Lemière F, Sensitive and specific quantification of the anticancer agent ZD1839 (Gefitinib) in plasma by on-column focusing capillary liquid chromatography–tandem mass spectrometry, J Chromatogr A, 1082(1), 2005, 2–5.