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# Anticancer Drug Discovery – From Serendipity to Rational Design

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Additional information is available at the end of the chapter

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## 1. Introduction

Cancer is nowadays used as a generic term describing a group of about 120 different diseases, which can affect any part of the body and defined as the state characterized by the uncontrolled growth and invasion of normal tissues and spread of cells [1]. According to WHO reports cancer is a leading cause of premature death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) only in 2008 [2]. The deaths from cancer worldwide are projected to continue rising, reaching an estimated 13.1 million in 2030 (WHO 2012). The number of all cancer cases around the world reached 12.7 million in 2008 and is expected to increase to 21 million by 2030. Approximately one in five people before age 75 will suffer from cancer during their lifetime, while one in ten in this age range is predicted to die due to cancer [2]. About 70% of all cancer deaths occurred in low- and middle-income countries. Cancer statistics indicate that most common new cancer cases (excluding common non-melanoma skin cancer) include lung, breast, colorectum, stomach, prostate and liver. These statistics are affected by a few factors including the increase in the number of carcinogens in daily life conditions (food, alcohol, tobacco etc.; high levels of chemicals and pollutants in environment, exposure to UV and ionizing radiation and viruses), genetic disposition [1] but also higher effectiveness of the treatment regimes. The number of recognized carcinogens (agents, mixtures, oncoviruses, environmental factors) increased from 50 in 1987 to 108 in 2012. Although it seems small, this number increases continually with the evidence of new (probably and possibly) carcinogens (64 and 271 in 2012) to humans [3]. What is significant only one compound is listed as probably not carcinogenic - caprolactam. Moreover, since not all chemicals have been tested yet, the number of human carcinogens is undervalued and will increase in the near future. Although mortality rates for some cancers (e.g. leukaemia, testicular or ovarian cancer) are reduced and

the overall survival time increased significantly, especially in high-developed countries, but in fact the metastases, not the cancer itself, are the major cause of death. In 1971, only 50% of people diagnosed with the cancer went on to live at least five years, while nowadays, the five-year survival rate is 63% [2]. However if a cancer has spread the chances of survival are only scarcely better than in the 1970s. These numbers indicate that although the knowledge about cancer in the last two decades raised, even to a larger degree than in all preceding centuries, but the problem of cancer diseases persists and our knowledge is still insufficient to solve it. Despite the remarkable progress in cancer prevention, early detection, and treatment, made during the last few decades, the methods of cancer diagnosis and treatment are still not sufficiently specific and effective thus cancer still takes a heavy toll.

Not so long ago in the beginning of 20<sup>th</sup> century, neither carcinogens nor cellular targets were identified while the treatment was carried out exclusively by surgeries or natural products selected by trial and error. Modern cancer therapy based on the so-called holistic approach - the combined use of surgical methods, radiotherapy, chemotherapy, hormonal therapy and immunotherapy - is applied in the treatment of cancer at most stages. In fact this approach originated from the ancient Sumerian, Akkadian, Babylonian, Assyrian and Egyptian medicine, and was largely influenced by the Roman and Greek ideas concerning anatomy, physiology as well as the achievements of practical medicine and natural science. The chemotherapy, hormonal therapy, immunotherapy and radiotherapy as the methods of cancer treatment joined to the oldest surgical one only in the 20<sup>th</sup> c. An important component of the combined therapy, but sometimes when cancer had already metastasised, the only available therapeutic method, is chemotherapy using natural or synthetic anticancer drugs and treated as curative, palliative, adjuvant or neoadjuvant. Over the centuries anticancer drugs evolved from natural products, discovered mainly from green plants and minerals to fully chemically synthesized chemotherapeutic agents. However, even today drugs of natural origin play an important role in the treatment of cancer as 14 of them were on the list of the top 35 drugs worldwide sales [4]. The process of anticancer drug discovery leading from natural products to chemotherapeutic agents, often illicitly limited only to cytostatic and antiproliferative, has evolved from serendipity to rational design based on advances in chemistry, physics and biology in a long and complicated process. Nowadays both cancer itself and anticancer drugs are investigated at the molecular level thus methods of drug discovery have changed diametrically. The dominant direction of contemporary anticancer drug discovery is the search for the possibilities to influence the pathogenetic mechanisms specific of the tumour structures at the cellular and molecular levels, which require the knowledge of cancer origins.

This chapter will focus on the factors which influenced the direction of anticancer drug discovery methods from guessing to the targeted search i.e. from serendipity to rational design.

## 2. Cancer origins

Cancer (proper medical name - malignant neoplasm) commonly considered to be a civilization disease, has in fact been traced to occur even before the ancestral species of man [5]. The oldest

evidence of cancer dates back to several million years ago and has been found in fossilized remains (bones) of a dinosaur in Wyoming. The oldest specimens of cancer, a hominid malignant tumour (probably Burkitt's lymphoma) and bone cancer - were found in the remains of a body of either *Homo erectus* or an *Australopithecus* and in the remains of a female skull dating to the Bronze Age (1900-1600 B.C.), respectively. Bone cancers have been also discovered in mummies in the Great Pyramid of Giza and in mummified skeletal remains of Peruvian Incas. The earliest written records differentiating between benign and malignant cancers date back to ancient times (3000-1500 B.C., Mesopotamia and Egypt). Seven Egyptian Papyruses including the Edwin Smyth (2500 B.C.), Leyde (1500 B.C.), and George Ebers (1500 B.C.) described not only the symptoms but also the first primitive forms of treatment, i.e. the removal of a malignant tissue. The Hindu epic, the Ramayana (500 B.C.), mentioned not only cancer cases but also the first medicines in the form of arsenic pastes, for treatment of cancerous growth. Ancient Greek physician Hippocrates of Kos (ca. 460-370 B.C.) described many different types of cancer (breast, uterus, stomach, skin, and rectum) recognised the difference between benign and malignant tumours and formulated the humoral theory of cancer genesis. As the veins surrounding the tumour resembled the crab claws, he named the disease after the Greek word *carcinos*. Cornelius Celsus (ca. 25 B.C.-50 A.D.), who described the first surgeries on cancers, translated Greek *carcinos* into now commonly used Latin term cancer. Claudius Galen (129-216 A.D.), the most famous Roman Empire physician, who wrote about 500 medical treatises, left a comprehensive descriptions of many neoplasms. He introduced the Greek word *oncōs* (swelling) to describe tumours. Nowadays the use of Hippocrates and Celsus term is limited to describe malignant tumours, while Galen's term is used as a part of the name of the branch of medicine that deals with cancer - that is oncology. Followers of his works in Constantinople, Alexandria, Athens explained the appearance of cancer as a result of an excess of black bile. This idea prevailed through up to the 16<sup>th</sup> century.

The intensive studies in the field of anatomy and physiology during the Renaissance, resulted in advancement of surgery and development of rational therapies based on clinical observations. Based on autopsies William Harvey (1578-1657) described the systemic circulation of blood through the heart and body. Although cancers were still incurable, their temporary inhibition was often observed thanks to complementary remedies including the most common arsenic-based creams and pastes. In the beginning of the 16<sup>th</sup> c. Zacutus Lusitani (1575-1642) and Nicholas Tulp (1593-1674) formulated the contagion theory and proposed isolation of patients in order to prevent the spread of cancer. Throughout the 17<sup>th</sup> and 18<sup>th</sup> centuries, this theory was so popular that the first cancer hospital founded in Reims, France, was forced to move outside the city. Nowadays, we know that their certain viruses, bacteria, and parasites can increase a risk of developing cancer. Gaspare Aselli (1581-1625), who discovered the lymphatic system, suggested a connection between the lymphatic system and cancer. Georg Ernst Stahl (1660-1734) and Friedrich Hoffman (1660-1742) proposed a concept that tumours grow from degenerating lymph constantly excreted by the blood. This idea was accepted by John Hunter (1728-1793), who described methods to identify surgically removable tumours. At that time the so-called humoral theory of cancer was replaced by the lymph theory. Claude-Deshasis Gendron (1663-1750) was convinced that cancer arises as a solid and growing mass untreatable with drugs, and must be completely removed. The discovery of a microscope by

Antonie van Leeuwenhoek (1632-1723) in the late 17<sup>th</sup> century extended the knowledge about the cancer formation process and accelerated the search for the origin of cancer. It was realised that the progress in cancer treatment critically depends on the ability to distinguish between normal and malignant cells. Giovanni Battista Morgagni (1682-1771), father of pathomorphology, related the illness to pathological changes that laid the foundation for scientific oncology. This observation in connection with discovery of anaesthesia in 1844 by Horace Wells (1815-1848) enabled development of precise diagnosis of cancer and modern radical cancer surgery. In 1838, Johannes Peter Muller (1801-1858) indicated cells as basic units of tumours and proposed the blastema theory that cancer cells developed from budding elements (blastema) between normal tissues. In 1860, Karl Thiersch (1822-1895), showed that cancers metastasize through the spread of malignant cells and described establishment of secondary cancer as a result of their spread by lymph. Rudolf Virchow (1821-1902), the founder of cellular pathology, recognized leukaemia cells. He showed that cancer cells can be differentiated from surrounding normal cells from which they originated and the stage of cancer can be determined using microscopic images. Virchow also properly recognized chronic irritation as one of the factors favouring cancer development. Nowadays, we are aware that cancers arise from sites of infection, chronic irritation and inflammation. The next key step in understanding the mechanism of cancer development was the discovery of chromosome and mitosis credited to German botanist Wilhelm Hofmeister (1824-1877). In 1902 Theodor Boveri (1862-1915) reasoned that a cancerous tumour begins with a single cell, which divided uncontrollably, while David Paul von Hansemann (1858-1920), included multipolar mitoses among the factors responsible for the arise of abnormal chromosome numbers in cells leading to tumour formation. In fact Hansemann formulated chromosomal theory of cancer, while Boveri proposed the existence of cell-cycle checkpoints, tumour suppressor genes, and oncogenes and speculated that uncontrolled growth might be caused by physical (radiation), chemical (some chemicals) or biological (microscopic pathogens) factors. Thomas Hunt Morgan (1866-1945) made a key observations of chromosomal changes and demonstrated in 1915 the correctness of this theory. But still the carcinogens like chemical agents or irradiation could not explain the fact that sometimes cancer seemed to run in families. Already in the 17<sup>th</sup> c. Lusitani and Tulp observed the appearance of breast cancer in whole families.

A rapid progress in understanding the cancer origins was possible thanks to the scientific progress and appearance of instruments required to solve complex interdisciplinary problems of chemistry and biology. The turning points in the research on cancer were the mapping of locations of the fruit fly (*Drosophila melanogaster*) genes by Alfred Sturtevant (1891-1970), the discovery that DNA is the genetic material by Oswald Avery (1877-1955), Colin Munro MacLeod (1909-1972) and Maclyn McCarty (1911-2005) and the resolution of the exact chemical structure of DNA, the basic material in genes, by James Watson and Francis Crick (1916-2004). Their results indicated that DNA was the cellular target for carcinogens and that mutations were the key to understanding the mechanisms of cancer. In 1970 the first oncogene (SRC, from sarcoma) a defective proto-oncogene i.e. gene which after mutation, predispose the cell to become a cancerous (stimulate cell proliferation) was discovered by G. Steve Martin in a chicken retrovirus. One year later, but long before the human genome was sequenced, Alfred George Knudson identified first tumour suppressor gene, the Rb gene, located on a region of

long arm of chromosome 13 at position 14.2 in humans. Its mutation results in retinoblastoma juvenile eye tumour. On the basis of the earlier (dated to 1953) findings of Carl Nordling, he has formulated the accepted till now “two-hit hypothesis” which assumes that both alleles coding a particular protein must be affected before an effect is manifested. Knudson provided an explanation of the relationship between the hereditary and non-hereditary origins of cancer and predicted the existence of tumor suppressor genes that can suppress cancer cell growth. It was later discovered that both classes of genes proto-oncogenes and tumor-suppressor genes encode many kinds of proteins controlling cell growth and proliferation and the mutations in these genes can contribute to carcinogenesis.

In 1976 John Michael Bishop and Harold Elliot Varmus discovered the presence of oncogenes in many organisms including humans. Nowadays, after human genome sequencing in 2004, we know that human DNA contains approximately 20,500 genes [6]. About 50 of them are known to be proto-oncogenes, while 30 tumour suppressor genes. The proto-oncogenes (*c-onc*) initiate the process of cell division and code enzymes which control growth and division of cells. Proto-oncogenes can be activated to oncogenes by many factors including chromosome rearrangements, gene duplication, mutation or overexpression. For example, a chromosome rearrangement results in formation of BCR-ABL gene which leads to chronic myeloid leukaemia [7], acquired mutation activate the KIT gene which results in gastrointestinal stromal tumour [8], while inheritance of BRCA1 or BRCA2 increase the risk of breast, ovarian, fallopian tube, and prostate cancers [9]. To cause cancer most oncogenes require an additional step, for example mutations in another gene or introduction of foreign DNA (e.g. by viral infection). Infection and inflammation significantly contribute to about 25% of cancer cases. During the inflammatory response to viral infection the free radicals - reactive oxygen and nitrogen species - are generated as a physiological protective response. During chronic inflammation the mechanism is different - free radicals induce genetic and epigenetic changes including somatic mutations in cancer-related genes and posttranslational modifications in proteins involved in DNA repair or apoptosis. However, irrespective of the origins, the tumour microenvironment created by inflammatory cells, is an essential factor in the whole neoplastic process. It facilitates proliferation and survival of malignant cells, promotes angiogenesis and metastasis, subverts adaptive immune responses, and alters responses to chemotherapeutic agents. If a cell accumulates critical mutations in a few of these proto-oncogenes (five or six), it will survive instead of undergoing apoptosis, will proliferate and become capable of forming a tumour. The protecting mechanism involves the tumour suppressor genes, "anti-oncogenes", which protect from developing or growing cancer by repairing DNA damages (mutations), inhibiting cell division and cell proliferation or prevent reproduction by stimulating apoptosis. Mutation of these genes may lead to cessation of the inhibition of cell division. As a result the cell will divide uncontrollably, and produce daughter cells with the same defect. For example, mutation in the TP53 gene (initially after discovery in 1979 by Arnold Levine, David Lane and William Old incorrectly believed to be an oncogene), one of the most commonly mutated tumour suppressor genes which encoding tumour protein - so called p53 protein, a key element in stress-induced apoptosis, is involved in the pathophysiology of leukaemias, lymphomas, sarcomas, and neurogenic tumours [10,11]. Homozygous loss of p53 is found in 70% of colon cancers, 50% of lung cancers and 30–50% of breast cancers. Other important tumour suppressor

genes include p16, BRCA-1, BRCA-2, APC or PTEN [12]. Mutation of these genes may lead to melanoma (p16), breast and ovarian cancer in genetically related families (BRCA), colorectal cancer (APC) or glioblastoma, endometrial cancer, and prostate cancer (PTEN).

In general cancerogenesis is a multistep process thus it is usually a combination of proto-oncogene activation and tumour suppressor gene loss or inactivation is required. However, in a few cases (only 5-10% of cancer cases) this abnormal change in gene can be inherited, passed from generation to generation, in most cases it is a result of sporadic or somatic mutation acquired during a person's lifetime. Although cancer is generally believed to arise as a result of slow accumulation of multiple mutations, but in some cases (2-3%) massive multiple mutation can also arise in a single event. Thus cancer is described as a disease of abnormal gene function, genetically caused by the interaction of two factors: genetic susceptibility and environmental mutagens and carcinogens. Of key importance for the recognition of the molecular mechanism underlying cancer treatment - cell apoptosis - was the discovery of telomeres and telomerase. In the early 1970s, Alexei Olovnikov, on the basis of the Leonard Hayflick's concept of limited somatic cell divisions (Hayflick limit), suggested that chromosomes cannot completely replicate their ends. In 1978 Elizabeth Blackburn discovered the unusual nature of stretches of DNA in the ends of the chromosomes of protozoan *Tetrahymena* - the so-called telomeres. The sequence of human telomere was established 10 years later, in 1988, by Robin Allshire. Blackburn described telomere-shortening mechanism which limits cells to a fixed number of divisions and protect chromosomes from fusing each other or rearranging that can lead to cancer. Shortened telomeres have been found in many cancers, including pancreatic, bone, prostate, bladder, lung, kidney, and head and neck. In 1985, Carol Greider isolated the enzyme telomerase, controlling the elongation of telomeres. Four years later, in 1989, Gregg B. Morin reported the presence of telomerase in human tumour cells and linked its activity with the immortality of these cells (inability to apoptosis), while Greider discovered the lack of active telomerase in normal somatic cells apart from stem cells, keratinocytes, intestines, and hair follicle. It was discovered that deactivation of telomerase prompts the apoptosis of human breast and prostate cancer cells. These results indicated the important role of telomerase in the process of oncogenesis.

Carcinogenesis have been found a complex and multi-step (preinitiation, initiation, promotion and metastasis) biological process characterised by independence from growth factors, insensitivity to inhibitors of growth, unlimited potential for replication (reactivation of telomerase), invasiveness, the ability to metastasis and to sustain angiogenesis, and resistance of apoptosis [13]. DNA mutation inherited and caused by exposure to carcinogens (chemical: compounds including drugs, physical: radiation, or biological: the introduction of new DNA sequences by viruses) have been found to be the true origin of uncontrolled growth of cells coupled with malignant behaviour: invasion and metastasis (Fig. 1).

The knowledge of the molecular mechanisms involving the above mentioned factors, especially apoptosis and cancer resistance to it, can improve cancer therapy through resensitization of tumour cells. Fundamental method of cancer treatment - classical chemotherapy (and radiotherapy) which is harmful also to normal cells, act primarily by inducing cell apoptosis either locally, in tumour, or globally, when cancer metastasize. Any disturbance in apoptosis



results in a decrease in the effectiveness of the therapy. The recent targeted therapies instead of interfering with rapidly dividing cells, interfere with selected targets in the cell and use small molecules to interfere with abnormal proteins (required for carcinogenesis and tumour growth) or cell receptors, or use monoclonal antibodies, which destroy malignant tumour cells and prevent tumour growth by blocking specific receptors. Targeted cancer therapy, which may be more effective and less harmful than classical chemotherapy but still are based on the use of chemical compounds is perceived as modern chemotherapy or chemotherapy of the future.



**Figure 1.** Multi-step process of carcinogenesis.

### 3. Cancer risk — Carcinogens and co-carcinogens

Currently we are aware that apart of inherited mutations, an important role in carcinogenesis play the factors connected with the expression to carcinogens [14]. This includes environmental factors (pollutions), lifestyle factors (tobacco smoking, diet, alcohol consumption, obesity, sedentary life), occupational factors (e.g. synthesis, dyes, fumes) and other factors (excessive exposure to sunlight, radiation, viruses, etc). Carcinogens (of chemical, physical or biological origin) include chemicals or non-chemical agents, which under certain conditions are able to induce cancer. Co-carcinogens, are not carcinogenic themselves but with other chemicals or non-chemical carcinogens, such as for example UV or ionizing radiation, promote the effects of a carcinogen in carcinogenesis. Carcinogens as well as co-carcinogens can be of natural or synthetic origins. In general, their carcinogenic action relay on direct or indirect action in the cellular DNA. Carcinogens acting directly can initiate the carcinogenesis by yielding highly reactive species that bind covalently to cellular DNA, while those acting indirectly can induce mutations to cellular DNA. Thus carcinogens are able to distort the conformation or function (replication/transcription) of DNA, which results not only in oncogene activation but also DNA amplification, gene transposition or chromosome translocation. Carcinogens may induce carcinogenesis directly by mutational activation of protooncogenes and/or inactivation of tumour suppression genes. Indirect action is realised through the mechanisms that generate chemical species (free radicals, reactive oxygen species, carcinogenic metabolites) which are capable of entering the nucleus of the cell.

Over 80% of carcinogenic substances are of environmental origins [15]. Restriction of the exposition to carcinogens can substantially reduce the risk of cancers also those of occupational type, which make approximately 4-5% of all human cancers. Thus evaluation and classification of the carcinogens is required from the cancer prevention point of view. Although there are many international and national organizations that classify carcinogens, but only a few are

highly influential, the oldest and setting the standards, World Health Organization of the United Nations (WHO) International Agency for Research on Cancer (IARC) headquartered in Lyon, France, established in 1965; United Nations initiative from 1992 called Globally Harmonized System of Classification and Labelling of Chemicals (GHS), National Toxicology Program of the U.S. Department of Health and Human Services established in 1978, professional organization American Conference of Governmental Industrial Hygienists, founded in 1938 in Washington and reorganized in 1946, European Union directives Dangerous Substances Directive (67/548/EEC) and the Dangerous Preparations Directive (1999/45/EC) and Safe Work Australia (Independent Statutory Agency) which evolved from National Occupational Health and Safety Council (NOHSC) established in 1985. One of the prime roles of these organizations is to evaluate and classify the chemical, physical and biological carcinogens targeted to develop strategies for cancer prevention and control used by international and national health and regulatory agencies to protect public health. Since 1971 IARC evaluated the carcinogenicity of approximately 400 and collected data about 900 agents and published them in a series (101 till 2012) of Monographs on the Evaluation of Carcinogenic Risks to Humans [3]. Up to now IARC has identified 108 definitely, 66 probably, 284 possibly carcinogens, 515 not classifiable as carcinogens and 1 probably not carcinogenic, Table 1. Alternative to IARC, a complex GSH classification system, collects data from tests, literature, and practical experience [16]. Since 2003 four editions of GHS have been published, but only most recent one, dated to 2011, has the form convenient for worldwide implementation. GSH delivers a global system of classification of chemicals (substances, alloys, mixtures) divided into three groups of hazards: physical (16 classes), health and environmental (12 and 2 classes, respectively) and a unique system of labelling and collecting the information in the form of safety data sheets (SDS). GSH requires the use of the harmonized classification scheme and the harmonized label elements for any carcinogenic chemical. Within the GSH system, the class of carcinogens was clearly separated as health hazard risk factor and divided to two categories: known and presumed carcinogens (subcategories 1A and 1B, respectively) and suspected carcinogens (category 2), Table 2. Irrespective of the classification system, the epidemiological evidence indicates that many drugs, including antineoplastic, sex hormones, antithyroid, antibacterial, antiparasitic, immunosuppressive ones used as single agents or in combinations as well as radiation ( $\gamma$ , X or UV) are known carcinogens.

### 3.1. Carcinogens of chemical/environmental origins

As early as in 16<sup>th</sup> c. Phillippus Aureolus Theophrastus Bombastus von Hohenheim (1493-1541) known as Paracelsus suggested that the “wasting disease of miners” might be linked to exposure to realgar (tetra-arsenic tetra-sulphide). Since the 17<sup>th</sup> c. cancer was associated with the presence of some chemicals. For example John Hill (1716-1775) linked tobacco use with nasal cancer, while Percivall Pot (1814-1788) described occupational risk of epithelial cancer of the scrotum connected with soot, in chimney sweepers. In 1795 Samuel Thomas von Soemmerring (1755-1830) cautioned that pipe smokers were excessively prone to cancer of the lip. Since then epidemiological evidence has been important in detecting carcinogens. In 1858, a Montpellier surgeon Etienne-Frédéric Bouisson (1813-1884) found that 63 of his 68 patients suffering from oral cancer were pipe smokers. Shortly after replacement of natural dyes by




synthetic aromatic amine dye in German industry, Ludwig Rehn (1849-1930) reported an increased incidence of bladder cancer in workers exposed to it. Many years later exact carcinogen, 2-naphthylamine, was recognized. In the 1930s the first company in American dye industry, DuPont, reported first cases of occupational cancer connected with the use of dyes, bladder cancer, at the Chambers Works plant. In 1935, Takaoki Sasaki and Tomizo Yoshida (1903-1973) induced malignant tumours (hepatoma) in a digestive organ by feeding rats by one of the azo dyes - *o*-aminoazotoluene.

IARC classification	Effect	Criteria	No	Agents and groups of agents/mixtures/ the exposure circumstance
Group 1	definitely carcinogenic	sufficient evidence of carcinogenicity to humans, epidemiologic evidence, occupational exposure, and animal studies; strong evidence that the agent acts through relevant mechanisms of carcinogenicity to humans	108	chlorambucil, cyclophosphamide, chlornaphazine, melphalan, tamoxifen5, thiotepa, sulfur mustard  ultraviolet radiation (UV-A, UV-B, UV-C), X-radiation, $\gamma$ -radiation, radiation, radionuclides, neutron radiation, solar radiation
Group 2A	probably carcinogenic	limited evidence of carcinogenicity to humans, but sufficient evidence of carcinogenicity in experimental animals; strong evidence that the carcinogenesis is mediated by mechanisms that are also operate in humans	66	azacitidine, cisplatin, nitrogen mustard, doxorubicin
Group 2B	possibly carcinogenic	limited evidence in humans; less than sufficient evidence in experimental animals; inadequate evidence in humans but sufficient or limited in experimental animals	284	aziridine, dacarbazine, daunomycin, thiouracil, bleomycin
Group 3	not classifiable as carcinogenic	inadequate evidence in humans; inadequate or limited to experimental animals; mechanisms of carcinogenesis in animals does not operate in humans.	515	ifosfamide, isophosphamide, actinomycin D
Group 4	probably not carcinogenic	negative evidence of carcinogenicity, not used	1	caprolactam

**Table 1.** Classification of carcinogens according to IARC, 2012

But the first chemical carcinogen - coal tar - was identified as early as in 1915 by Katsusaburo Yamagiwa (1863-1930) and Koichi Ichikawa (1888-1948) who induced cancer in laboratory

animals by prolonged application of coal tar to rabbit skin. Inflammation accompanying the coal tar application and cancer formation was in a good agreement with Virchow findings. The search for specific chemical carcinogens led to the discovery of pure carcinogenic chemicals including polycyclic aromatic hydrocarbons PAHs (e.g. benzo[a]pyrene, 1,2,5,6-dibenzanthracene) by Ernest Lawrence Kennaway (1881-1958) and Izrael Hieger (1901-1986), which were shown to be carcinogenic in mouse skin by Hieger et al. in 1933 [17]. Nowadays, we know that PAHs, mainly benzo[a]pyrene and heterocyclic amines (HCAs) belong to definite carcinogens which appear in smoke as a result of incomplete combustion [18] and thus are present not only in tobacco smoke but also in a fried/smoked meat as well as barbeque. PAHs often induce stomach cancer.

Category	Effect	Criteria	Signal word	Hazard statement	Symbol/Pictogram
1A	known human carcinogen	known to have carcinogenic potential for humans – largely based on human evidence	Danger	may cause cancer	
1B	presumed human carcinogen	presumed to have carcinogenic potential for humans – largely based on animal evidence	Danger	may cause cancer	
2	suspected human carcinogen	evidence from animal and/or human studies is limited	Warning	suspected of causing cancer	

**Table 2.** Classification of carcinogens according to GSH, 2011

Although cancer-causing substances are often considered to be exclusively synthetic, there are numerous natural carcinogens, chemical compounds that occur in environment, and in food plants [19]. Isaac Berenblum (1903-2000) discovered the potent inflammatory agent, croton oil extracted from *Croton Tiglium* L. native or cultivated in Asia (India, Ceylon, China), Malay Argipelago and Africa (Zanzibar, Tanzania), and its most active ingredient, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 1941 [20]. Both agents now belong to classic tumour promoters. In 1956, John Barnes (1913-1975) and Peter Magee, reported an example of synergistic interaction of chemical carcinogens with proinflammatory agents. i.e. liver tumors in rats induced by *N*-nitrosdimethylamine (NDMA) [21]. In 1972, another case, the influence of chronic respiratory infection with influenza virus on the development of lung cancer in rats induced by carcinogenic *N*-nitrosamine was reported [22], which occurs in some foodstuffs, latex, cosmetics. Since then about 90% of nitrosamine derivatives including hydrazines from raw mushrooms *Agaricus bisporus* (Lange) Imbach and *Gyromitra* (Pers.) Fr. have been deemed to be carcinogenic and promoters of benign hepatomas, liver cell carcinomas, angiomas and angiosarcomas of blood vessels, adenomas and adenocarcinomas of lungs.

One of the most potent naturally occurring microbial carcinogen is Aflatoxin B<sub>1</sub>, which is produced as secondary metabolite by the fungi *Aspergillus flavus* and *Aspergillus parasiticum* [23]

growing on stored grains, nuts and peanut butter and found worldwide as a contaminant in food. The discovery of Aflatoxin B<sub>1</sub> followed upon "Turkey X Disease" (a liver disease) which killed over 100,000 turkeys in the UK in the early 1960s. The major metabolite of Aflatoxin B<sub>1</sub>, Aflatoxin B<sub>1</sub>-8,9-epoxide, exerts hepatotoxic effect, but synergistic interaction between Aflatoxin B<sub>1</sub> and hepatitis B virus results in hepatocellular carcinoma. Another fungal contaminant is mycotic toxin Ochratoxin-A (OTA) produced by *Penicillium viridicatum* discovered during laboratory studies in the mid-1960s and encountered as a natural contaminant in maize in 1969 in the USA [24]. Large group of carcinogens are tannins and tannic acid, which occur widely in plants (tea, coffee, and cocoa) but in concentrated doses reveal hepatocarcinogenic properties in both animals and humans. They have been found capable of causing liver tumours in experimental animals and oesophageal, throat & mouth cancers in humans. Cycads, important food sources in tropical regions, contain unique toxins cycasin and macrozamin that cause liver and kidney tumours in rats [25]. Safrole, 5-Allyl-1,3-benzodioxole found in sassafras tea, cinnamin, cocoa, nutmeg, black pepper, and other herbs and spices as well as isosafrole, 1,2-(Methylenedioxy)-4-propenylbenzene belong to liver carcinogens in rats, they produce liver tumours following their oral administration [26]. Dihydro-safrole is also carcinogenic in rats and mice, in which it produces tumours of the oesophagus, and liver tumours in males and lung tumours in both males and females, respectively. There is an evidence of the carcinogenic properties of estragole from anise, star anise, basil, bay, tarragon, fennel, marjoram or American wood turpentine oil, which proceeds through a genotoxic mechanism identical to that of safrole and also induce liver cancer in mice [27]. Black pepper (*Piper nigrum* L.), apart of tannic acid and safrole contains secondary amines piperidine and alpha-methylpyrrolidine, which can be nitrosated to N-nitroso-piperidine, a strong carcinogen, carcinogenic to experimental mice. It has been known since the 1960s that Comfrey (*Symphytum officinale* L.) contains carcinogenic hepatotoxins belonging to pyrrolizidine alkaloids (PAs) e.g. lasiocarpine and symphatine, which can interfere with RNA and DNA synthesis within the liver cells and cause liver damage, cancer, and death [28].

Some chemical carcinogens have been discovered as a result of industrial or environmental accidents. For example in 1976, notoriety was gained by Seveso disaster - an explosion occurred in a TCP (2,4,5-trichlorophenol) reactor at the ICMESA chemical plant located about 20 km north of Milan, Italy. A mixture of different chemicals including dioxin was released into the atmosphere. This industrial accident caused the highest known exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in residential populations and linked dioxin exposure to chloracne, genetic impairments and excessive risk of lymphatic and hematopoietic tissue [29-32]. Another environmental disaster related to dioxines was contamination of a landfill of Love Canal in the Niagara Falls, New York, USA. This region was turned in 1920 to municipal and industrial chemical dumpsite by Hooker Chemica and in 1942-1953 it was contaminated by eleven highly toxic carcinogens including TCDD. In 1978 a record amount of rainfall in Love Canal resulted in leaching the chemicals from corroding waste-disposal drums in this area which caused environmental disaster, a drastic increase in birth defects, nervous disorders, high white-blood-cell counts in residents, a possible precursor of leukaemia and cancers [33-35]. Many years later probable carcinogenic action of triclosan, an antibacterial agent added to soaps, toothpastes etc., has been linked to its degradation to TCDD in chlorinated water [36]. In the

early 1980s the high risk of lung, skin, kidney and bladder cancer due to chronic low level arsenic poisoning of water in different countries (Bangladesh, Vietnam, Cambodia, Tibet, Argentina, Chile, China, India, Mexico, Thailand, and US) caused by contamination of water by pesticides and various alloys containing arsenic, which resemble and thus substitute phosphorus in chemical reactions, was discovered [37]. In 1980 it was realized that exposure to formaldehyde (a hazard in embalming and production of plastics and vinyl chloride, from which PVC is manufactured) could cause nasal cancer in rats [38]. In the early 1970s, the carcinogenicity of vinyl chloride was linked to occupational angiosarcoma cancers in workers in industry. A few years later PVC was classified as a carcinogen [39-41]. Since then specific substances: aniline and benzidine, asbestos, wool/wood/leather dust have been linked to different types of cancer in humans bladder cancer, sinuses and lung cancer, mesothelioma, nasal sinuses, respectively [3]. Many drugs, including chemotherapeutic anticancer agents, diuretics, hormones have been recognized as a source of secondary cancers and thus classified as definite carcinogens. Most of anticancer drugs is classified as group 1 agents in IARC classification [3].

### 3.2. Carcinogens of biological origins (Oncogenic viruses/bacteria/parasites)

The hypothesis that cancer can originate from a virus comes from Danish scientists Oluf Bang (1881-1937) and Vilhelm Ellerman (1871-1924), who was the first to show, in 1908, that avian erythroblastosis (chicken leukaemia) can be transmitted by cell-free extracts. In 1911, Francis Peyton Rous (1879-1970), American pathologist, described a solid cancer, sarcoma, in domestic chickens caused by exposing the healthy bird to a cell-free filtrate containing retrovirus later became known as the *Rous sarcoma virus* [42]. Abbie Lathrop (1868-1918) and Leo Loeb (1869-1959) described breast cancer in mice caused by a transmissible agent as early as in 1915 [43]. Since then several oncoviruses have been linked to different types of cancer [44]. In 1933 Richard Edwin Shope (1901-1966) discovered the first mammalian tumour caused in cottontail rabbit by fibroma virus and papilloma virus (*Shope papilloma virus*). Shortly later, in 1936, a geneticist and cancer biologists John Joseph Bittner (1904-1961) discovered a mouse mammary tumour virus (MMTV), the so-called Bittner virus, causing a breast cancer, which is a promoter in models of human breast cancer [45]. In 1957, Sarah Elizabeth Stewart (1905-1976) and Berenice E. Eddy (1903-1989), pioneers in the field of viral oncology research, discovered the Stewart-Eddy polyoma virus, which produced several types of cancer in a variety of small mammals [46]. John J. Trentin (1908-2005) and others were the first to report of cancer (sarcoma) produced in animals (hamsters) by inoculation of virus of human origin (*Adenovirus*) [47]. Michael Anthony Epstein, Bert Achong (1928-1996) and Yvonne Barr identified the first human cancer virus (Epstein-Barr Virus or EBV) from Burkitt lymphoma cells in 1964 [48]. Baruch Blumberg (1925-2011) isolated Hepatitis B virus (HBV), a cause of hepatitis, and suggested that it contributed to liver cancer hepatocellular carcinoma. It was confirmed to be an oncovirus in the 1980s. Hepatitis C virus (HCV) was shown to be a major contributor to liver cancer (hepatocellular carcinoma) by Michael Houghton and Daniel W. Bradley in 1987. The first human retroviruses, Human T-lymphotropic virus 1 (HTLV 1) and 2 (HTLV 2), linked to T-cell lymphoma/T-cell leukaemia and Hairy-cell leukaemia, respectively, were discovered by Bernard J. Poiesz, Robert Charles Gallo and Mistuaki Yoshida. In 1984 Harald zur Hausen and Lutz Gissman discovered

that the human papillomaviruses HPV16 and HPV18 were responsible for approximately 70% of cervical cancers, while Alan Storey, Kit Osborn and Lionel Crawford in 1990 indicated that HPV types 6 and 11 were responsible for 90% of genital warts. Valerie Beral, Thomas A. Peterman, Harold W. Jaffe related Kaposi's sarcoma-associated herpesvirus (KSHV) with AIDS [49], which prompted Patrick S. Moore, Yuan Chang, Frank Lee and Ethel Cesarman to isolate Kaposi sarcoma-associated herpesvirus (KSHV or HHV8) in 1994 [50]. Very recently in 2008, Chang and Moore developed a new method to identify oncoviruses called digital transcriptome subtraction (DTS) and isolated DNA fragments of Merkel cell polyomavirus from a Merkel cell carcinoma, considered to be responsible for 70–80% of these cancers [51].

There is also evidence of a link between the bacteria *Helicobacter pylori* (HP) responsible for development of gastric and duodenal ulcers and cancer risk [52,53]. The human oncogenic viruses, which include HBV, HCV, HIV, HPVs, EBV, KSHV, HTLV-I and HTLV-II and HP are associated with nearly 20% of the human cancer cases. The elimination of these pathogens would decrease by 23.6% the cases of cancer in developing countries and by 7.7% in developed countries [54]. The commonly omitted advantage of the discovery of oncoviruses was the possibility of transplantation of carcinogen-induced tumour systems in mice, which delivered models for the studies on anticancer drugs.

Rare source of cancer are also parasitic diseases caused by *Clonorchis sinensis* (Japan, Korea, Vietnam) and *Opisthorchis viverrini* (Thailand, Laos, and Malaysia) or *Schistosomas species* (Africa, Asia). All of them are known to be carcinogenic and linked with biliary tract cancer (cholangiocarcinoma) and bladder cancer, respectively [55]. Most of the biological carcinogens are classified as group 1 agents in IARC classification [3].

### 3.3. Carcinogens of physical origins (Radiation)

Shortly after the discovery of chemical carcinogens, i.e. factors that suppress and activate the cell growth and division, the first physical carcinogens were identified. After discovery of X-rays by Wilhelm Roentgen (1845-1923) in 1895 and radioactive radiation by Henri Becquerel (1852-1908) in 1896, the exposure to radiation has been identified as one of the causes of cancer. Working with early X-ray generators resulted in the acute skin reactions and the first radiation-induced cancer arising in an ulcerated area of the skin was reported in 1902. In 1910 to 1912, Pierre Marie, Jean Clunet and Gaston Raulot-Lapointe reported the induction of sarcoma in rats by the application of X-irradiation. As early as in 1911 the first report of leukaemia in radiation workers appeared [56]. The 20<sup>th</sup> century pioneers in X-Ray/radium studies fell victims to their work; surgeon Robert Abbe (1851-1928), physicist Marie Skłodowska-Curie (1867-1934) and physician Jean Bergonie (1857-1925) died due to leukaemia. The use of uranium/plutonium based bombs against Hiroshima/Nagasaki during World War II revealed that ionising radiation irrespective of its origin is a cause of cancer [57]. Increased incidence of cancer of bone marrow and essentially all organs was noted in Japan years to decades later. Some physical carcinogens have been discovered as a result of nuclear disasters. In 1957, the cooling system failed and the radioactive wastes chemical explosion of at Mayak nuclear fuel reprocessing plant, Ozyorsk/Mayak, Russia caused radiation contamination which spread over hundred kilometres and pollution of the Techa River. This accident called Kyshtym disaster

belongs to three most serious nuclear accidents ever recorded, although it was revealed only in 1976 [58]. The scarce epidemiological studies suggest very different numbers of cancer deaths among residents associated to radiation exposure. In 1979 the cooling system of Three Mile Island nuclear power plant near Harrisburg, Pennsylvania failed and the reactor core was partially melted. Radiation from the reactor contributed to the premature deaths and cancers in local residents, but the disaster was relatively small [59]. There is still vivid discussion about the carcinogenic effects of nuclear power plant explosion in 1986 in Chernobyl located 80 miles from Kiev, Ukraine, which was the greatest source of long-lived radioactive plutonium and short-lived radioactive caesium ( $^{137}\text{Cs}$ ), iodines (particularly  $^{131}\text{I}$ ) and strontium ( $^{90}\text{Sr}$ ). The major health effect of Chernobyl was an elevated thyroid-cancer incidence due to iodine absorption by the thyroid gland in adolescents and children some of whom were not yet born at the time of the accident, and drastic increase in leukaemia cases caused by distribution the strontium incorrectly recognized by the body as calcium throughout the bone structure [60]. Radioactive isotopes of barium, caesium, iodine and tellurium were detected in a radiation plume released by damaged nuclear reactors at the nuclear plant in Fukushima, Japan in 2011. Fukushima Daiichi disaster was the most serious accident in global scale. As the prolonged exposure to radiation in the air, ground and food can result in leukaemia and other cancers thus about 160,000 people were evacuated from the region surrounding the plant. According to theoretical 3-D global atmospheric models this nuclear disaster may cause as many as 2,500 cases of cancer, mostly in Japan. Only recently, in the 1990s, much-less energetic UV radiation has been also recognized as carcinogen causing not only genetic mutations but also melanoma or non-melanoma cancers. In 2011 WHO/IARC classified radiofrequency electromagnetic fields as possibly carcinogenic to humans (Group 2B), on the basis of an increased risk of glioma, a malignant type of brain cancer, associated with wireless phone use [3]. Nowadays we are aware that exposure to radiation can be incidental like in Hiroshima, Nagasaki, Chernobyl, Fukushima [1,61] or systematic due to repeated doses of radiation like UV during sun-bathing or MW during phone-cell use. Anyway the most common radiation induced cancers are basal cell carcinoma and squamous carcinoma of the skin, leukaemia and thyroid cancer. The first two can arise from excessive exposure to UV radiation, while the other are mainly result of ionising radiation e.g.  $\gamma$ , X-Ray [1]. The controlled use of ionising radiation in medicine and industry and annual limits of doses for each individual [62] has reduced the risk connected with ionising radiation but the awareness of UV or MW related risk is still low. Common feature of cancers induced by physical factor is late onset and long period of risk persisting. Most of the physical carcinogens are classified as group 1 or 2 agents in IARC classification [3].

## 4. Chemotherapeutical agents

### 4.1. Drugs of natural origin

In the second half of the 20<sup>th</sup> century, one more type of cancer therapy was added to surgery, irradiation and hormonotherapy, which was chemotherapy. Nowadays this term primarily refers to the treatment of cancer with an antineoplastic drug or a combination of drugs, but when it was introduced in 1909 by Paul Ehrlich (1854-1915) it had a broader meaning as it



referred as well to antibacterial chemotherapy and treatment of autoimmune diseases, in general use of chemicals to treat disease. Chemotherapy, generally assumed as the youngest method of cancer treatment, is in fact rooted in ancient times. Although cutting out the cancer changed tissue was early found as the main treatment, it was not always effective. Thus various substances of natural origin were applied as complementary medications. Many even ancient cultures had proposed theories explaining the cause of cancer. These theories influenced the search for medicaments. For example Egyptians believed that natural substances similar in look or function to human organs can be used to treat ailments in those organs, thus the use of mixtures of pigs eyes or ears was popular.

Although the products of animal and mineral origin had made an important contribution to drug development, the main source of drugs for millennia have been green plants. The most frequently used included castor oil plant (*Ricinus communis* L.), exploding cucumber (*Ecballium elateritum* L.), belladonna (*Atropa belladonna* L.), myrrh (dried sap from trees *Commiphora Myrrha* L.), incense (dried sap from trees *Boswellia thurifera* L., *Boswellia frereana* Birdw., *Boswellia bhawdajiana* Birdw.), stinging nettle (*Urtica dioica* L.), gingers (*Zingiber Boehm.* L.), red clover (*Trifolium pratense* L.) and autumn crocus (*Colchicum autumnale* L.). Although, in nature most of them cause sickness, but in small doses or after chemical modifications, they revealed therapeutic effects. Some of them were rediscovered by modern medicine. For example from *Colchicum autumnale* L. described by Pedanius Dioscorides (40-90 A.D.) in *De Materia Medica* a toxic alkaloid colchicine was extracted in 1820 by Pierre Joseph Pelletier (1788-1842) and Joseph Bienaimé Caventou (1795-1877). Albert Pierre Dustin (1914-1993), described its antymitotic properties in 1934 [63]. In 2009 it was accepted by Food and Drug Administration (FDA) as a drug for gout and Familial Mediterranean Fever. Another interesting case described by Dioscorides is red viscous sap called the dragon's blood mostly collected from *Dracaena cinnabari* Balf. f or *Croton lechleri* L. and used as a dye and anti-inflammatory, antimicrobial and anticancer folk remedy not only by ancient Greek but also Romans and Arabs. Recently methanolic extract of *Croton lechleri* was shown to exert cytotoxic effects on HeLa (Human epithelial carcinoma cell line) cells and its antitumor effect in HeLa tumour in mice was documented [64]. Two other commonly used antileukemic drugs, vinblastine and vincristine, were extracted in 1950 from the species of Madagascar periwinkle (*Catharanthus roseus* L.), for centuries known as folk remedy, and shortly after approved by FDA. Nowadays vinblastine, which binds tubulin, thereby inhibiting the assembly of microtubules, is an important component of a number of chemotherapy regimens, including ABVD for Hodgkin lymphoma, advanced testicular cancer, breast and lung cancers, and Kaposi's sarcoma [65]. Realgar widely used in Chinese traditional medicine because of its anti-inflammation, antiulcer, anticonvulsion, and anti-schistosomiasis activity was recently found capable to induce cell apoptosis and thus effective in the treatment of hematological malignant diseases [66]. As early as in 1021, Avicenna described the medicinal use of *Taxus baccata* L. (Zarnab) as cardiac remedy in *The Canon of Medicine*. Various parts of *Taxus brevifolia* Peattie, *Taxus Canadensis* Marshall, *Taxus baccata* L. have been used by several Native American Tribes mainly for the treatment of non-cancerous diseases [65] but the use for the treatment of cancer was noted only in the Hindu Ayurvedic medicine. Paclitaxel (Taxol®), used in treatment for breast, ovarian, small and non-small cell lung cancer and Kaposi sarcoma, was isolated in 1967 by Monroe E. Wall and

Mansukh C. Wani from the bark of the 200-years old Pacific yew (*Taxus brevifolia* Nutt.) tree [67]. Its structure was elucidated in 1971 [67,68]. Its cytostatic mechanism of action (mitosis inhibition) was discovered by Susan B. Horowitz in the late 1970s, but only the discovery of total chemical synthesis of Paclitaxel in 1994 by Robert A. Holton widespread its use [69]. Camptothecin isolated from the Chinese and Tibetan ornamental joy tree Decne (*Camptotheca acuminata* var. *rotundifolia* B. M. Yang & L. D. Duan), *Nyssaceae* Arnott family, was discovered in 1966 by Wall and Wani in systematic screening of natural products [70]. Although it is a potent topoisomerase inhibitor, it was dropped in the 1970s from clinical trials because of severe bladder toxicity [71]. But two of its semi-synthetic derivatives - topotecan and irinotecan are used for the treatment of ovarian and small cell lung and colon-rectal cancers, respectively [72,73]. Epipodophyllotoxines also belong to active anti-tumour agents derived from plants. Podophyllotoxin and deoxypodophyllotoxin were obtained from the roots of American mandrake or May apple (*Podophyllum peltatum* L.), Himalayan mayapple (*Podophyllum emodi* Wallich ex Hook. f. & Thomson) and Chinese or Asian Mayapple (*Podophyllum pleianthum* L.), respectively [74], all belongs to *Berberidaceae* Juss. family. *Podophyllum peltatum* and *Podophyllum emodii* were used by the Native American Tribes for the treatment of cancer including skin-cancers. Podophyllotoxin was isolated from the rhizome in 1880 by V. Podwysotski [75]. More cytotoxic 4-deoxypodophyllotoxin was isolated from Cow Parsley (*Anthriscus sylvestris* L.) and Korean pasque flower (*Pulsatilla koreana* Y.Yabe ex Nakai). Although native epipodophyllotoxines are not used but its synthetic analogues - etoposide and teniposide, which belongs to topoisomerase II inhibitors, are effective in the treatment of lymphomas and bronchial and testicular cancers [65,76]. Another example is bruceantin isolated from a tree, *Brucea antidysenterica* Mill from *Simaroubaceae* DC family, used traditionally for the tumour treatment in Ethiopia [77]. Recently it was discovered that bruceantin can be an effective agent for the treatment of hematological malignancies (leukaemia, lymphoma and myeloma). Its activity has been linked with the down-regulation of a key oncoprotein. Omacetaxine mepesuccinat (Homoharringtonine), alkaloid isolated from the Cowtail Pine called Japanese Plum Yew (*Cephalotaxus harringtonia* Koch), is one more example of plant-derived anticancer agent [78,79]. Its racemate (harringtonine mixed with homoharringtonine) which induces apoptosis by inhibition of protein synthesis, particularly Mcl-1 (induced myeloid leukemia cell differentiation protein), is used for the treatment of chronic leukaemia - acute lymphoblastic leukaemia and chronic myelogenous leukaemia [65]. Elliptinium acetate, a derivative of ellipticine, which was isolated from a Fijian plant *Bleekeria vitensis* A.C. Sm., is used for the treatment of breast cancer [65]. Recently numerous potential anticancer compounds have been isolated from different plants. A few of them are currently in clinical or preclinical trials but most require further investigation. A case of considerable interest is indirubine extracted from Mu Lan (*Indigofera tinctoria* L.) from *Leguminosae* Lindl. family called Indigo plant a main component of traditional Chinese herbal remedy called Dang Gui Long Hui Wan used to treat chronic myelogenous leukaemia. Synthetic agents flavopiridol derived from the indirubins - plant alkaloid rohitukine, which was isolated from *Dysoxylum binectariferum* Hook. f. (*Meliaceae* Juss.) [80] and roscovitine derived from olomucine, which was isolated from *Raphanus sativus* L. (*Brassicaceae* Burnett), are respectively in Phase I and II of clinical trials [65,81] against a broad range of cancers including leukaemia, lymphomas and solid tumours [82]. Both are belongs

to inhibitors of cyclin-dependent kinases (Cdks), key regulatory proteins in the cell cycle. Most recent studies indicate that drugs of the indirubin family may block brain tumour and thus improve survival in glioblastoma. Other synthetic derivatives of indirubins (3'-monooxime and 5-bromo) reveal comparable activity to other Cdk inhibitors and thus are promising for drug development [83]. Unique source of indirubins are gastropod molluscs: *Bolinus brandaris* L. and *Hexaplex trunculus* L. (*Muricidae* L.) used for over 2,500 years to obtain purplish red dye known as "Tyrian Purple". The 6-bromoindirubine treated as impurity to indigo dye and its synthetic derivative show selective inhibition of glycogen synthase kinase-3 (GSK-3) [81]. The discovery of GSK-3 functions resulted in the search for its inhibitors as potential drugs against neurodegenerative diseases, inflammation and cancer. Combretastatin isolated from the bark of the South African "bush willow", tree *Combretum caffrum* (Eckl. & Zeyh.) Kuntze (*Combretaceae* Loefl. family) [84] belongs to the most cytotoxic phytomolecules isolated so far [85,86] and is promising in the treatment of colon, lung cancers, lymphomas and leukaemias. Combretastatins belongs to stilbenes, which are anti-angiogenic agents, causing vascular shutdown resulting in tumour necrosis. *Combretum* was widely used in African and Hindu medicine for the treatment of a variety of diseases, but *Terminalia* L. flowering plant from the same family *Combretaceae* Loefl., have been used traditionally for cancer treatment. Another promising stilbene is trans-Resveratrol natural phenol produced by several plants (eg. *Vitis vinifera* L., *Vitis labrusca* L., *Vitis rotundifolia* Michx.), when under attack by pathogens (bacteria or fungi like *Botrytis cinerea* (De Bary) Whetzel). It was extracted from False Helleborine (*Veratrum Album* L.) by Michio Takaoka in 1939. More than 60 years later in 1997, Ming-Hua Jang reported that trans-Resveratrol prevented skin cancer development in mice treated with a carcinogen, which gain attention to its potential anticancer applications. It was shown that trans-Resveratrol acts on all steps of the process of carcinogenesis [87]. It triggered apoptosis in uterine, colon cancer cell line, colon, human breast, prostate, lung cancer and pancreatic cancer cell lines in vitro, but is also able to arrest the cell cycle or to inhibit kinase pathways. The inhibition in the development of oesophageal, intestinal, and breast cancer after oral administration of resveratrol was revealed in studies on animal models. The human clinical trials for cancer have not been reported. A few promising substances betulinic acid, lupeol have been obtained from white part of *Betula species* (*Betulaceae* Gray) bark. The alcohol precursor of betulinic acid - betulin - was isolated as long ago as in 1788 by Tobias Lowitz (1757-1804). Betulinic acid, a pentacyclic triterpene, is a common secondary metabolite of plants, it was isolated also from *Ziziphus zizyphus* L. H. Karst. species, e.g. *Ziziphus Mauritiana* Lam., *Ziziphus Rugosa* Lam. and *Ziziphus Oenoplia* (L.) Mill. [88,89], while lupeol was found in a variety of plants, including mango (*Mangifera* L.) and acacia visco (*Acacia visite* Griseb.). All of them are potent anti-inflammatory agents and displayed selective cytotoxicity against human melanoma cell lines [90]. A case of considerable interest is birch polypore fungus Chaga (*Inonotus obliquus* Pers. Pill.), which belongs to *Basidiomycetes* R.T. Moore. It forms black perennial woody growth called a conk on birch trees. It is traditionally used in Russia for the treatment of a number of conditions including cancer, gastritis and ulcers. Two phenolic compounds, hispidin and hispolon extracted from Chaga but also from Japan, Chinese and Korean medicinal fungus *Phellinus linteus* (Japanese *meshimakobu*, Chinese *song gen*, Korean *sanghwang*) were reported to be cytotoxic against human cell line HeLa [91], while the poly-

saccharides  $\beta$  (1 $\rightarrow$ 3)-D glucopyrans and  $\beta$  (1 $\rightarrow$ 6)-D-glucosyl, found also in ornamental plant *Pteris ensiformis* Burm., originating from tropical Africa, Asia and Pacific region, have promising anticancer activity against a number of different cell lines.

Claims for another efficient plant derivative - *Tabebuia* Gomes (*Bignoniaceae* Juss.) used traditionally by the indigenous people in the Amazonian region for the treatment of variable diseases, appeared in the 1960s. Numerous bioactive compounds including naphthaquinones, particularly lapachol and  $\beta$ -lapachone have been isolated from the stem bark and wood of *Tabebuia impetiginosa* (Mart. Ex DC.) Standl., *Tabebuia rosea* Bertol., and *Tabebuia serratifolia* (Vahl) Nicholson. Lapachol revealed potent in vivo anti-tumour activity, but was dropped out because of unacceptable level of toxicity [92].  $\beta$ -lapachone was recently found active against breast cancer, leukaemia, prostate tumour and several multidrug resistant (MDR) cell lines and more promising than lapachol [93]. It is a potent inhibitor of Cdc25 phosphatases enzyme that play a key role in cell cycle progression [83]. Another potent and promising in the field of MDR is pervilleine A, aromatic ester tropane, selectively cytotoxic against oral epidermoid cancer cell line which was isolated from the roots of the Madagascar tree *Erythroxylum pervillei* Baill. from *Erythroxylaceae* Kunth family [94,95]. In the early 1970s another plant originated substance, maytansine was isolated from the Ethiopian plant, *Maytenus serrata* (Hochst. Ex A. Rich.) Wilczek from *Celastraceae* R. Br. family. Although the results of preclinical animal tests were very promising but the lack of efficacy in clinical trials in the early 1980s resulted in dropping it out from further study. However, related compounds, the ansamitocins, isolated from actinomycete *Actinosynnema pretiosum* shed some light on its possible microbial origin [96]. Its synthetic derivative - cytotoxic Mertansine is a component of humanized monoclonal antibodies: Cantuzumab mertansine, Bivatuzumab mertansine, Lorvotuzumab mertansine and Trastuzumab emtansine effective in colorectal, squamous cell carcinoma, small-cell lung or ovarian cancer and breast cancer, respectively. Another case of considerable interest is thapsigargin isolated from the umbelliferous plant, *Thapsia garganica* L. (*Apiaceae* Lindl.) from Mediterranean island of Ibiza [97]. Thapsigargin, induces apoptosis in prostate cancer cells and synthetic prodrug derived from it called "G-202" is in Phase II clinical trials. Silvestrol isolated from the fruits of *Aglaila sylvestre* Roemer from *Meliaceae* Juss. family [98], exhibit cytotoxicity against lung and breast cancer cell lines [65]. Its synthetic analogue 4'-desmethoxyepisilvestrol is cytotoxic against lung and colon cancer cell lines. Two alkaloids, schischkinnin and montamine isolated recently from the seeds of *Centaurea schischkinii* Tzvelev and *Centaurea montana* L. [99,100] exhibit significant cytotoxicity against HCCLs (human colon cancer cell lines). The essential oil of *Salvia officinalis* L., most popular folk remedy in Middle East known for its antitumor effects, which contains monoterpenes thujone,  $\beta$ -pinene, and 1,8-cineol was shown to be cytotoxic against squamous human cell carcinoma cell line of the oral cavity [101]. There are many other natural substances like extracts of unknown composition from *Colubrina macrocarpa* (Cav.) G. Don., *Hemiangium excelsum* (Kunth) A.C. Sm., *Acacia pennatula* (Schltdl. & Cham.) Benth., *Commiphora opobalsamum* Jacq., *Astragalus* L., *Paris polyphylla* Sm., *Teucrium polium* L., *Pistacia lentiscus* L. used as anticancer remedies on folk medicine in China, Israel, Plestina, Saudi Arabia etc.

In general, over 120 currently prescribed drugs including anticancer ones being the basis of modern chemotherapy were first extracted from plants. About 60% of the anticancer drugs available prior to 1983 were of natural origin [65]. As much as 40% of anticancer drugs developed from 1940 to 2002 had natural or natural-product origins, while another 8% were natural-product mimics. Although nowadays about 300,000 different plant species are known but less than 5,000 have been studied for their potential drug usefulness. Since 1989, the National Cancer Institute (NCI) has screened up to 10,000 potential anticancer agents per year including minerals, exotic plants from tropical rain forests and animal venoms and toxins.

Animal venoms and toxins which has been used as therapeutics in ancient Ayurvedic, Unani, Chinese folk medicine as well as in Homeopathy are also screened. Venoms of snakes, scorpions, toads, frogs and their derivatives protein or non-protein toxins, peptides, enzymes are promising and show some potential in cancer treatment. Léon Charles Albert Calmette (1863-1933) a French physician, bacteriologist and immunologist, was the first to describe an antitumor effect of the venom of Indian cobra *Naja naja* sp. on adenocarcinoma cells. Thereafter many reports have established the anticancer potential of venoms of different species of Elapidae, Viperidae, Crotalidae snakes [102-107] and *Hydrophis spimlis* sea snake [108,109] and assigned it to phospholipase activity. Scorpions venom has been used by traditional and folk medicine in India, China, Africa and Cuba. Chinese red scorpion (*Buthus martensi* Karsch) venom and skin extracts, known as Chan Su in China and Senso in Japan, have been used by traditional Chinese medicine for as long as 2000 years also as anti-leukaemia agents. 4',6-diamidino-2-phenylindole extracted from *Buthus martensi* Karsch induced cell apoptosis in malignant glioma cells in vitro [110], while serine proteinase and hylauronidase have promising anticancer activity against a number of different cell lines including breast ones [111]. Bengalin protein isolated from Hindu black scorpion is suspected to have anti-leukemic properties [112]. Chlorotoxin and Charybdotoxin, 36- and 37-amino acid peptides, respectively isolated from the venom of death stalker scorpion (*Leiurus quinquestriatus* Hebraeus) are promising for the treatment of several types of cancers including glioma and human breast cancer [113,114]. The anticancer effect of the venom of Cuba red scorpions (*Rhopalurus junceus*) was discovered 20 years ago in Guantanamo, but after 15 years of studies Vidatox drug was announced in 2011. The skin extract from Hindu toads (*Bufo melanostictus*, *Bufo gargarizans* Cantor), Chan Su used by Chinese traditional medicine, was discovered to contain a few bufadineolides showing specific activity against human leukemic, liver carcinoma and melanoma cell lines. Species belonging to the families Bufonidae (toads), Lampyridae (fireflies) and Colubridae (snakes) as well as mammalian tissues contain bufadienolides, but the richest source of them are toad species. Although all the bufadienolides showed potent cytotoxicity in vitro, but the evidence of their activity in vivo is limited to human hepatocellular carcinoma and HeLa human cancer cells in mice and require further investigation.

Some hope rises with the use of minerals as a source of anticancers drugs. Most important example is sodium bicarbonate,  $\text{NaHCO}_3$ , which was originally derived from Nahcolite (thermokalite) carbonate mineral. The ancient Egyptians used natural natron, a mixture of sodium carbonate decahydrate, and sodium bicarbonate as a soap and embalming tool. Recently it has been shown that sodium bicarbonate administered orally causes a selective



increase in the pH of tumour and reduces the formation of spontaneous metastases in mouse models of metastatic breast cancer [115,116]. Another interesting case is selenite known since ancient times but recently revealed as a promising anticancer agent capable of inducing apoptosis in malignant mesothelioma and sarcoma cells [117].

As yet none of the new natural venom, toxin or minerals derived anticancer agents have reached the status of the clinical drug, but a number of agents are still in study or in preclinical development.

## 4.2. Synthetic drugs

The first steps toward chemical synthesis of drugs were undertaken by iatrochemistry, a branch of chemistry and medicine concerned with seeking chemical solutions to diseases and ailments. Paracelsus pioneered the use of chemicals and minerals in medicine. He introduced alcohol, arsenic, copper, lead and silver salts into medicine, and developed rules for drug administration and dosages of drugs. Paracelsus also devised methods of extracting the arcanum (active ingredient) from plant materials. For this reason he is considered to be the father of phytochemistry and pharmacognosy. Ehrlich, the father of chemotherapy, developed the animal model to screen a series of chemicals for their potential activity against diseases, which had a major influence on the direction of cancer drug development. He also studied the usability of aniline dyes and the first primitive alkylating agents in cancer treatment. The first overall cancer treatment programme was the work of another pioneer of modern chemotherapy - George Clowes (1915-1988). He developed the first transplantable carcinogen-induced tumour systems in mice, which allowed the standardization of models for cancer drug testing. These early model systems including Sarcoma 37 (S37), Sarcoma 180 (S180), Walker 256, and Ehrlich's ascites tumour have been used for several decades [118]. In 1935 Murray Shear developed the most organized program for cancer drug screening. About 3,000 compounds including natural ones, were screened with S37 as a model system. The reason for the failure of this first systematic attempt to search for anticancer drugs - only two drugs have been subjected to clinical trials, but finally dropped because of unacceptable toxicity - was the lack of knowledge on how to test cytotoxic effects in humans. An extension of the number of tumour systems available for studies by the Yoshida's ascites sarcoma model and a murine leukaemia induced by a carcinogen, Leukaemia 1210, described by Lloyd Law allowed fast progress.

## 4.3. Cell Cycle Non Specific Agents (CCNSA)

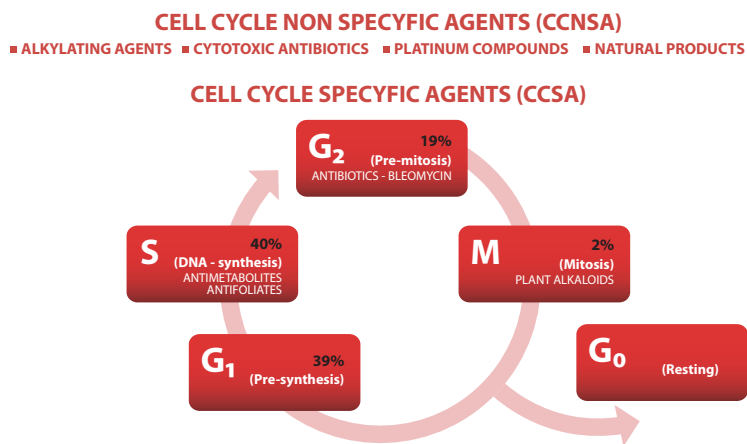
### 4.3.1. Alkylating agents

The first real breakthrough in the search for chemotherapeutics was the chemical synthesis of nitrogen mustards [4,119]. Sulphur mustard was synthesised much earlier, in 1822, but its harmful effects were not known until 1860. It was first used as chemical warfare weapon agent during the latter part of the First World War but its therapeutic activity against squamous cell carcinoma was discovered by accident. In fact most of the first so-called true synthetic chemotherapeutics, were discovered by serendipity, the special term *Serendipity* for accidental discoveries was introduced by Horace Walpole (1717-1797) in the 18<sup>th</sup> c. Nitrogen mustard, an



analogue of the highly toxic sulphur mustard gas, was introduced in 1942 as the first alkylating agent and a true chemotherapeutic. Alfred G. Gilman, Louis S. Goodman and Thomas Dougherty, examined the potential therapeutic effects of nitrogen mustard in rabbits and mice bearing a transplanted lymphoid tumour, while Gustaf E. Lindskog (1903-2002), a thoracic surgeon, administered it to patients with non-Hodgkin's lymphoma. Many cases of cancer regression succeed intensive screening of related alkylating compounds and discovery of busulphan by L.A. Elson, G.M. Timmis, and David A. G. Galton (1922-2006) in 1951, Chlorambucil by James Everatt in 1953, melphalan by Frank Bergel and John Stock in 1954, Cyclophosphamide by Herbert Arnold, Friedrich Bourseaux and Norbert Brock in 1956, Lomustine and Carmustine by John A. Montgomery, George S. McCaleb, Thomas P. Johnston in 1966. While many different classes of alkylating agents (nitrogen mustards, nitrosoureas, alkyl sulphonates, triazines, and ethylenimines) are known, the chemical mechanism of their action is common and based on three different mechanisms all of which achieve the same end result - disruption of DNA function and apoptosis. The first mechanism of DNA alkylation results in its fragmentation by repair enzymes to prevent DNA synthesis and RNA transcription from the affected DNA. The second mechanism is the formation of intrastrand or interstrand cross-links by an alkylating agent, which prevents DNA from being separated for synthesis or transcription. The third mechanism of action is the induction of mispairing of the nucleotides, which leads to mutations, even permanent ones.

Alkylating agent acts on a cancer cell in every phase of its life cycle, Fig. 2, thus can be used in the treatment of a wide range of cancers from various solid tumours to leukaemia. However strong adverse effect is their ability to induce secondary cancers, which is reflected by their classification as definite carcinogens by IARC [3].



**Figure 2.** Cell replication occurs in the cell cycle (G<sub>0</sub>, G<sub>1</sub>, S, G<sub>2</sub> and M). The cell cycle nonspecific agents (alkylating agents, platinum compounds, cytotoxic antibiotics) are able to kill a cell during any phase of the cycle, while cell cycle specific (antimetabolites, antifolates, planta alkaloids, some cytotoxic antiniotics line bleomycin) are only able to kill only during a specific phase.

#### 4.3.2. Cytotoxic antibiotics

A number of cytotoxic antibiotics that have been derived from natural sources such as gram-positive bacteria in soil and water, belonging to genus *Streptomyces* (phylum *Actinobacteria*) [4,119]. They produce secondary metabolites, many of which have been successfully isolated and used as antifungals, antibiotics and anticancer drugs. The large-scale screening of fermentation products by the pharmaceutical industry which resulted in the discovery of antibiotics to treat wound infections is one more example of finding anticancer drugs by serendipity. Although penicillin, which was the basic compound for the above mentioned studies has no antitumor properties itself, but the chromo oligopeptide actinomycin D, isolated from *Streptomyces antibioticus* by Selman Abraham Waksman (1888-1973) and Boyd Woodruff in the 1940s as a result of search for drugs to treat tuberculosis, has significant antitumor properties and was applied in the 1950/1960s in paediatric oncology. This antibiotic was approved by the U.S. Food and Drug Administration (FDA) in 1964. In 1950, the search for anticancer compounds from soil-based microbes in the area of Castel del Monte, Italy, resulted in the discovery of an antibiotic - Daunorubicin (red pigment) - independently by Aurelio di Marco, Arpad Grein and Celestino Spalla from bacterium *Streptomyces peucetius* and by M. Dubost from *Streptomyces caeruleorubidus*. It was found to be active against murine tumours (Yoshida sarcoma). Although clinical trials which began in the 1960s suggested its significant activity against acute leukaemia and lymphoma, but shortly after, in 1967, it was recognized that daunorubicin had significant cardiac toxicity. In general, many antibiotics produced by *Streptomyces* are too toxic for use as antibiotics in humans, but their activity towards specific cells lines makes them useful in chemotherapy. The search for more effective antitumor antibiotics over 2,000 analogues of slightly modified structures yielded in a series of compounds, some of which are in common use till today. In 1969, Federico Arcamone developed a derivative of Doxorubicin which in the same year was tested against animal tumours by di Marco. Daunorubicin and Doxorubicin belongs to inhibitors of the topoisomerase II, one of two enzymes that regulate overwinding/underwinding of DNA. Inhibition of the topoisomerase II block cleavage of both strands of the DNA which ultimately leads to cell death. An important antibiotic of a wide spectrum of anticancer activity is Mitomycin C isolated from *Streptomyces caespitosu* in 1955 and *Streptomyces lavendulae* in 1958 and clinically used since the first successful trials against childhood leukaemia reported by Charlotte Tan in 1965. Mitomycin C belongs to bifunctional alkylating agents, whose biological activity mode is DNA alkylation and cross-linking. It has a broad activity against a range of tumours. In 1966 Hamao Umezawa discovered an important unique antibiotic in this group - bleomycin - a glycopeptide showing anticancer activity, while screening a culture filtrates of *Streptomyces verticillus*. Bleomycin is used to treat many types of cancer, including testicular cancer, non Hodgkin's lymphoma, Hodgkin's lymphoma and cancers of the head and neck. Anticancer antibiotics, apart from Bleomycin, act on a cancer cell in every phase of its life cycle and prevent cell divisions, but Bleomycin is considered as cell cycle agent specifically working in G2 and M phase, Fig. 2. However, the risk connected with the use of cytotoxic antibiotics classified as group 2 or 3 agent in IARC classification is smaller than that related to alkylating agents [3].

### 4.3.3. Platinum compounds

Cisplatin was synthesized in 1845 but its potential as an antitumour agent was not recognized until 1965 when its capabilities were discovered by Barney Rosenberg, Loretta van Camp and Thomas Krigas. The inhibition of growth caused by platinum complex of ammonia and chloride (Peyrone's salt i.e. Cis-platinum) was discovered by serendipity during the studies of the influence of electric current on bacterial growth. The positive result during the studies of murine tumours in vivo confirmed its antitumor activity and prompted the studies of other compounds from this class [4,56,119]. It was introduced into clinical practice one decade later in the 1970s [120]. By 1978 about 1,000 platinum complexes had been screened, but only seven were selected for detailed pharmacological evaluation on rats and only two - Carboplatin and Oxaliplatin - were non-toxic at effective antitumour dose. Although Cisplatin belongs to three most commonly used chemotherapeutics, progress made to improve its use since its discovery is in fact limited. The mechanism of action of Cisplatin and other platinum compounds resemble that of the alkylating agents. They interact covalently with DNA and form intrastrand (within the same DNA molecule; >90%) or interstrand (between two different DNA molecules; <5%), cross links between adjacent guanine molecules [121]. The formation of DNA adducts results in an inhibition of DNA synthesis and transcription. Platinum compounds act on a cancer cell in every phase of its life cycle, Fig.2. Their use is widespread and includes the treatment of bladder and colorectal cancer, upper gastrointestinal disease, germ cell tumours, head and neck malignancies, lung and ovarian cancer. Their ability to induce secondary cancers reflected by their classification as definite carcinogens by IARC is high [3].

## 4.4. Cell Cycle Specific Agents (CCSA)

### 4.4.1. Antifolates

Antimetabolites (folic acid, pyrimidine or purine analogues), which structurally resemble naturally occurring molecules necessary for DNA and RNA synthesis and either inhibit enzymes needed for nucleic acid production or induce apoptosis during the S phase of cell growth, Fig. 2, were among the first effective chemotherapeutics discovered. In the early 1940s, Sidney Farber (1903–1973) studied the effect of folic acid (pteroylglutamic acid; Vitamin B9) first isolated from spinach leaves. In 1945, Rudolf Leuchtenberger reported that folic acid inhibited tumour growth in mice, while Richard Lewisohn reported complete regression of spontaneous breast in mice observed with folic acid. Farber, Robert D. Heinle, and Arnold D. Welch tested folic acid in leukaemia and concluded spuriously that deficiency of Vitamin B9 accelerates leukaemia cell growth. Efforts to treat leukaemia resulted in pharmacological folic acid analogues with effects antagonistic to those of Vitamin B9. Shortly after Sidney Farber and Harriet Kille developed a series of foliate antagonists including highly active aminopterin (4-amino-pteroylglutamic acid). Its analogue 4-amino-4-deoxy-10-N-methyl-pteroylglutamic acid, known nowadays as Methotrexate, was discovered by Yellapragada Subbarao (1895-1948) and successfully applied by Sidney Farber in 1947 to induce remissions in children with leukaemia. In 1958 Min Chiu Li, reported fully effective treatment of a very rare tumour of the placenta, choriocarcinoma with Methotrexate, which was the first-ever intentionally

discovered synthetic anticancer drug i.e. first true chemotherapeutic. Starting from the 1950s, Methotrexate has replaced the more toxic aminopterin and is still in widespread clinical use. In general folate antagonists mechanism of action is linked with competing with folates for uptake into cells and inhibition of the formation of folate co-enzymes or reactions that are mediated by them. Only one of those mechanisms is clinically important, it is the prevention of formation of tetrahydrofolate by inhibition of the enzymes: dihydrofolate reductase (DHFR) or thymidylate synthase (TS). Methotrexate inhibits only DHFR, while a new-generation antifolate Pemetrexed developed by Edward C. Taylor in 1992 inhibits DHFR, TS and also transformylases (GAR and AICAR), but primarily acts as a TS inhibitor. Thus both act similarly by hindering enzymes needed for de novo synthesis of the thymidine and purine nucleotides but show different spectrum of activity. Methotrexate is effective mainly in the treatment of leukaemia, lymphoma and choriocarcinoma but also cancers of breast, head and neck, colorectal, osteosarcoma and bladder, while Pemetrexed is approved for the treatment of mesothelioma and non-small cell lung cancer, active in solid tumours treatment, especially those drug resistant. The risk connected with the use of Methotrexate classified as group 3 agent in IARC classification is smaller than that of alkylating agents [3].

#### 4.4.2. Antimetabolites

The discovery of nitrogen mustard and Methotrexate and their success in medical applications was a breakthrough and stimulated the search for the other antimetabolites as well as new classes of cell cycle specific synthetic anticancer drugs [4,56,119]. In 1944, Richard O. Roblin and James W. Clapp synthesised 8-azaguanine (8-AZA), while 5 years later George W. Kidder and Virginia C., reported that 8-AZA was a guanine antagonist in the metabolism of *Tetrahymena geleii* (S) (*Colpidium campyllum* L.) and inhibited the growth of transplanted mammary adenocarcinoma in mice. Since 1944, George Herbert Hitchings (1905-1998) and Gertrude Belle Elion (1918-1999) have investigated the role of purines in nucleic acid metabolism and methods to prevent them from being incorporated to DNA synthesis along the metabolic pathway that would lead to interruption of cell reproduction. By the early 1950s, Hitchings & Elion synthesized more than 100 purine analogues including 2,6-diaminopurine, 6-thioguanine (6-TG), 6-mercaptopurine (6-MP) and Azathioprine (AZA) as a result of their rational approach to drug development. 6-TG, 6-MP and AZA although categorized as growth inhibitory antimetabolites, exert their functions more like a genotoxic methylating agents, such as alkylating drug Temozolomide, which methylates DNA. 6-MP, one of the early analogues, is widely used not only for acute leukaemias but also in gout and herpes viral infections, and as immunosuppressive agents in the organ transplantations, 6-TG is predominantly used as antileukaemic agent, while AZA as an immunosuppressive. The discovery of a few purine analogues Fludarabine (FLU), Cladribine (2CDA), and Pentostatin (DCF) was a result of further targeted studies performed within a NIH programme. Fludarabine discovered by John Montgomery and Kathleen Hewson in 1968 was the first halogenated ribonucleotide reductase inhibitor, a new-generation pro-drug of the purine class successfully used in treating refractory chronic lymphocytic and chronic B cell leukaemias, non-Hodgkin's lymphoma and T-cell lymphoma. Cladribine, resembling deoxyadenosine and remarkably active in hairy cell leukaemia was synthesised in 1972 by L.F. Christensen, A. Broom, M.J. Robins, and A.J. Bloch and in 1978

selected by Dennis A. Carson as the most potent enzyme adenosine deaminase (ADA) inhibitor from many candidate congeners. Pentostatin, which also inhibits ADA and similarly to 2CDA is active in hairy cell leukaemia and chronic lymphocytic leukaemia was synthesised by Hollis D.H. Showalter and David C. Baker in 1983. Recently, three new purine antimetabolites nelarabine, clofarabine, and forodesine have been found highly promising. Although these compounds belong to purine antimetabolites and reveal activity against specific types of leukaemia, they differ in metabolic properties and mechanism of action. As long ago as in 1964 Elmer Reist and Leon Goodman synthesized 9- $\beta$ -Darabinofuranosyl guanine (ara-G), which despite of its antitumor properties in in-vitro canine leukaemia models evaluated by Elion & Kurtzberg was rejected because of inadequate solubility. In 1988, nelarabine, the 6-methoxy derivative of Ara-G, which is 10-fold more soluble than Ara-G, was synthesized by Thomas A. Krenitsky. In 2012, 48 years after synthesis of Ara-G, Nelarabine entered phase II of clinical studies with indication to T-cell acute lymphoblastic leukaemia or T-cell lymphoblastic lymphoma treatment. Clofarabine, a hybrid of Fludarabine and Cladribine, was synthesised by Montgomery in 1992. It also recently entered phase II of clinical studies with indication as antileukemic agent active in acute lymphoblastic leukaemia as well as in myeloid disorders in paediatrics. The third intensively studied purine antimetabolite is forodesine, synthesised by Peter C. Tyler and Vern L. Schramm in 1998. Forodesine, which is not incorporated into DNA and has unexplored mechanism of action is effective for the treatment of relapsed B-cell chronic lymphocytic leukaemia.

In the 1950s Robert Duschinsky synthesised the first pyrimidine analogue, 5-fluorouracil (5-FU). His discovery was based on the observation of the role of greater uptake of uracil in rat hepatoma metabolism, thus it was the first known case of “targeted” studies. 5-FU introduced into the clinic in 1957 by Charles Heidelberger has broad-spectrum activity against non-hematologic cancers, thus is now widely applied for treatment of many kinds of solid tumours of breast, head and neck, adrenal, pancreatic, gastric, colon, rectal, oesophageal, liver and G-U (bladder, penile, vulva, prostate). Even nowadays, 5-FU apart of its analogue Floxuridine, remains a fundamental drug in the treatment of colorectal cancer. Discovery of 5-FU was not only the first example of targeted studies but also the first targeted therapy, which later attracted much attention in current cancer drug development. However, the target in this case was understood not as a molecular target but as a biochemical pathway. In 1950 two spongocytosides (spongouridine and spongothymidine) were isolated by Werner Bergman and Robert Feeney from a Caribbean sponge *Cryptotethya crypta*. It inspired Richard Walwick, Walden Roberts, and Charles Dekker to synthesise Cytarabine and Vidarabine in 1959. In 1964, John Evans tested activity of Cytarabine using in-vitro murine S180 model while four years later Rose Ruth Ellison introduced it into clinic for the B-cell leukaemia treatment. Cytarabine is effective in acute non-lymphocytic, lymphocytic, myelogenous, chronic myelocytic leukaemias, as well as leptomeningeal carcinomatosis and non-Hodgkin’s lymphoma, Other pyrimidine antagonists include Capecitabine, which is an oral 5-FU pro-drug adjuvant in colon and breast metastasis therapy, Gemcitabine which is a prodrug of Cytarabine, effective in pancreatic, metastatic breast, bladder, ovarian and non-small cell lung cancers and Decitabine, used in myeloplastic syndrome. The antimetabolites of purines and pyrimidine compounds

acts on a cancer cell in S phase of its life cycle, Fig.2 and are classified as a group 3 agents in IARC classification.

#### 4.4.3. Plant alkaloids

A true breakthrough in chemotherapeutics came by in the 1950s as the discovery of the activity of plant alkaloids from Madagascar periwinkle plant *Vinca rosea* (*Catharanthus roseus* (L.) G. Don) by Canadian scientists Robert Laing Noble, Charles T. Beer and Gordon Sloboda [56]. The vinca alkaloids extracted from *Vinca rosea* consist of a subset of structurally similar compounds comprising two multiringed units, vindoline and catharanthine. Initially, they were investigated because of putative hypoglycaemic properties, but strong marrow suppression observed in rats and significant antileukaemic effects *in vitro* decided about their clinical use shortly after the discovery of their properties, in 1959. Nowadays, vinca alkaloids are produced synthetically and only four major ones - Vinblastine, Vincristine, Vinorelbine and Vindesine - are in the oncological clinical use. Vinblastine is most often applied in Hodgkin's disease, non-Hodgkin's lymphoma, breast cancer, and germ cell tumours, Vincristine is effective against leukaemia and Hodgkin's, Vinorelbine has significant antitumor activity in patients with breast cancer and antiproliferation effects on osteosarcoma, while Vindesine is used in the treatment of leukaemia, lymphoma, melanoma, breast cancer, and lung cancer. All vinca alkaloids have a unique mechanism of action; they bind to the microtubular proteins of the mitotic spindle, which leads to crystallization of the microtubule and mitotic arrest or apoptosis.

Another group of novel cytotoxic agents from plant alkaloids, taxane diterpenes, was discovered during long-term screening in the 1970s. The main compound in this class, Paclitaxel, was the first taxane introduced into clinical practice for the treatment of recurrent ovarian cancer metastatic, breast cancer, often in combination with Cisplatin. Nowadays, Paclitaxel is totally synthesized, but less popular than vinca because of its poor solubility. Both plant alkaloids vinca and taxanes are mitotic inhibitors M phase specific, Fig. 2, but they act in different ways. Their principal mechanism of action is the disruption of microtubule function, but in contrast to the vinca alkaloids, taxanes do not destroy mitotic spindles. The plant alkaloids are classified as a group 3 agents in IARC classification [3].

#### 4.4.4. Combination regimens

In 2005, conventional chemotherapeutics still made the majority of the Top 20 Cancer Therapeutics. Their popularity was dictated not only by wide spectrum of activity and also long history of use in oncology but also their key role in the multidrug treatment programs. In the early 1960s, single alkylating agents were basic for all cancer treatment programmes. Although the remissions were observed for example up to 25% in advanced Hodgkin's disease but they were still incomplete and temporary. Increasing resistance of the cancer cells to classical drugs and numerous side effects forced new strategies. After Jacob Furth and Morton Kahn discovery that a single leukemic cell was sufficient to cause the death of an animal, Howard E. Skipper formulated "Cell Kill" hypothesis, according to which a given dose of drug is able to kill only a constant fraction of tumour cells. This hypothesis favoured search for more aggressive



chemotherapeutics but also resulted in the use of drug combinations. In 1965 Emil Frei and Emil J. Freireich developed the new treatment program for children leukaemia known as "VAMP" (Nethotrexate, 6-MP, Vincristine and Prednisone). The use of multiple drugs: Methotrexate, which disrupt folic-acid uptake, 6-MP which inhibits synthesis of purine, both critical in cell division, Vincristine which interfered with cell division by binding to spindle protein and Prednisone, anti-inflammatory steroid resulted in the remission rate level as high as 60%. Further modifications like "MOMP" (nitrogen mustard, Vincristine, Methotrexate, and Prednisone), "MOPP" (Procarbazine, nitrogen mustard, Vincristine, and Prednisone) and C-MOPP (Procarbazine, Cyclophosphamide, Vincristine, and Prednisone) resulted in the 80% complete remission rate in advanced Hodgkin's disease in the 1970s. On the basis of the above mentioned first programs dedicated exclusively to leukaemias many modifications - e.g. four drug EBVP (Epirubicin, Bleomycin, Vinblastine, Prednisone) and ABVD (Adriamycin, Bleomycin, Vinblastine and Dacarbazine) and six-drug STANFORD-V (Cyclophosphamide/ Mechlorethamine/Ifosfamide, Doxorubicin, Vinblastine, Vincristine, Bleomycin, Etoposide) - have been developed. Nowadays, some cancer diseases like Hodgkin's or acute lymphocytic leukaemia are curable in 90% within the modern protocols using aggressive chemotherapy programs. Despite the reasonable position of classical chemotherapeutics in multidrug combined regimens, their capabilities inevitably decrease because of multidrug resistance (a major factor in the failure of many forms of treatment) and secondary effects (adverse or paradoxically carcinogenic). Significant limitation is also the need for multiple chemotherapy in long-term, sequential multidrug regimens and in-hospital administration.

#### 4.4.5. Modern chemotherapeutics

At the beginning of the 20<sup>th</sup> c., Paul Ehrlich postulated the idea of a "Magic Bullet" (Zauber-kugel) i.e. drugs that reach directly intended cell-structural targets. To some extent this idea is the driving force behind the development of modern targeted chemotherapeutics. Conventional chemotherapeutics are cytotoxic, but affect all cells and work in the so-called statistical manner. Because cancer cells multiply faster than normal, the cancerous cells are killed by the drug with a higher ratio than the normal ones, which are not spared. Therefore the principal criterion applied in the modern anticancer drug design is the principle of selective toxicity, which require activity restricted exclusively to the cancer cells. From the point of view of selective activity directed on tumour cells and mechanisms of carcinogenesis, a few different classes of modern drugs can be distinguished: inhibitors of cytokine stimulating cell proliferation, dissociation, motility; cytokine receptor blockers; intracellular kinases inhibitors; transcription factors inhibitors; cell cycle inhibitors; cell adhesion inhibitors and proteasome inhibitors.

Short-lived hopes were raised in the 1990s at the discovery of the inhibitors of angiogenesis, which hold back the growth of capillary vessels in cancer. Avastin, humanized monoclonal antibody, discovered by Napoleone Ferrara in 1997 was the first drug from this class approved by FDA in 2004 to use for several types of metastatic cancer, but the approval of the breast cancer indication was revoked in 2011. Two other inhibitors of angiogenesis include Cetuximab invented by Joseph Schlessinger, Michael Sela in 1988, approved for by FDA in 2009 to

use in colorectal cancer therapy and Sunitinib discovered by Joseph Schlessinger and Axel Ullrich approved by FDA in 2006 to use for renal cell carcinoma and gastrointestinal stromal tumour. Recent progress in genetic sequencing has led to the discovery of Vemurafenib by Fritz Hoffmann, approved by FDA in 2011. It targets the B-Raf gene that signals the growth of new blood cells in melanoma tumours, which are extremely difficult to treat.

Recently widespread attention has been given to inhibitors of protein kinases, enzymes that catalyze phosphorylation reactions, a principal mechanism of signal transduction governing various cellular processes including growth, division, migration and apoptosis. Imatinib, developed in the late 1990s by Nicholas Lydon, introduced to clinic by Brian Druker and approved to treat chronic myelogenous leukaemia by FDA in 2001 was the first drug of this new class of small active molecules. It inhibits the oncogene BCR-ABL1 and blocks the signals for cell proliferation, controlling tumour growth. Many imatinib analogues: including Nilotinib, Dasatinib, Bosutinib, Ponatinib, Bafetinib were obtained further by rational drug design. Gefitinib invented by ASTRA/ZENECA (approved by FDA in 2003 but withdrawn in 2005) and Erlotinib invented by OSI (approved by FDA in 2004), were the first selective inhibitors of epidermal growth factor receptor among the kinase inhibitors used for treatment of lung cancer.

Monoclonal antibodies that allow the cytotoxin to reach a target required to kill the malignant cells (induce apoptosis), without harming normal cells belong to the unique class of chemo-immunotherapeutics. In contrast to small molecule drugs which have a direct impact on their targets, the monoclonal antibodies stimulate the immune system i.e. re-direct targets to the immune system. Most popular includes: Gemtuzumab ozogamicin invented by Wyeth Ayerst and used for the treatment of acute myelogenous leukaemia, but withdrawn from the market in 2010 due to its toxicity, Ibritumomab tiuxetan developed by IDEC Pharmaceuticals used for the treatment of non-Hodgkin's lymphoma, but known to cause serious side effects, Panitumumab used for the treatment of colon cancer but ineffective, Rituximab developed by IDEC Pharmaceuticals and still used for the treatment of B-cell non-Hodgkin's lymphoma and tositumomab developed by Mark Kaminski and Richard Wahl used for the treatment of non-Hodgkin's lymphoma (mainly follicular lymphoma), currently in the clinical trials.

Although modern targeted therapies provide a new approach to cancer therapy and similarly to conventional ones are able to suppress tumour growth, but they also have drawbacks and limitations, while true cancer treatment is still a challenge for oncology.

## 5. Summary

Throughout history of medicine the process of new drug discovery has been based on natural sources and drugs have been discovered by serendipity (sheer luck) or in a trial-and-error process. While until the mid-1980s new drugs were discovered mainly by serendipity, over the next decade, till mid-1990s, the knowledge of structure was the basis for research, then the starting point was to identify a target and a relationship between structure and function [122]. Nowadays a few major classes of drugs useful in cancer treatment have been defined: (1)

General Chemotherapy Drugs (the alkylating agents, anti-neoplastics, anti-metabolites), (2) Steroids, (3) Bisphosphonates, (4) Hormone therapies and (5) Biological therapies/Immunotherapy. This modern classification reflects the fact that anticancer drugs evolved from classical chemotherapeutics discovered mostly by serendipity to drugs acting directly against abnormal proteins in cancer cells designed by rational drug design. All of them have remarkable influence on the growth of cancer cells and on the mechanisms whereby cells replicate, transmit, and translate genetic information.

Current research are so multidirectional that it is impossible to discuss all of them in a short chapter. New directions in this field include the search for the improved pharmaceutical forms, new analogues of currently used drugs, new chemical compounds (natural or synthetic) of anticancer activity, selective anticancer agents (acting on the basis of pathophysiological mechanisms), the search for drugs among old-known drugs currently used for other indications than cancer, search for the methods of precise delivering the anticancer drugs to cancer tissue and stroma or to stimulate the immune system to generate anti-tumor immune responses and protect against cancer.

Even nowadays precursors or generic drugs are frequently discovered by serendipity, while their analogues are developed by purely rational design. Often the newly synthesised drug proved effective in quite different than expected applications. For example Aminoglutethimide was found to be effective in breast cancer treatment instead of being an antiepileptic, Cisplatin, an electrolysis product, was discovered to be cytotoxic or Tamoxifen antiestrogenic activity of *cis*-isomer was discovered as unexpected bonus in the search for drugs to treat mania in bipolar disorder. Sometimes surprisingly a new field of activity is revealed for a long known drug. For example potassium-sparing diuretic Amiloride is effective in glioma; sedative Thalidomide, linked to birth defects, slows the propagation prostate cancer; Tebropfen, antiviral drug, slows the propagation of breast cancer; S-dimethylarsin-gluthathione, an organic form of arsenic, slow solid tumours expansion; anti-epileptic Valproic and Rapamycin, immunosuppressor in organ transplantation are valuable in antitumor therapy; Gossypol, potential male contraceptive overcome resistance to Cisplatin; antimalarial chloroquine may address a critical cell nutrition issue with proliferating cancer cells while insecticide benzoyl-phenylurea helps understand the microtubule assembly process important for growth of the pancreatic cancer cell. Anticancer properties have been ascertained to be shown by aspirin used commonly as analgesic, antipyretic and an anti-inflammatory medication, 9-aminoacridine used as an antiprotozoa and antibacterial agent and Quinacrine commonly used antimalarial drug. The use of long know drugs fasten modern process of drug discovery, which involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy and proceeds through many stages including discovery, product characterisation, formulation, pharmacokinetics, preclinical toxicology testing and IND (Investigational New Drug) application, bioanalytical testing and clinical trials. The new drugs (new completely or a long known one) often have many adverse effects. Sometimes in the final step the new drug proves toxic, ineffective or even carcinogenic. Many widely applied chemotherapeutic anticancer agents (nitrogen mustards, HN2 and HN3, treatamine, Chlorambucil,

Sacrolysin, Melphalan, and Busulphan), have been recognized as a source of secondary cancers and thus classified as definite carcinogens.

Sometimes known carcinogens have become invaluable drugs widely applied in clinical oncology. The most impressive example is arsenic - a component of the well known Poison of Kings ( $\text{As}_2\text{O}_3$ ). It was used in traditional Chinese medicine in the treatment of promyelocytic leukaemia and acute myelogenous leukaemia. Reaglar containing arsenic was used by Hipocrates as a component of antitumor liniment. Avicenna in the 11<sup>th</sup> c. recommended it for cancer, both internally and topically. In the 16<sup>th</sup> century Paracelsus used it as a drug but linked with a cancer disease. In 1786 Thomas Fowler discovered Fowler's Solution (a 1% aqueous solution of potassium arsenite,  $\text{KAsO}_2$ ), which was applied as first chemotherapeutic in chronic myeloid leukaemia treatment in 1865 by Lissauer and persisted till the introduction of the first modern cytotoxic drugs in the 1940s. In 1931, its use in chronic myeloid leukaemia was described. In the late 1960s in China, an arsenic containing liniment was rediscovered for use as an effective anticancer treatment in melanoma. First reports of the intravenous administration of Fowler's Solution in acute promyelocytic leukaemia appeared in the 1990s, also in China. But in the 1990s IARC classified arsenic compounds as definite carcinogens. Despite this, in 2001 Fowler's Solution was accepted by FDA for the treatment of relapsed or refractory acute promyelocytic leukaemia in children. After being abandoned for decades, arsenic trioxide in the 21st c started to be prescribed as a drug for acute promyelocytic leukaemia, and still it is classified as definite anticancer. Recently some hope rises with reaglar as well as new arsenic-based compounds (e.g. C-glycosides), which have been intensively studied. On the other hand, a very recent studies performed by Peter S. Nelson et al. indicated that chemotherapy can damage healthy cells which secrete a protein WNT16B that sustains tumour growth and results in a resistance to further treatment [123]. This proves that chemotherapy itself can boost cancer growth. The paradox drug/carciogen concerns not only chemotherapeutics but also the methods, which revolutionized the treatment of cancer, being on the other hand carcinogenic, like X-Ray widely used in the diagnosis of cancer cells, cancer treatment and anticancer drug design, UV radiation being a basis of the photodynamic therapy. Paracelsus, father of toxicology already wrote "*All substances are poisons: there is none which is not a poison. The right dose differentiates a poison and a remedy.*" Paraphrasing him - the method and conditions of the use differentiates a carcinogen and anticancer drug. Thus the search for new drugs among carcinogens seems quite reasonable, while the protection against contact with or exposure to a carcinogen is a necessity.

The recent advances in genomics and proteomics deliver a promise of understanding the true internal mechanisms of cancerogenesis - a basis for cancer diseases. They cover the knowledge of genes alteration caused by cancer, its influence on the proteins encoded by them, the interaction of these proteins with each other in living cells, the resulting changes in the specific tissues and finally the effect on the entire body. The achievements in this field delimit new fully rational directions in anticancer drug discovery and development of drugs addressing the specific needs (targeted drugs).

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