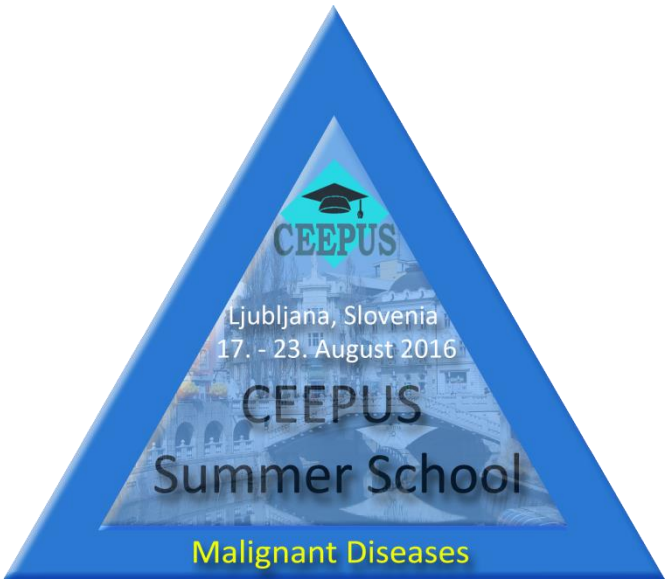

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Malignant Diseases

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Table of Contents

Molecular mechanisms in carcinogenesis

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Cancer is a complex genetic disease and it develops as a multi-step process during which accumulation of nonlethal mutations results in malignant transformation of the cells. Due to the fact that each cancer is characterized by distinctive set of mutations and consequently it is phenotypically specific, it should be considered as a unique disease. That genetic/phenotypic specificity of cancers makes lot of troubles for clinicians and seeks for personalized approach in treatment and therapy. This is why, understanding of molecular mechanisms underlying carcinogenesis is of utmost importance for development new drugs and personalized therapeutic approaches. Consequently, personalized medicine at the moment achieves the biggest success in oncology.

Carcinogenesis is complex, multi-step process that leads to transformation of normal cells which acquire physiological characteristics that together determine malignant phenotype. Accumulation of mutations may last for years, thus the tumor incidence increases with age and the prevalence of tumors is much higher in population over 60 years of age. Mutations are the consequence of the action of environmental agents (*e.g.* chemicals, radiation, or biological agents) or may be inherited in the germ line. In some cases, mutations may be spontaneous and stochastic. Thus, it is difficult to point out the precise cause of cancer, yet it is know that the tumor initiation is a result of genetic change that leads to proliferation and uncontrolled growth. Tumor progression continues and by time particular cells may acquire additional mutations which make them more malignant. Finally, the cell with highest capacity to proliferate and to invade, in addition to other characteristics, become a source of all cells which lately form tumor (clonal selection), *i.e.* tumors are monoclonal. In addition, tumor cells acquire ability to escape from immune system attack.

As it was mentioned above, nonlethal mutations are the primary cause of carcinogenesis. There are three main classes of carcinogenic agents: (*i*) chemicals; (*ii*) radiation (*iii*) microbial agents. While chemicals and radiation are well documented causes of cancer in humans, oncogenic viruses are involved in development of at least some human tumors.

Chemical carcinogens: Most chemical carcinogens are mutagenic and considered to be *initiators* of carcinogenesis. They contain highly reactive groups that interact with DNA (as well as proteins and RNA). Important targets of chemical carcinogens are oncogenes and tumor suppressors (such as *Ras* and *p53)* (*e.g.* aflatoxin B1 causes characteristic mutations in the *p53* gene). Another group of chemicals, *promotors* (*e.g.*, hormones, phenols, and drugs), which by themselves are non-tumorigenic (mutagenic), may induce cell proliferation and thus augment carcinogenicity of some chemical carcinogens. It seems that an initiator may cause the mutational activation of an oncogene while subsequent application of promoters leads to clonal expansion of initiated (mutated) cells.
There are two types of chemical carcinogens (*i*) direct-acting carcinogens which require no metabolic conversion to become carcinogenic. They are in general weak carcinogens but are important because some of them are cancer chemotherapeutic drugs (e.g., alkylating agents); (*ii*) indirect-acting agents - chemicals that require metabolic conversion to an ultimate carcinogen (polycyclic hydrocarbons e.g. benzopyrene, formed in the high-temperature combustion of tobacco in cigarette smoking or during the process of broiling meats; aromatic amines and azo dyes e.g. β-naphthylamine; aflatoxin B1 produced by some strains of *Aspergillus*, a mold that grows on improperly stored grains and nuts; vinyl chloride, arsenic, nickel, chromium, and insecticides are potential carcinogens in the workplace and about the house; nitrites - used as food preservatives.

Radiation: Radiation (UV rays of sunlight, x-rays, nuclear fission, radionuclides) causes chromosome breakage, translocations, and, less frequently, point mutations. Biologically, DNA breaks seem to be the most important form of DNA damage caused by radiation. UV light has the ability to damage DNA by forming pyrimidine dimers, which can be repaired by the nucleotide excision repair pathway. In case of extensive UV light exposure, due to overwhelmed repair systems, it can cause skin cancer.

Microbial agents: Several viruses have been linked with human cancer (*i*) oncogenic RNA viruses *e.g.* human T-cell leukaemia virus-1 (HTLV-1)**,** associated with a form of T-cell leukaemia/lymphoma; (*ii*) oncogenic DNA viruses (human papillomavirus (HPV), Epstein-Barr virus (EBV), Kaposi sarcoma herpesvirus (human herpesvirus 8, HHV8), hepatitis B virus (HBV); and *(iii) Helicobacter pylori*. The precise molecular mechanisms underlying the carcinogenetic effects of different virus type are complex and still under investigation.

Once transformed to malignant form, cells comprise several fundamental phenotypic characteristics:

Autocrine stimulation of growth and/or self-sufficiency in growth signals: Tumours have the capacity to proliferate without external stimuli, usually as a consequence of oncogene activation or they can produce all needed growth factors, by them self.

Insensitivity to growth-inhibitory signals: Tumors may not respond to the molecules that inhibits normal cells growth such as transforming growth factor β (TGF-β) and direct inhibitors of cyclin-dependent kinases (CDKIs).

Evasion of apoptosis: Tumours may avoid programmed cell death, by inactivation of tumor suppressor p53 or activation of anti-apoptotic genes.

Limitless replicative potential and disturbed cell differentiation: Tumor cells have unrestricted proliferative capacity, avoiding cellular senescence and mitotic catastrophe. In some cases, they lose ability to differentiate, so undifferentiated cells uncontrolledly proliferate.

Sustained angiogenesis: Tumor cells, like normal cells, need nutrients and oxygen for their growth, thus they have to stimulate angiogenesis.

Ability to invade and metastasize: Spreading into environment is essential for tumor growth, so tumor cells produce high quantity of proteases. Tumor metastases depend on processes that are intrinsic to the cell or are initiated by signals from the tissue environment. Due to a loss of contact inhibition and reduced adhesiveness tumor cells can easily detach from tumor mass and enter into the lymph or blood circulation, thus spreading around the body.

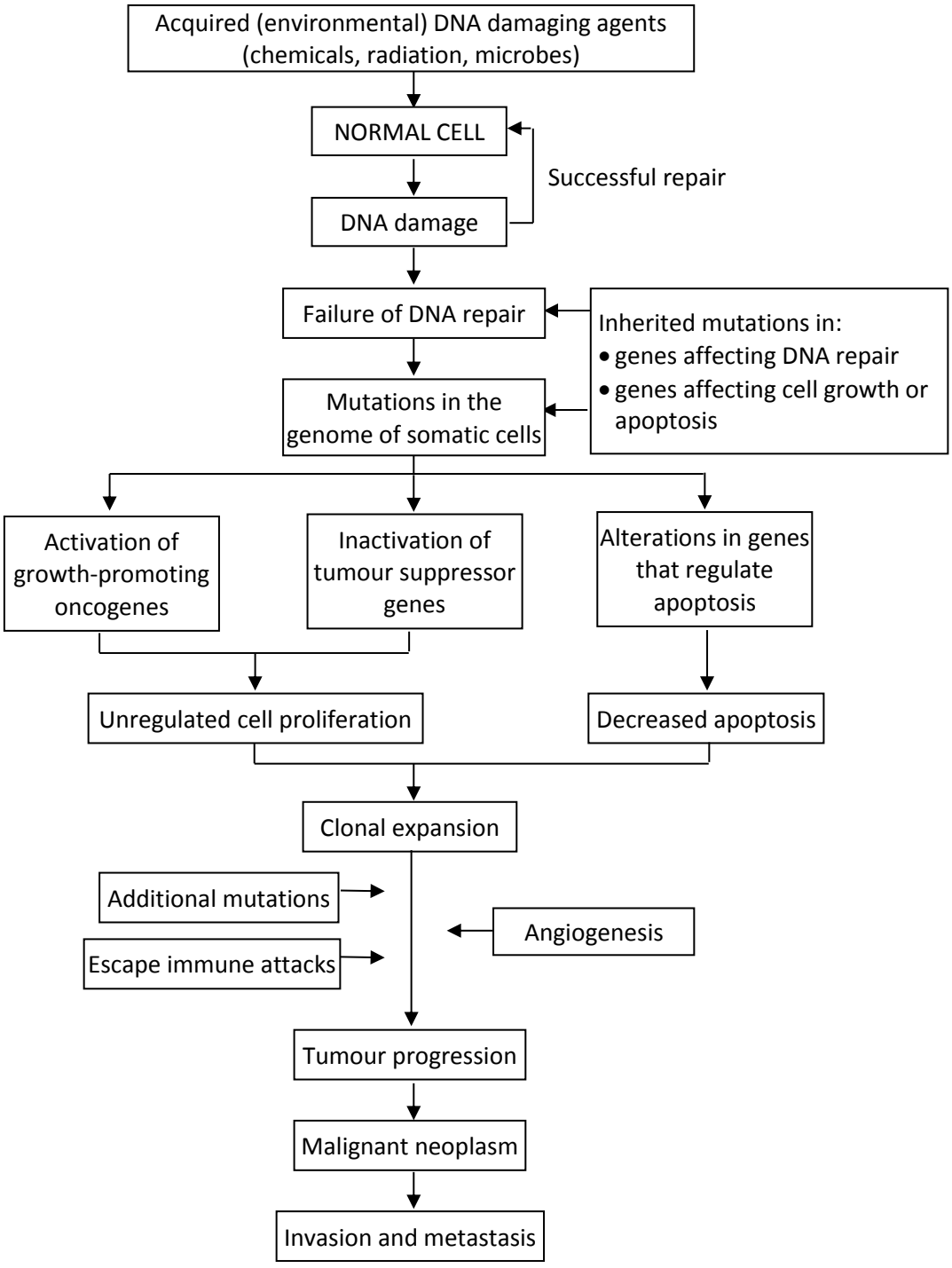
Defects in DNA repair: Tumours may fail to repair DNA damage caused by carcinogens or incurred during unregulated cellular proliferation, leading to genomic instability and mutations in proto-oncogenes and tumor suppressor genes.

Four classes of normal regulatory genes are the principal targets of genetic damage.

- proto-oncogenes
- tumor suppressor genes
- genes that regulate programmed cell death (apoptosis)
- DNA repair genes.

While mutant alleles of proto-oncogenes are considered dominant, both alleles of the tumor suppressor genes must be damaged before transformation can occur. However, in some cases loss of

a single allele of a tumor suppressor gene reduces level or activity of the protein enough that the brakes on cell proliferation and survival are released. Genes that regulate apoptosis may behave as proto-oncogenes or tumor suppressor genes. Mutations of DNA repair genes do not directly transform cells by affecting proliferation or apoptosis. Instead, DNA-repair genes affect cell proliferation or survival indirectly by influencing the ability of the organism to repair nonlethal damage in other genes, including proto-oncogenes, tumor suppressor genes, and genes that regulate apoptosis. Damage of DNA-repair genes can predispose cells to widespread mutations in the genome and thus to neoplastic transformation. Here, we should also mentioned microRNAs (miRNAs), molecules recognized to be involved in tumor development process, which can act as either oncogenes or tumor suppressors by affecting the translation of other genes.



Genes that promote autonomous cell growth in cancer cells are called *oncogenes*, and their unmutated cellular counterparts are called *proto-oncogenes*. Oncogenes are created by mutations in proto-oncogenes and are characterized by the ability to promote cell growth in the absence of normal growth-promoting signals. Their products, *oncoproteins*, resemble the normal products of proto-oncogenes except that oncoproteins are often devoid of important internal regulatory elements, and their production in the transformed cells does not depend on growth factors or other external signals. In this way cell growth becomes autonomous, freed from checkpoints and dependence upon external signals. Several growth factors, such as PDGF-β chain, fibroblast growth factors, and TGF-α can be mutated in a way that they act as oncoproteins as well as their receptors, *e.g.* EGF-receptor family members, PDGF receptor, receptor for neurotropic factors. Some growth factor receptors are transmembrane proteins with an external ligand-binding domain and a cytoplasmic tyrosine kinase domain. In the normal forms of these receptors, the kinase is transiently activated by binding of the specific growth factors, followed rapidly by receptor dimerization and tyrosine phosphorylation of several substrates that are a part of the signalling cascade. The oncogenic variants of these receptors are associated with constitutive dimerization and activation without binding to the growth factor. Hence, the mutant receptors deliver continuous mitogenic signals to the cell, even in the absence of growth factor in the environment.

There are several examples of oncoproteins that mimic the function of normal cytoplasmic signal-transducing proteins. Most such proteins are strategically located on the inner leaflet of the plasma membrane, where they receive signals from outside the cell (*e.g.*, by activation of growth factor receptors) and transmit them to the nucleus. Biochemically, the signal-transducing proteins are heterogeneous. The most well-studied example of a signal-transducing oncoproteins is the RAS family of guanine triphosphate (GTP)-binding proteins (G proteins). It becomes activated by binding of ATP molecules, while the hydrolysis of ATP to ADP turns it into inactivated form. The activated RAS stimulates downstream regulators of proliferation, such as the mitogen-activated protein (MAP) kinase cascade, which floods the nucleus with signals for cell proliferation. Several distinct point mutations of *RAS* have been identified in cancer cells. The affected residues lie within either the GTP-binding pocket or the enzymatic region essential for GTP hydrolysis, and thus markedly reduce the GTPase activity of the RAS protein. Mutated RAS is trapped in its activated GTP-bound form, and the cell is forced into a continuously proliferating state.

Mutations that release latent oncogenic activity also occur in several non-receptor-associated tyrosine kinases, which normally function in signal transduction pathways that regulate cell growth. As with receptor tyrosine kinases, in some instances the mutations take the form of chromosomal translocations or rearrangements that create fusion genes encoding constitutively active tyrosine kinases. An important example of this oncogenic mechanism involves the c-ABL tyrosine kinase. In chronic myeloid leukaemia (CML) and some acute lymphoblastic leukaemias, the *ABL* gene is translocated from its normal position on chromosome 9 to chromosome 22, where it fuses with the *BCR* gene. The resultant chimeric gene encodes a constitutively active, oncogenic BCR-ABL tyrosine kinase.

Whereas oncogenes drive the proliferation of cells, the products of tumor suppressor genes apply brakes to cell proliferation. Many tumor suppressors, such as Rb and p53, are part of a regulatory network that recognizes genotoxic stress from any source, and responds by shutting down proliferation. There are many other proteins involved in regulation of cell proliferation as transcription factors, cell cycle inhibitors, signal transduction molecules, cell surface receptors, and regulators of cellular responses to DNA damage (e.g. TGF-β receptor, E-cadherin, *APC*/β-catenin, *PTEN, SMAD2* and *SMAD4*, *BRCA1* and *BRCA2*). Mutated forms of these genes were found to be present in many tumors resulting in reduced level or disturbed activity of these proteins. For instance, p53 protein functions as a critical gatekeeper against the formation of cancer. It acts as a

“molecular policeman” that prevents the propagation of genetically damaged cells. p53 is a transcription factor, positioned in the centre of a large network of signals that sense cellular stress, such as DNA damage, shortened telomeres, and hypoxia. It prevents neoplastic transformation by three interlocking mechanisms: activation of temporary cell cycle arrest (quiescence), induction of permanent cell cycle arrest (senescence), or triggering of programmed cell death (apoptosis). In response to DNA damage, p53 is phosphorylated by proteins that sense the damage and are involved in DNA repair. p53 contributes to DNA repair by causing G1 arrest and inducing DNA-repair genes. A cell with damaged DNA that cannot be repaired is directed by p53 to undergo apoptosis. This is why, p53 has been rightfully called a “guardian of the genome.” With loss of p53 function, DNA damage goes unrepaired, mutations accumulate in dividing cells, and the cell continues to malignant transformation.

Accumulation of neoplastic cells may result not only from activation of growth-promoting oncogenes or inactivation of growth-suppressing tumor suppressor genes, but also from mutations in the genes that regulate apoptosis. A cell with genomic damage can be induced to die, preventing the accumulation of cells with mutations. Consequently, mutations in genes encoding proteins involved in regulation of apoptosis may also result in impaired apoptosis and allow propagation of mutated cells.

Taken together, many molecular events underlie the initiation and promotion of malignant transformation and proliferation of mutated cells. This is why it is of utmost importance to elucidate them in details with aim to provide the platform which will contribute to better diagnostic and therapeutic approaches for malignant diseases in the future.

Biochemical and inflammatory aspects of tumorigenesis

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Metabolism of the cancer cells

Cancer cells certainly have abnormal metabolism, govern by oncogenic mutations in several metabolic enzymes which affects the whole metabolic pathway. There are several existing and emerging therapies aim to target this abnormal metabolism in various ways.

Cell metabolism describes the group of intracellular chemical reactions that convert nutrients and endogenous molecules into the energy and biomolecules (proteins, nucleic acids and lipids) thatsustain organisms survival. Adenosine triphosphate (ATP), the principal molecule that drives all energy dependent cellular processes, is mainly generated by two metabolic pathways: glycolysis andoxidative phosphorylation (Fig. 1). In glycolysis, glucose is converted to pyruvate, generating two net ATP molecules. When oxygen levels are low, anaerobic glycolysis continues, turning pyruvate into lactate. If oxygen is plentiful, however, the much more efficient processof oxidative phosphorylation occurs, in which pyruvate is routed to the tricarboxylic acid (TCA), or Krebs, cycle in the mitochondria, generating theoretically 36 ATP molecules per molecule of glucose (Figure 1).

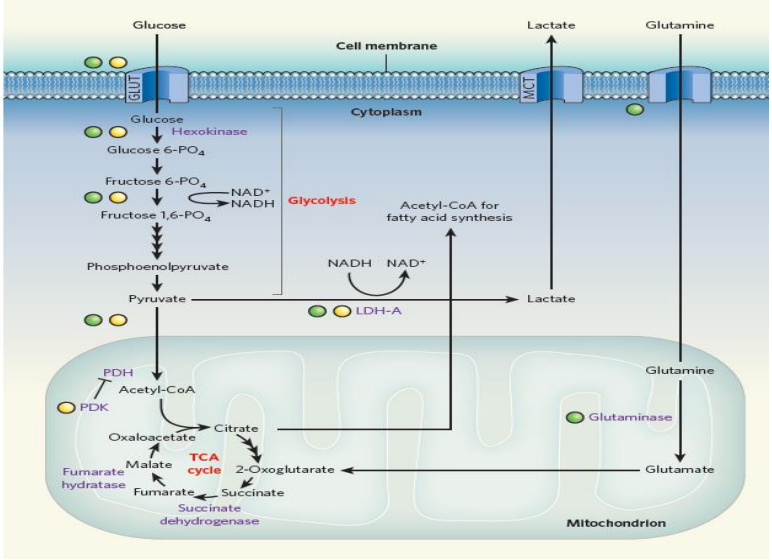


Figure 1. Main metabolic pathways in glucose metabolism of normal cells
GLUT, glucose transporter, MCT – monocarboxylate transporter

If oxygen levels are low pyruvat is converted to lactate even in the cytoplasm. Cancer cells drive pyruvat conversion to lactate even in the presence of oxygen. Metabolism of the nutrient glutamine is also mi+odified in cancer. The transcription factors HIF (yellow) and MYC (green) seem to affect these metabolic pathways at various steps.

Cancer cells are wasteful. Compared with normal cells, they use a disproportionate share of the nutrients in their environment. This is partly because cancer cells metabolize glucose by anaerobic glycolysis (that is, they avoid oxidative phosphorylation even when oxygen is abundant — the Warburg effect). Moreover, cancer cells make inefficient use of glutamine, the most common amino acid. In normal cells, glutamine is used to donate amine and amide groups for the synthesis of amino acids, andnitrogen for *de novo nucleotide formation.*

Cancer cells, by contrast, secrete a significant fraction of glutamine-derived nitrogen and carbon as waste rather than incorporating them into macromolecule synthesis.

Normal cells can carry out anaerobic glycolysis and inefficient glutamine breakdown when they are induced to proliferate. But if their supply of intracellular nutrients becomes depleted, the cells adapt by reactivating oxidative phosphorylation and ceasing proliferation. This ability of normal cells to switch between proliferation and steady state, according to their nutritional status depends on the integrity of a number of tumor suppressors, including the p53 and LKB1 proteins. Cancer cells, which often lack these tumor-suppressor proteins, exhibit 'addiction' to glucose and/or glutamine consumption. Increased activity of the PI3K–AKT signaling pathway — due, for example, to activating mutations in receptor tyrosine kinases— mediates excessive nutrient uptake. AKTactivates the HIF and MYC transcription factors, which enhance the expression of glucose transporters (GLUTs) and glycolytic enzymes(Fig. 1) in order to preserve survival of the cells even in a devastating conditions such as hypoxia. Moreover, HIF diverts pyruvate from the TCA cycle. Some of these actions can be reinforced by the loss of p53. Oncogenic MYCcan also directly induce glutamine uptake in excess of nitrogen or carbon demand. Cells must eliminate the breakdown products of this metabolism by secreting the excess carbon and/or nitrogen as lactate, alanine and ammonia.

As anaerobic glycolysis is inefficient, cancer cells maintain ATP levels by burning more glucose. Besides, too high a yield of ATP is probably not a good thing for a cancer cell. Cell growth depends on a continuous glycolytic and mitochondrial flux of metabolites to generate supplies of the macromolecular precursors NADPH, acetyl-CoA, ribose and glucose-derived nonessential amino acids. To maintain this high rate of glycolysis, a ready supply of the phosphate acceptor ADP is needed. If cellular metabolism is too efficient, all the ADP will be phosphorylated to ATP, and further glucose metabolism will be inhibited. Cancer cells therefore divert glucose-derived carbon into aerobic glycolysis and uncouple ATP production from mitochondrial electron transport. This means that they can consume the metabolic cofactor NADH without producing ATP, and promote the diversion of glycolytic intermediates into non-ATP-generating bypass pathways. Cancer cells are engaged extensivelyin net lipid synthesis — another feature of cancer metabolism — to generate phospholipids for membrane production and lipid-derived signalling intermediates such as prostaglandins. It would be inefficent to simultaneously break down their newly synthesized fatty acidsto generate energy.A fundamental problem for solid tumors is that they often outgrow their blood supplies,and face low oxygen levels (hypoxia). Under hypoxic conditions, HIF accumulates and triggersthe transcription of about 200 genes, many of which support cell survival. These includegenes that promote glycolysis and suppress oxidative phosphorylation. HIF accumulation is suppressed by the activity of oxygen-dependent PHD (prolyl hydroxylase domain) enzymes(Fig. 2). The HIF response can be further magnified by mutations that promote HIF translation, such as those leading to AKT activation, or by mutations that compromise HIF degradation, such as those leading to loss of the tumor-suppressor protein pVHL.Furthermore, changes in the levels of reactive oxygen species or TCA-cycle metabolites. Glutamine is essential for cell growth - abnormal increase in biomass is a hallmarkof cancer. It is the metabolite that acts as an intermediate in the transport of reduced nitrogen through the bloodstream. To produce nucleotides and non-essential aminoacids, cancer cells need a robust supply of reduced nitrogen. Furthermore, glutamine plays a crucial part in the uptake of essential amino acids, can maintain the TCA cycle when glucose levels are limiting (Fig. 1), and can support NADPH production, which is necessary for lipid and nucleotide biosynthesis. Oncogenic activation of MYC promotes glutamine use but at the expense of glutamine addiction. Otto Warburg and colleagues were the first to report that cancer cells continue to engage in glycolysis and lactate production even when oxygen levels are abundant. This is known as the Warburg effect. Despite extensive efforts, however, Warburg and others could not find mutations that impaired oxidative metabolism in cancer cells. Consequently, many biologists questioned whether Warburg's observations truly reflected tumor metabolism or were instead an artefact of inadvertent hypoxia arising during his in vitro experiments. Imaging studies described below support the *in vivo* relevance of the Warburg effect. The Pasteur effect describes the reciprocal relationship between anaerobic glycolysis and oxidative phosphorylation: when oxygen is present, glucose

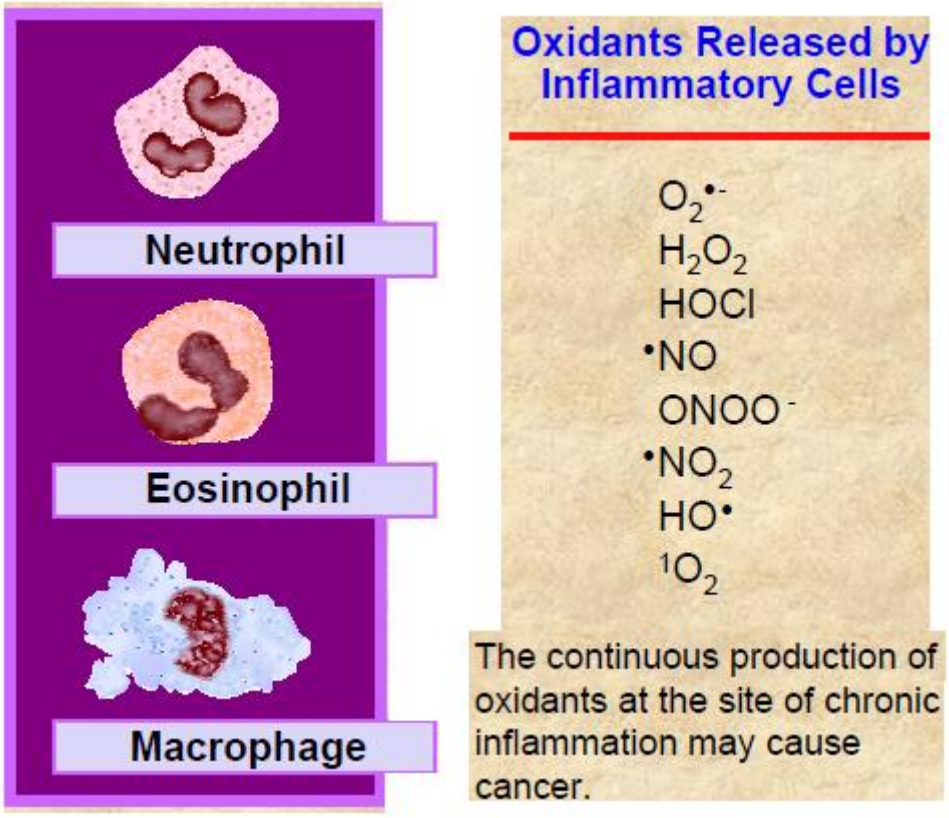
consumption to produce lactate should be suppressed in favour of oxidative phosphorylation. The Warburg effect is a loss of the Pasteur effect. It is clear that HIF is essential for both the Pasteur and the Warburg effects. In normal cells, oxygen suppresses HIF activity, leading to diminished glycolysis and increased entry of pyruvate into the TCA cycle. In cancer cells, oxygen can no longer effectively decrease HIF activity. Moreover, mutations affecting crucial cancer targets such as MYC and p53 can conspire with HIF activation to maintain a highly glycolytic state. Metabolic pathways are independently regulated at various steps, and mutations in a single enzyme are usually insufficient to affect a nutrient's uptake or metabolism. Rather, it seems that tumors seize control of their own metabolism by selecting for mutations that affect metabolic pathways rather than individual pathway components. *In vitro, many cancer cells die in response to* glucose withdrawal. But blocking glycolysis has not been useful in the clinic. For instance, the hexokinase enzyme mediates the first step of glycolysis (Fig. 1), but clinical trials with the hexokinase inhibitors 2-deoxyglucose and lonidamine were terminated, presumably owing to their untoward toxicity or inefficacy. The specificity of other hexokinase inhibitors under investigation, such as 3-bromopyruvate, has also been questioned. Clinical trials on TLN-232 (or CAP-232), an inhibitor of the glycolytic enzyme pyruvate kinase, are continuing. A theoretical concern with glycolysis inhibitors is that glucose is an essential fuel source for red blood cells and, under non-starvation conditions, for the brain. Drugs that indirectly target glycolysis are also being pursued. mTOR inhibitors, for example, indirectly reduce glycolysis by decreasing levels of the HIF-1α subunit and perhaps other HIF-α family members. Several agents that block mTOR or receptor-tyrosine-kinase signalling upstream of mTOR have been approved for cancer treatment. The efficacy of these drugs can be rapidly assessed by their ability to reverse tumor-associated 18FDG uptake. Whether their antiproliferative and pro-cell-death effects are also due to changes in metabolism is unclear, however.

An exciting finding is that germline mutations that affect the TCA-cycle enzymes fumarate hydratase and succinate dehydrogenase can produce cancer, by causing HIF stabilization and a state of pseudo-hypoxia (Figs 1, 2). Defective succinate dehydrogenase can also mediate the development of neural-crest tumors independently of HIF. Similarly, mutations in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) have been reported in brain tumors and acute leukaemia. Instead of their normal product (the TCA-cycle intermediate 2-oxoglutarate), these mutant enzymes produce 2-hydroxyglutarate, which might alter the activity of 2-oxoglutarate-dependent enzymes such as those that modify HIF levels. Several histone demethylase enzymes also seem to work as 2-oxoglutarate-dependent dioxygenases, raising the intriguing possibility that oxygen, reactive oxygen species, and metabolites such as 2-oxoglutarate may have relatively direct roles in influencing DNA-dependent processes including transcription and cell behaviour. Clearly, metabolic pathways are highly interconnected with pathways that govern the hallmarks of cancer, such as unrestrained proliferation and resistance to cell death. The many metabolic enzymes, intermediates and products involved could be fertile ground for improving cancer diagnostics and therapeutics.

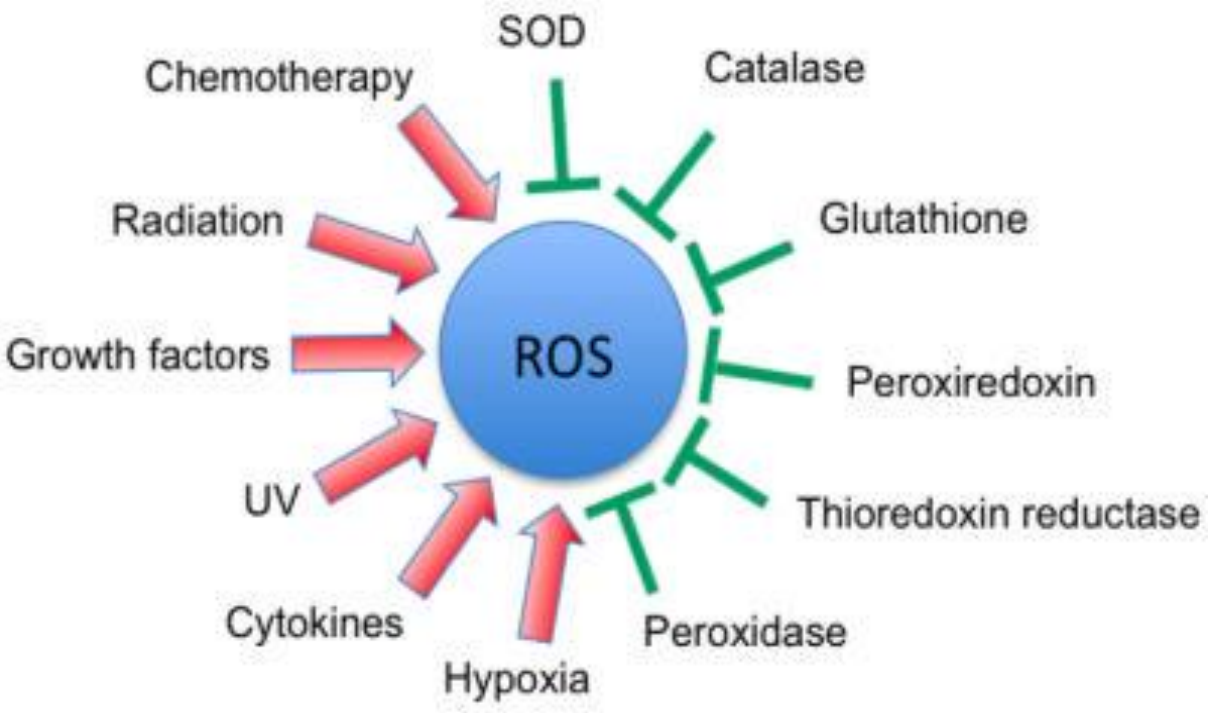
Regulation of HIF function

HIF is a heterodimer consisting of an unstable α-subunit and a β-subunit. In the presence of oxygen, HIF-α is hydroxylated on one (or both) of two prolyl residues by members of the PHD enzyme family. In addition to oxygen, these enzymes require the TCA-cycle intermediate 2-oxoglutarate and reduced iron (Fe^{2+}), and are inhibited by high concentrations of some other TCA-cycle metabolites such as fumarate, succinate and perhaps reactive oxygen species (ROS). A ubiquitin ligase enzyme complex containing the tumour-suppressor protein pVHL recognizes prolyl-hydroxylated HIF-α and earmarks it for degradation by proteasomes by adding a chain of ubiquitin (Ub) proteins. Dashed lines depict components that regulate PHD enzymes. In several congenital disorders, such as Fanconi anaemia, xeroderma pigmentosum, ataxia telangiectasia, Bloom syndrome, Down syndrome and cystic fibrosis the cells show evidence of increased oxidative stress. Affected individuals show an increased incidence of cancer. Chromosomal instability is also a common feature of the first four disorders. Taken together these data suggest that the increased oxidative stress may contribute to

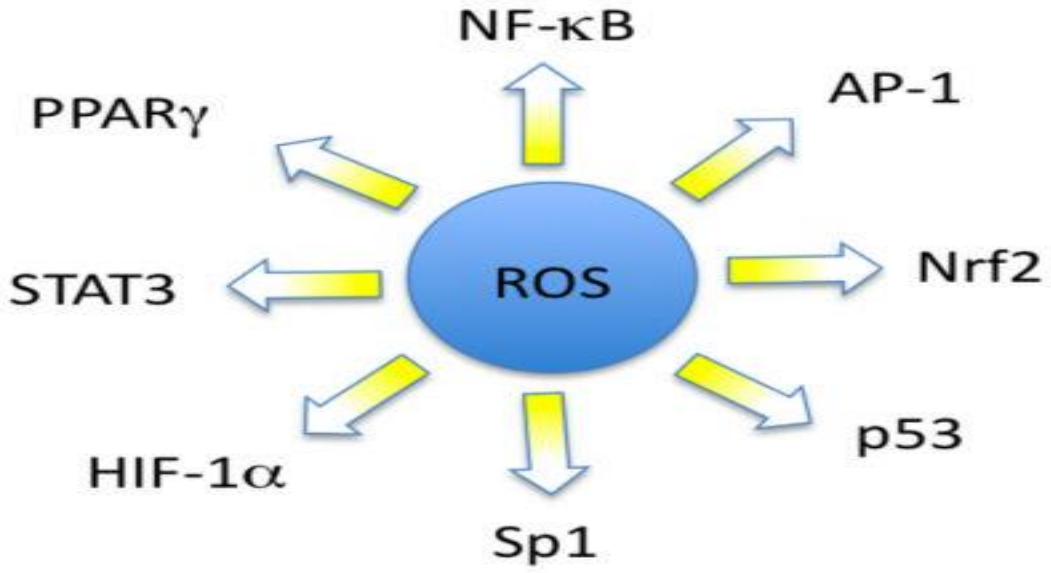
development of genomic instability that is a hallmark of cancer cells. The continuos production of oxidants at the site of chronic inflammation may cause cancer.



Under a sustained environmental stress, ROS are produced over a long time, and thus significant damage may occur to cell structure and functions and may induce somatic mutations and neoplastic transformation. Indeed, cancer initiation and progression has been linked to oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation. The skin, for example, is chronically exposed to both endogenous and environmental prooxidants due to its interface function between the body and the environment, and to protect the skin against this overload of oxidant species, it needs a well-organized system of both chemical and enzymatic antioxidants. The lungs, which are directly exposed to oxygen concentrations higher than in most other tissues, are protected against these oxidants by a variety of antioxidant mechanisms. Furthermore, aging, which is considered as an impairment of body functions over time, caused by the accumulation of molecular damage in DNA, proteins and lipids, is also characterized by an increase in intracellular oxidative stress due to the progressive decrease of the intracellular ROS scavenging. Acting to protect the organism against these harmful pro-oxidants is a complex system of enzymatic antioxidants [e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, catalase] and nonenzymatic antioxidants [e.g., glutathione (GSH), vitamins C and D.



On the other hand, inflammatory cells also produce soluble mediators, such as metabolites of arachidonic acid, cytokines and chemokines, which act by further recruiting inflammatory cells to the site of damage and producing more reactive species. These key mediators can activate signal transduction cascades as well as induce changes in transcription factors, such as nuclear factor kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor-1α (HIF1-α), activator protein-1 (AP-1), nuclear factor of activated T cells (NFAT) and NF-E2 related factor-2 (Nrf2), which mediate immediate cellular stress responses (Figure 2). Induction of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), aberrant expression of inflammatory cytokines [tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6 and chemokines [IL-8; CXC chemokine receptor 4 (CXCR4)], as well as alterations in the expression of specific microRNAs, have also been reported to play a role in oxidative stress-induced inflammation. This sustained inflammatory/oxidative environment leads to a vicious circle, which can damage healthy neighboring epithelial and stromal cells and over a long period of time may lead to carcinogenesis.



Under normal conditions, anti-oxidants outbalance pro-oxidants, but under oxidative conditions, pro-oxidants prevail over anti-oxidants, which can lead to many inflammatory diseases including cancer.

To control the balance between production and removal of ROS, a variety of DNA repair enzymes exist, although antioxidants are more specific and efficient in protecting cells from radicals. This antioxidant system includes both endogenous and exogenous and enzymatic and non-enzymatic antioxidants. Glutathione (GSH), is a tripeptide and the major endogenous antioxidant produced by the cells, which helps to protect cells from ROS such as free radicals and peroxides. It is now well established that ROS and electrophilic chemicals can damage DNA, and that GSH can protect against this type of damage. GSH can also directly detoxify carcinogens through phase II metabolism and subsequent export of these chemicals from the cell. On the other hand, elevated GSH levels are observed in various types of cancerous cells and solid tumors, and this tends to make these cells and tissues more resistant to chemotherapy. Cytotoxicity of different anticancer-agents depend almost exclusively on ROS production. Treatment succes depends on tumor cells sensitivity to oxidative stress. Chemo-therapeutics which have this kind of mechanism of action are: doxorubicin, daunorubicin, mitomycin C, etoposid, cisplatin, imatinib, ionizing radiation and photodynamic therapy.

Tumor markers in clinical practice

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In recent years, research on cancer has focused on the discovery of new tumor markers that would be useful in clinical practice, and could contribute to the early detection of cancer (1). Measurement of these markers in the serum of patients is today one of the fastest developing areas of laboratory biomedicine (2).

Tumor marker was first described 160 years ago, when Bence Jones noticed the presence of abnormal proteins in the urine of patients with multiple myeloma. Tumor markers are described as products of malignant cells, or substances occurring in other cells under the influence of malignant cells (5). They are useful for the diagnosis of cancer, monitoring the progression of the tumor mass, predicting prognosis and for monitoring the success of therapy 4).

The ideal tumor marker comprises a protein, which is synthesized only in a malignant tumor, and is characterized by a certain organ and the type of tumor. It can be demonstrated in all patients with the same type of tumor forms in sufficient quantities early in the development of malignant disease and its serum concentrations reflect the size of the malignant tissue amended (3).

Despite much research in this area could be used in clinical practice, only a few of them (2). The main reason is their lack of specificity and increased production in the physiological and non-malignant conditions. The false-positive results may contribute inflammatory processes, benign tumors, liver disease, extensive tumor necrosis, the consequences of diagnostic tests and treatments, pregnancy and renal dysfunction. False negative results can lead to poor blood flow to the tumor, the formation of immune complexes with tumor antigens or rapid elimination of tumor antigen in the serum (3).
Today known tumor markers are less suitable for the detection of malignant change, but they are potentially useful for patients with a known diagnosis for predicting the prognosis thereof. They help in the choice of treatment, and are used for the prediction of therapeutic outcome and are of great importance for the early detection of relapse (2, 3).

Clinically useful markers of malignant transformation of the α-fetoprotein (AFP) for the control of patients with hepatocellular carcinoma, carcino embryonic antigen (CEA), to monitor patients diagnosed with cancer of the colon and rectum, CA 125 to control therapy in female patients with cancer of the ovary and hCG to control patients with trophoblastic and non-seminoma germ tumors. In breast cancer estrogen receptors are used for prediction of response to hormonal therapy, while HER-2, to enable the identification of patients who are suitable for treatment with trastuzumab (2). Maximum usability expresses prostate specific antigen (5).

The first tumor marker for prostate cancer was the enzyme acid phosphatase. Serum acid phosphatase may originate from different organs of the bone, prostate, liver, kidneys, the red blood cells. Diseases of other organs may therefore cause elevated levels of an enzyme in the blood, which leads to poor specificity of the analyte. In 1966 we can find the first known record for gama semino - protein, protein in seminal fluid, which is today known as prostate specific antigen (6). In 1980, we find for the first time documented the presence of elevated levels of PSA in the serum of patients with prostate cancer (4). In 1994, FDA approval was recorded for the use of PSA for early detection of prostate cancer (7).

The role and usefulness of individual tumor markers and methods for a given type of cancer is defined by the statistical concepts of sensitivity and specificity (4). The sensitivity of the test is the%

of patients with certain tumors, which have a positive test. The specificity of the test represents% healthy, with negative results (3).

For optimal cancer treatment we need reliable prognostic and predictive markers. Prognostic markers are factors that predict the final outcome of the disease in the absence of therapy. Predictive markers are associated with the response or resistance to a particular therapy. Traditional prognostic factors for malignancy are the tumor size, stage of disease and the number of metastases in regional lymph nodes (2).

Traditional tests for the evaluation of tumor markers are ELISA - type tests for serum indicators and immunohistochemical methods for markers of malignant tissue of transformation (2).

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Targeted therapies and predictive markers in cancer

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Specific differences in cancer cells (or other cells in their vicinity) help them grow and thrive. Targeted therapy is a special type of chemotherapy that takes advantage of (i.e. „targets“) these differences between normal cells and cancer cells.

Cancer cells typically have many changes in their DNA/ genes, some of these gene changes might allow the cell to stop working the right way and/or grow and divide very quickly. These changes are what make it a cancer cell, but is a plethora of different types of cancer, and they vary significantly. For example, lung cancer, melanoma and colon cancer cells have different gene changes that help them grow and/or metastasize, even if the same signalling pathway. Another example is breast cancer with various histopathological and genetic subtypes. Basically, we can say that each tumour is unique.

Most standard chemo drugs do not consider genetic or subsequent metabolic and signalling changes, they work by killing cells in the body that grow and divide quickly. Cancer cells divide quickly, which is why these drugs often work against them. But chemotherapy drugs also affect other cells in the body that divide quickly, which can sometimes lead to serious side effects.

Although targeted drugs are technically considered chemotherapy like other drugs used to treat cancer, they don’t work the same way as standard chemotherapy drugs. These drugs also tend to have side effects different from standard chemotherapy drugs. To choose the most appropriate treatment for an individual patient/ tumour, predictive markers are the best help where they exist. A predictive marker is a particular protein or gene that indicates sensitivity or resistance to a specific therapy. The use of predictive markers is becoming increasingly relevant in cancer therapy as it allows for better identification of patients who will respond positively to the therapy. And frequently, predictive marker (or its product) is the target at the same time.

In August 2016, the FDA has already approved dozens of targeted drug cancer therapies, and many more are being studied in clinical trials either alone or in combination with other treatments.

In general targeted drugs work to:

- block or turn off grow /proliferation signals that tell the cancer cell to grow and divide
- stop making new blood vessels to nurture the cancer cells
- stimulate the immune system to kill the cancer cells
- carry a toxin to kill cancer cells, but not normal cells
- change proteins within the cancer cells to induce apoptosis

There are there major classes of targeted drugs:

- small molecules
- antibodies
- vaccines

Of course, some targeted drugs are more “targeted” than others, affecting only a single change in cancer cells, while others can target several different changes. Others enhance the way the organism fights the cancer cells itself. Besides that, targeted therapy is only rarely used alone, most often it is applied with other cancer treatments such as chemotherapy, surgery and/or radiation therapy.

As an example, R-CHOP immunochemotherapy regimen which is used to treat both indolent and aggressive forms of non-Hodgkin lymphoma, consists of rituximab, cyclophosphamide, hydroxydaunorubicin hydrochloride (doxorubicin hydrochloride), vincristine (Oncovin) and prednisone.

Targeted cancer agents are broadly classified as either monoclonal antibodies or small molecules. Therapeutic monoclonal antibodies target specific antigens found on the cell surface, such as transmembrane receptors or extracellularly, such as growth factors. In some cases, monoclonal antibodies are conjugated to radio-isotopes or toxins to allow delivery of these cytotoxic agents specifically to the intended cancer cell target.

Small molecules can penetrate the cell membrane to interact with targets inside a cell, typically a cell signalling cascade proteins. Small molecules are usually designed to interfere with the enzymatic activity of the target protein.

As with any drug, targeted cancer therapies typically have several different names. One or more is used to designate the chemical compound during development; afterwards the drug receives a generic name and then a brand name used by the pharmaceutical company for marketing. E.g. the small molecule STI-571, known as imatinib (generic name) is marketed by Novartis under the brand name Gleevec™.

The generic name of a targeted agent provides clues to the type of agent and its cellular target:
- small molecules end with the stem "-ib" (indicating protein inhibitory properties)
- monoclonal antibodies end with the stem "-mab" (monoclonal antibody) and have an additional substem designating the source of the antibody, e.g "-ximab" for chimeric human-mouse antibodies, "-zumab" for humanized mouse antibodies, and "-mumab" for fully human antibodies.

Both monoclonal antibodies and small molecules contain an additional stem in the middle of the name describing their target; e.g. for monoclonal antibodies include "-ci-" for a circulatory system target and "-tu-" for a tumour target, or for small molecules include "-tin-" for tyrosine kinase inhibitors and "-zom-" for proteasome inhibitors. At the beginning of the generic name there is a unique prefix for each agent.

Examples:
bevacizumab = humanized monoclonal antibody with a circulatory system target (VEGF-A)
cetuximab = chimeric monoclonal antibody with a tumour target (EGFR)
ipilimumab = fully human antibody with an immune system target (CTLA-4)
bortezomib = small molecule proteasome inhibitor
imatinib = small molecule tyrosine kinase inhibitor
seliciclib = small molecule cyclin-dependent kinase inhibitor

There are many important targeted therapies in the clinical use, to present some examples:
Rituximab - a chimeric mouse-human anti-CD20 antibody destroys B cells (CD20 is primarily found on their surface) and is therefore used to treat diseases which are characterized by overactive, dysfunctional, or excessive numbers of B cells. This includes many lymphomas, leukaemia, autoimmune disorders and transplant rejections.
Monoclonal antibodies cetuximab and panitumumab are epidermal growth factor receptor (EGFR) inhibitors used for treatment of metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer in cases where downstream signalling protein of the EGFR pathway called K-RAS is not constitutively activated by mutation (otherwise blocking of the receptor above would not be effective).

Vemurafenib (**V**600**E** mutated B**RAF** inhibitor) causes programmed cell death in melanoma by interrupting the B-Raf/MEK step on the B-Raf/MEK/ERK pathway − if the B-Raf has the V600E or V600K mutation (about 60% of melanomas do). Melanoma cells without these mutations are not inhibited; vemurafenib paradoxically stimulates normal BRAF and may promote tumour growth in such cases!

Ipilimumab is a new agent in melanoma therapy for enhancing the immune response by targeting CTLA-4, a protein receptor that downregulates the immune system.
CML – chronic myelogenous leukaemia is best treated with tyrosine-kinase-targeted inhibition. The first of this new class of drugs was imatinib mesylate, approved by FDA in 2001. Since then, other drugs, e.g. dasatinib, nilotinib, radotinib and bosutinib have been introduced in practice.
EML4-ALK translocation (predictive marker), which is present in 3-5% of NSCLC (non-small cell lung cancer), leads to formation of a fusion gene coding for an abnormal tyrosine kinase receptor (target) which exaggerated activity can be inhibited by crizotinib or alectinib. There is a list of new drugs being tested.

About 25% of breast cancer cases are associated with an amplification of genes coding for a cell surface receptor called HER2/neu (receptor tyrosine-protein kinase erbB-2, also known as CD340 (cluster of differentiation 340) or proto-oncogene Neu). HER2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family. Amplification or over-expression (predictive marker) of this oncogene plays an important role in the development and progression of aggressive types of breast cancer. These patients have poor prognosis, however, the tumours can be targeted by trastuzumab (Herceptin), monoclonal humanized antibody against overexpressed (target) surface HER2 receptor.

Side effects of targeted cancer therapy drugs

Although targeted therapy drugs don't affect the patients the same way that standard chemotherapy drugs do, they still cause various side effects. As there are many different types of targeted drugs, the side effects from these drugs depend largely on what each drug targets. The side effects have also variable incidence and severity.

Because many targeted drugs are still quite new, it's hard to say how long you can expect side effects to last. We do know that some of the side effects from standard chemo drugs can last a lifetime, such as when the drug causes long-term damage to the heart, lungs, kidneys, or reproductive organs. In many cases we still don't know if targeted therapy drugs cause these kinds of long-term changes.
Common and serious side effects of targeted drugs are: hypertension, bleeding or blood clotting problems, bruising and bleeding (namely with anti-angiogenic drugs). Bleeding, such as from the stomach and intestines, can be severe and even life threatening. Some drugs can also cause blood clots in the lungs and legs, as well as heart attacks and strokes, skin rash, hives, slow wound healing, gastrointestinal perforations, heart damage, autoimmune reactions, swelling, nausea and vomiting, diarrhoea or constipation, mouth sores, shortness of breath or trouble breathing or cough, dizziness, fatigue, headache, hair loss, damage to organs such as the thyroid gland, liver, or kidneys, allergic reactions, increased risks of certain infections and secondary cancers!

Recommended literature:

My Cancer Genome website by Vanderbilt University (https://www.mycancergenome.org/content/)
ClinicalTrials.gov - a service of the U.S. National Institutes of Health, provides a registry and results database of publicly and privately supported clinical studies of human participants conducted around the world (www.clinicaltrials.gov)
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Therapeutic approaches to different cancer diseases

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Possibilities of treatment of cancer diseases are just in tendency of spreading. Despite of bulky investigations stratification of importance of different approach was not substantilal changed. Surgery is the best choice up to date for treatment of solid tumors. Irradiation as treatment's possibility was reestablished in recent twenty years. Chemotherapeutics are most common in use with many mechanisms of action, but pharmacological classification was not substantial changed for the long time. Many new chemotherapeutic drugs were synthesized in recent years, but patient's survival was not so much changed. In treatment of Non Small Lung Cancer (NSLC) survival was pronged for 3-4 mounts in past twenty years. It's necessary to establish new approach for lung cancer. Up to date discovered therapies are unsuccessful to be well strategy for the future. In calculation of cost-benefit no substantial improvement of general clinical status, or general improvement in the commonest cancers.

Widespread application of testing for chromosomal mutation, using PCR method was clarified the most important possible mechanism of tumor cells promotion. Many other dilemmas are resolved for the process of decision making for the of chemotherapy of cancer. Using PCR method it is possible to prognosis efficacy of chemotherapy for different cancers. So, many chemotherapeutic agents, now used for some cancer, proved ineffective. Guidelines for cancer chemotherapy quite accurately suggest the effectiveness of chemotherapy after chromosomal analysis by PCR technique. But guidelines are not literally administered in all countries. Therefore, a lot of money was invested in ineffective chemotherapy, but also a variety of side effects of these drugs are present.

The chemotherapeutic drugs can be divided as follows: alkylation agents, antimetabolites, mitotic inhibitors, antibiotics, hormones and hormonal antagonist and enzymes and other antitumor agents. All of these drugs affect cell division or DNA synthesis and function in some way.

Alkylating agents, or their reactive intermediates, form covalent bonds with deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein to form an adduct in which a methyl or ethyl group is added. Some of the drugs of this group: (Busulfan (Myleran), Carboplatin (Paraplatin), Chlorambucil, Cisplatin, Cyclophosphamide (Cytoxan), Dacarbazine (DTIC-Dome), Estramustine Phosphate, Ifosphamide, Mechlorethamine (Nitrogen Mustard), Melphalan (Phenylalanine Mustard), Procarbazine, Thiotepa, Uracil Mustard.

Antimetobolites alter the synthesis of DNA or RNA. Antimetabolites that are structural analogues of nucleotides are incorporated into cell components as if they were the essential pyrimidine or purine, and as a consequence, disrupt the synthesis of nucleic acids. (Molecular mimicries). Other antimetabolites disrupt essential enzymatic processes of metabolism like the folate antagonist, 5-fluorouracil, which disrupts vital folic acid metabolism. Some of antimetabolites: Cladribine, Cytarabine (Cytosine), metotrexsate, 5-fluorouracil, mercaptopurin.

Mitotic inhibitors (Mitotic spindle inhibitors) bind to microtubular proteins and block their ability to polymerize or depolymerize, a process which halts nuclear division, but without impairment of DNA synthesis. DNA topoisomerase II inhibitors block religation of double strand DNA breaks (i.e., sister chromatid separation or cleaved DNA). Etoposide (VP-16), Teniposide (VM-26m Vumon), Vinblastine, Vincristine, Vindesine are the examples of mitotic inhibitors.

Antitumor antibiotics interfere between DNA base pairs and disturb the synthesis or function of nucleic acids. Some drugs: Aclarubicin, Bleomycin, Dactinomycin (Actinomycin D), Daunorubicin, Doxorubicin (Adriamycin), Epirubicin, Idarubicin, Mitomycin C, Mitoxantrone, Plicamycin (Mithramycin).

Hormones or hormone-blocking agents either exert a corticosteroid effect, such as prednisone, or manipulate the hormone environment in hormone-responsive tumors. The antiandrogenic agent, flutamide, which is used to treat prostate cancer, is believed to block androgen receptor sites. The antiestrogenic agent, tamoxifen, binds to intracellular estrogen receptors, then enters the nucleus where the tamoxifen-estrogen-receptor complex inhibits DNA and protein synthesis. Some of the drugs: Equine Estrogen (Premarin), Cortisone, Chlorotriansene , Dexamethasone , Diethylstilbestrol Ethinyl Estradiol, Fluoxymesterone, Hydroxyprogesterone Caproate, Medroxyprogesterone Acetate (Provera), Megestrol Acetate (Megace), Prednisone, Tamoxifen (Nolvadex), Testosterone.

Anti tyrosine kinases ((TK) was introduced after discovery of monoclonal antibody, and thereafter start era of ”anti TK” as targets for cancer therapy. The landscape was changed radically by the success of imatinib mesylate, an inhibitor of the BCR-ABL TK in chronic myeloid leukemia (CML) — a result heralded as a proof-of-principle and a triumph of targeted cancer therapy. TKs are now regarded as excellent targets for cancer chemotherapy, but reality lies somewhere between the extremes of triumph and tribulation.

Therapy against tumor stroma and vascularisation. EGFR is activated by binding specific ligands, including epidermal growth factor and transforming growth factor-α, endothelial vascular growth factor (EVGF). Activation of EGFR promotes cell proliferation and survival, as well as angiogenesis, leading to tumor growth and metastasis. Anti-hEGFR-hIgG4 (S228P) features the constant region of the human IgG4 (S228P) isotype and the variable region of cetuximab. Cetuximab is a chimeric human/mouse IgG1 monoclonal antibody that targets epidermal growth factor receptor (EGFR), a cell surface receptor over expressed in many types of cancer. VEGF overexpression in tumors is associated with increased angiogenesis, proliferation and metastasis. Phosphorylated VEGFR-2 (KDR) expression in numerous solid tumors including three lung carcinomas, three breast carcinomas, Non Hodgkin's lymphomas, and melanoma. Therapy against tumor stroma and vascularisation is oriented against primary against interleukins.

Induction of higher activity of interleukins is just opposite approach than previous one. Application of bacillus Calmette-Guérin (BCG) is introduced in antitumor therapy thirty years ago, and reestablished in some recent years. The goal of therapy is to stimulate production of Tumor necrosis factor (TNF), and interleukine-2 (IL-2) with idea to slow-down tumor growth. Actually this method is used mostly in treatment of urinary bladder tumors.

Different approachs were in use for antitumor therapy. There some substantial success in this treatment, like hormone-sensitive tumors, some type of leukemia, etc. But chemotherapy is to be revised in essential postulate if the good perspective of this therapy could be reestablished.

Clinical Pharmacy Services in Oncology

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Clinical pharmacy includes all services performed by pharmacists practising in clinics and hospitals, community pharmacies, community health centres, nursing homes and other settings where medicines are prescribed and used. Its main mission is to move the focus of attention from the drug to the single patient or population receiving drugs.

Clinical pharmacists are following the overall goal of clinical pharmacy activities, i.e., to promote the correct and appropriate use of medicinal products and devices. These activities aim to:

- maximize the clinical effect of medicines, i.e., using the most effective treatment for each individual patient,
- minimize the risk of treatment-induced adverse events, i.e., monitoring the therapy course and the patient's compliance with therapy, and
- minimize the expenditures for drug treatments, i.e., trying to provide the best treatment alternative accompanied by the cost-effective use of drugs for the greatest number of patients.

In this contribution two places of good clinical pharmacy services in oncology are presented, i.e. University Clinic Golnik and Institute of Oncology Ljubljana.

First, clinical pharmacists at **the University Clinic Golnik** are integrated in the treatment of patients at higher risks for adverse drug reactions (those with decreased renal function, prescribed with strong inhibitors or inducers of drug enzymes and drug transporters, prescribed with strong opioids, prescribed with drugs where TDM is performed and prescribed medicines with a feeding tube), oncology patients, patients with tuberculosis and patients with hereditary angioedema. Moreover, clinical pharmacists successfully implemented on a full scale a medication reconciliation model for all oncological patients admitted to the clinic. As anti-cancer drugs belong to a group of high-risk medications, in guaranteeing the quality, safety and efficacy of therapy with these drugs pharmacists' role should not be restricted to dispensing only.

A study has been designed at the University Clinic Golnik to describe the implementation of a new pharmacy service – chemotherapy prescription screening – and evaluate its benefits by analysing the recorded interventions. The study was conducted immediately after the centralized chemotherapy preparation was introduced and chemotherapy prescription screening implemented. The performed interventions were recorded over a five-month period in 2009. The results showed that chemotherapy prescription screening by pharmacists was integrated successfully into routine clinical practice and pharmacists' responsibilities and duties were defined. During the study period, 506 cancer prescriptions were reviewed by five pharmacists, who recorded 211 interventions. The high rate of interventions emphasizes the role that pharmacists play in oncology services. The interventions involved cancer drugs (31%), antiemetics (41%) and other support care drugs (12%). The identified problems were related to the following categories: "dose, frequency and regimen" (64%), "drug selection" (21%) and "administrative" (14%). Clinicians accepted most pharmacists' recommendations (76%), thereby confirming the need for the proposed interventions also from a medical point of view. However, the study provides no information on the clinical significance of the

recorded interventions, whether accepted or not. It turned out at the end that the high rate of interventions recorded in this study stresses the importance of integrating pharmacists' clinical roles into oncology services in order to maintain high quality standards of cancer treatment.

Another study has been performed at the University Clinic Golnik to show the importance of drug interactions for patient safety as cancer patients are prescribed numerous medications. The study was designed as a retrospective study and reviewed drug interactions in patients, where anticancer drug therapy was initiated in the year 2012. As part of routine clinical practice, in all patients, drug interactions were reviewed by a pharmacist, who discussed those judges to be clinically important with the patient's oncologist. As part of the study, drug interactions were reassessed using three different drug interaction databases (Lexi-comp, Stockley's Drug Interaction, Drugs.com) to record all possible interventions. Only drug interactions between drugs in systemic cancer therapy (including anticancer drugs and support care drugs) and prescription drugs for comorbidities were evaluated. Overall, the study included 223 lung cancer patients. Most patients were older (median 63 years), were taking a median of 4 drugs for treatment of comorbidities, and were prescribed a median of 6 drugs for cancer treatment. Review of drug interaction databases revealed 1416 drug interactions between drugs in systemic cancer therapy and other drugs, only 18 % of detected interventions involved anticancer drugs and only 19 % would affect the outcomes of anticancer treatment; the overwhelming number of possible drug interactions emphasises the importance of identifying clinically relevant drug interactions. The need for the critical appraisal of possible drug interactions is further evidenced by the low number of interactions (52/1416; 4 %), judged by the pharmacist as clinical important. Pharmacists more often identified as important interactions involving anticancer drugs (85 %) and those affecting the outcomes of anticancer therapy (79 %). To prevent the manifestation of drug interactions, pharmacists most often suggested a change in the treatment of comorbid conditions. To conclude, in the treatment with high risk drugs, all efforts should be invested to prevent adverse drug events and avoiding drug interactions falls within this aim. Review of drug interactions at initiation of anticancer drug treatment was successfully implemented into routine clinical practice. The study revealed a large number of possible drug interactions, showing the need for their critical appraisal in order to identify and prevent clinically relevant drug interactions.

Second, clinical pharmacists at **the Institute of Oncology Ljubljana** are involved occasionally in the treatment of patients at surgical ward. They are expected to check oncologic, supportive and chronic drug therapy and propose appropriate solutions. A clinical case of a patient who developed symptomatic urinary tract infection caused by yeasts after surgery due to colorectal carcinoma and relapse of the disease in abdomen is presented. Anidulafungin (a member of echinocandins) was indicated on the basis of microbiological review, however, its pharmacokinetics is not favourable as it is excreted in urine in less than 1%. As a result, symptoms of urinary tract infection persisted and treatment was not efficient. There were two options available: amphotericin B and fluconazole. Clinical pharmacist complained against the use of amphotericin B due to nephrotoxicity (patient had chronic kidney injury), high cost (1300 €/day) and better treatment alternatives. Understanding pharmacokinetics of fluconazole, one can see that the most of the drug is excreted in urine, fluconazole is concentrated in the urine – yielding urine levels > 100 μg/mL, the expected concentrations in urine exceeded the MIC not only for susceptible yeasts (MIC≤ 8 μg/mL), but also for organisms that are susceptible but dose-dependent (MIC 16-32 μg/mL) and sometimes even those that are resistant (MIC ≥ 64 μg/mL). Clinicians accepted pharmacist's recommendation, after initiation of fluconazole 400 mg/day, symptoms and signs of urinary tract infection improved, treatment was continued for 16 days.

Immunotherapy of cancer

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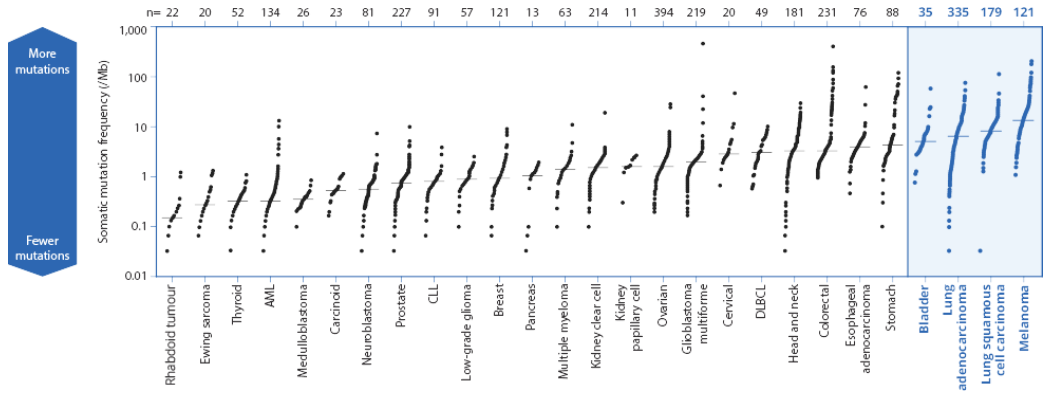
Immune system is involved in pathogenesis, maintenance and progression of cancer and therefore represents an important therapeutic target. Like cancer itself, the immune system is extremely complex. It reacts differently, according to (patho)-physiological conditions within the body. The key factors needed for induction of specific anti-tumor effector T cells are fully functional immune system and specific tumor (TA) and/or tumor-associated antigens (TAA) which may be more or less immunogenic and are often subjected to mutations, due to high genetic instability and deficient mismatched DNA repair in tumor cells. However, tumors, via numerous mechanisms, are able to establish microenvironments that actively protect them against specific anti-tumor immune responses.

In general tumors can be divided in those that are highly (melanoma), intermediately (prostate cancer) or weakly immunogenic (thyroid cancer), which corresponds to the degree of their heterogeneic mutational potential (1).



Picture1: *Somatic mutation frequencies detected in exomes from 3.083 tumor - normal pairs (1).*

Seen from another perspective, some tumors are considered to be inflammed and therefore more prone to immunological intervention, while others are not. Therefore microenvironment, especially of the latter should be first modulated to provide proinflammatory conditions in order to enhance the existing specific antitumor immune responses and to allow the adoptively transferred anti-tumor immune effector cells to be efficient (2, 3).

There are numerous factors and mechanisms that tumor cells are exploiting for prevention of anti-tumor immunity, for example, they (4, 5):

- can lose the capability to express certain major histocompatibility (MHC) molecules or even a whole MHC haplotype;
- lack costimulatory molecules (CD80, CD86, and others);
- produce immunosuppressive (anti-inflammatory) cytokines (VEGF, TGF-β, IL-10, IL-4), chemokine CCL22, which attracks and cumulates regulatory T cells (Treg), and indolamine-2,3-dioxygenase (IDO);
- are additionally protected against the immune system by various types of immunosuppresive cells:
  - besides Treg, also other $CD4^+$ (Tr1, Th3) and $CD8^+$ (Ts) T cells;
  - tumor-associated macrophages (TAMs or M2) and tolerogenic dendritic cells (DC2);
  - myeloid-derived suppressor cells (MDSCs) and even disregulated NK cells.

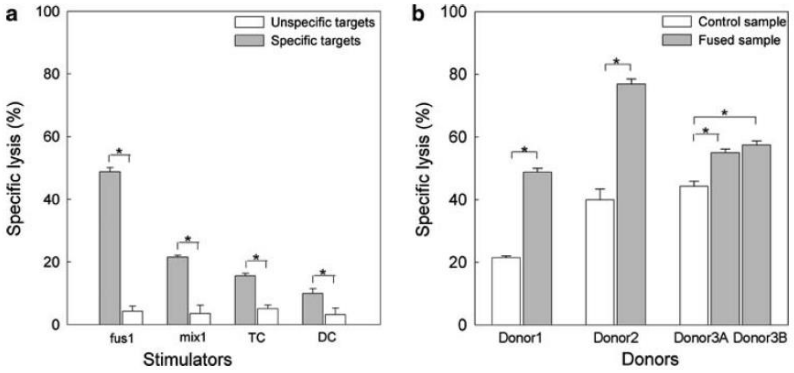
In order to change this tumor protective microenvironment and to improve the effects of immunotherapeutic cellular drugs, two main approaches are used, i.e. the non-myeloablative pre-conditioning and the immune checkpoint blockade. With the first one, by applying selected chemotherapeutic drugs (e.g. fludarabine, cyclophosphamide) and γ-irradiation, the numbers of immunosuppressive lymphocytes, MDSCs, TAMs and DC2 are greatly reduced. Consequently more favourable conditions are re-installed that enable maturation of effector DCs (DC1) and thereby effective activation of anti-tumor T cell clones with TA/TAA, as well as higher production of proinflammatory cytokines. Additionally, irradiation induces apoptosis of TCs which are better targets for specific anti-tumor effector $CD8^+$ cytotoxic T cells (CTLs) (5). On the other hand, for the immune checkpoint blocking, specific antibodies that prevent negative, immunosuppressing co-stimulatory receptor-ligand interactions, are used, e.g. anti-CTLA-4, anti-PD1 in case of T cells, and for example anti-KIR, anti-NKG2A in case of NK cells (3, 6, 7).

Different types of cellular anti-cancer immunotherapies enable personal, patient-specific treatments, and are based on:

- *ex vivo* selection and expansion of anti-tumor $CD8^+$ T lymphocyte clones (CTLs), isolated from tumor biopsies (TILs – tumor infiltrating lymphocytes) of patients and their subsequent adoptive transfer back to them, together with high dose human recombinant IL-2 (autologous adoptive immunotherapy) (8);
- *in vitro* preparation of TA/TAA-specific effector $CD8^+$ CTLs, using autologous DCs or artificial antigen presenting cells (APCs), and their subsequent adoptive transfer, together with high dose human recombinant IL-2 (autologous adoptive immunotherapy);
- *in vitro* preparation of anti-tumor cellular vaccines based on autologous peripheral blolod monocyte-derived DCs, loaded with TA/TAA;
- *ex vivo* isolation, expansion and activation of patient's autologous NK cells and their subsequent adoptive transfer back to him/her (7).

Adoptive immunotherapy with autologous TILs has proved to be effective especialy in non-myeloablatively pre-conditioned metastatic melanoma patients (8, 9).
The first ever approved (FDA) autologous cellular immunotherapy drug Sipuleucel-T for treating castrate-resistant prostate cancer, is based on the immunostimulatory capacities of DCs, presentig prostate acid phospatase (PAP) antigens, enhanced by granulocyte and monocyte colonies stimulating factor (GM-CSF) (10).

We have optimized two methods for providing TA/TAA to autologous DCs, i.e. their transfection with native or amplified total tumor RNA and their electrofusion with lethaly irradiated TCs (preparation of immunohybridomas) (11, 12). Immunohybridomas are wery efficient in presenting a large array of TA/TAA within the context of patient's MHC molecules and are able to evoke strong anti-tumor $CD8^+$ CTL responses *in vitro* (Picture 2). Currently we are performing a clinical study using autologous immunohybridomas for treating patients with castrate-resistant prostate cancer.



Picture 2: ***a*** *and* ***b*** *– electrofused immunohybridomas (grey columns) evoke statistically more potent anti-tumor-specific cytotoxic immune responses than mixed DCs and TCs (12).*

Currently, also other types of anti-cancer vaccinations are being evaluated, for example the Prostvac VF vaccine, using viral vectors packed with genes coding for specific TA (PSA - prostate specific antigen) and several costimulatory molecules, as well as T cells bearing TA/TAA-specific chimeric antigen receptors (CARs) (13, 14, 15).

It is expected that in the future cell-based immunotherapies will be used early in the onset of malignant diseases and will be applied together with combinations of different classical (cytotoxic drugs, irradiation) and new (immune checkpoint blockade) anti-cancer therapies.

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Life science research from a postdoc prospective

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Discovering new drugs, treatment possibilities, developing new methods or new models in various disorders that would benefit lives of the people all round the world is a dream every researcher in life science dreams off. Many groundbreaking advances in life science of the 20th and 21st century such as discovery of the penicillin, DNA helix and polymerase chain reaction as the basis for molecular biology and later on recombinant technology, have completely changed the way that the modern world lives and thrives. Life science is a broad field of diverse sciences that share researching live species, either humans (medicine) or animals (biology) with the ultimate aim to improve quality and standards of their lives. Since the discoveries in biology, i.e. basic science or preclinical studies aim to translate to humans, both branches intercross and the term biomedicine is used most of the time. When it comes to funding and money consumption, there is only space science that requires more resources and financial support than the life science. In comparison to a massive space science project such as generating international space station that costed 150 billion USA dollars, 5 billion every year are going into cancer research according to USA National cancer research institute (1). This should not be of any surprise as the highest levels of ethics (bioethics) and standards are required when researching live organisms in order to protect more lives. Given the fact that millions of people in developed countries die of cancer, cancer research is the most funded area in life science with billions of funding every year, most frequently split 50, 30 and 20% into basic, translational and clinical research pipelines (2).

United Kingdom (UK) has one of the strongest and most dynamic life science industries in the world with the annual turnover of 56 billion pounds (3). There is a solid and clear support from the government stating that life sciences is a priority sector for the UK economy with 2 billion pounds in public investment in health life science research via the Research Councils and National Institute for Health Research Programme. The final piece of the puzzle is the very supportive charitable sector consisting of over 130 medical research charities funding a third of all publicly funded research (4).The UK public rank top in the G7 (and fourth in the world) for charitable donations (4).The largest single non-government, non-business source of funds in the UK is the Wellcome Trust, which was established from a bequest by the co- founder of Wellcome, one of the first pharmaceutical businesses in the UK later being sold to Glaxo (5). Cancer Research UK is another importer supporter of research with an income of 621 million pounds in 2014, spending 393 million on research activities, funding over 4,000 scientists in the UK. UK Arthritis research, the fourth largest medical charity having budget of around 37 million pounds, presents the main charity funding musculoskeletal disorders research (6).

There comes a time in life of every life science researcher when you want to progress from either observational to functional studies or switching from vitro to in vivo experiments that can only be achieved in preclinical research using animal models. It might also be the case, in particularly if you are only part-time researcher, of striving for better research possibilities, such as protected time to do research only. However, most of the time it is just the matter of finding a job after finishing PhD in life science. The most common way to boost up your career as a life scientist is applying for a postdoctoral research position on a specific life science project. Having been given the chance to work as a life science researcher, there is an enormous potential to develop new skills, learn new methods and put your own ideas to life. On the other hand, life science is highly demanding, time-consuming and most of the times unrewarding, hence it requires strong motivation and perseverance. Creating cutting-edge technologies and producing high impact results are outcomes every postdoc endeavors for in order to apply for your own grants and funding that are becoming more and more competitive in the life science.

In the current talk my experience working as a UK Arthritis research postdoctoral research fellow at the Institute of medical Sciences University of Aberdeen, Scotland will be presented with the aim to stimulate young researchers eager enough to be challenged in a competitive field of life science.

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Luminal A and luminal B type of breast cancer

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Introduction

Cancer is one of the leading medical conditions in the western world, among which breast cancer has one of the highest percentage of occurrence [1, 2]. There are many subtypes of breast cancer: basal-like, Claudin-low, human epidermal growth factor 2 (HER2) overexpressing, Luminal A and Luminal B [1, 2]. The luminal A breast cancer is the most common subtype, representing 50–60% of all the subtypes [1, 2]. It is characterized by the expression of genes activated by the estrogen receptor (ER) transcription factor that are typically expressed in the luminal epithelium lining the mammary ducts [1, 2]. In addition, what is typical for luminal A is also the expression of progesterone receptor (PGR) and an absence of HER2 expression [3]. Moreover, the GATA binding protein 3 (GATA3) marker expresses its highest level in the luminal A subgroup [3]. GATA-3 belongs to the GATA family of transcription factors. It regulates luminal epithelial cell differentiation in the mammary gland [4]. GATA-3 is one of the three genes mutated in >10% of breast cancers [5].

Tumors with the luminal B molecular profile make up between 10% and 20% of all breast cancers [1, 2]. Compared to the luminal A they have a more aggressive phenotype, higher histological grade and proliferative index [3]. Usually, patients with luminal B type of breast cancer also have worse prognosis then patients with luminal A type [3]. The main biological difference between the subtypes A and B is an increased expression of proliferation genes in the luminal B subtype such as *MKI67* and cyclin B1, as well as overexpression of HER2 [6,7].

Molecular basis

ER signaling pathway is a complex biological pathway that controls a variety of functions such as cell proliferation, apoptosis, and angiogenesis which serve as a major survival pathway exploited by breast cancer cells [8]. ER modulates the expression of hundreds of genes, some by upregulation of expression and others by downregulation [8]. Upon estrogen binding, ER dimerizes with another receptor monomer and attracts a complex of co-activators and co-repressors to specific sites on DNA, called estrogen receptor elements (EREs). Consequence of the cascade is induction or modulation of gene transcription including coding growth factors (GFs) and receptor tyrosine kinases (RTKs) [9, 10, 11]. ER can also bind to other transcription factors such as activator protein 1 (AP-1) and specificity protein (SP-1) at their specific sites on DNA, thereby functioning as a co-regulator [10, 12]. ER may also work by non-transcriptional mechanisms. A small subset of the cellular pool of ER localized outside the nucleus and/or at the cell membrane associates in response to estrogen with growth factor RTKs and with additional signaling and co-activator molecules. This interaction activates multiple downstream kinase pathways which in turn phosphorylate various transcription factors (TFs) and co-regulators, including components of the ER pathway that enhance gene expression on EREs and other response elements (RE) [9]. The stress kinase pathway via p38 and c-Jun N-terminal kinases (JNK) can also modulate ER function by phosporilation of ER and its co-regulators [13,14]. The microenvironment and its associated integrin signaling may exert a similar activity [15]. The ER signaling pathway is also regulated by membrane receptor tyrosine kinases, which activate signaling pathways that eventually result in phosphorylation of ER as well as its co-activators and co-repressors influencing their specific function [16-19].

Estrogens could cause *de novo* breast cancer trough either receptor dependent or independent mechanisms [20]. The most widely accepted theory holds that estradiol (E2), acting through estrogen

receptor alpha (ERα), stimulates cell proliferation and initiates mutations arising from replicative errors occurring during pre-mitotic DNA synthesis. The promotional effects of E2 then support the growth of cells harboring mutations. Over a period of time, sufficient numbers of mutations accumulate to induce neoplastic transformation. Laboratory and epidemiological data also suggest that non-receptor mediated mechanisms resulting from the genotoxic effects of estrogen metabolites are involved in breast cancer development [21]. In addition, estrogen metabolites act as possible modulators of stem cell functionality and cancer progression [21].

Diagnosis

Luminal A subtype has been defined as ER+/PR+/HER2- [22]. Fewer than 15 % of luminal A tumors have p53 gene mutations [23, 24]. Detection of ER and HER2 is immunohistochemical, but the results are checked and compared with fluorescence in situ hybridization (FISH) [25]. Luminal A and B both express ER, but, since luminal B prognosis is worse, a strong effort to find biomarkers that distinguish between these two subtypes has been made [26]. One of the candidate markers for differencing between these two subtypes of breast cancer is Ki67 protein that may be necessary for cellular proliferation [22, 27]. The Luminal A subtype has been defined as ER+/PR+/HER2- and low level of Ki67, while the luminal B subtype has tumors with ER+/PR+/HER2- and high Ki67 or ER+/PR+/HER2+ [22]. One should bear in mind that this definition does not include all luminal B subtype tumors (up to 6% of the luminal B tumors are clinically ER-/HER2-). Also, technique used to determine Ki67 (cut-off point to distinguish luminal A and B set at 13.25%) has not been standardized which adds a variability factor in the assessment of this marker [22].

Therapy

Patients with luminal A subtype of cancer have a good prognosis; the relapse rate is 27.8% being significantly lower than that for other subtypes [3]. They have a distinct pattern of recurrence with a higher incidence of bone metastases (18.7%) and with respect to other localizations such as central nervous system, liver and lung which represent less than 10%. The treatment of this subgroup of breast cancer is mainly based on third-generation hormonal aromatase inhibitors (AI) in postmenopausal patients, selective estrogen receptor modulators (SERMs) like tamoxifen and pure selective regulators of ER like fulvestrant [28]. Luminal B also has a chance of relapse, but the pattern differs of that for Luminal A. It is still a tumor with relatively good prognosis in general, but much worse than Luminal A subtype [6, 7]. But on the bright side they respond better to neoadjuvant chemotherapy achieving pathological complete response (pCR) in 17% of the luminal B tumors (7% in luminal A).

For these reasons, treatment of this subtype of breast cancer is currently challenging. Many questions about what mechanisms lead to their survival, proliferation and metastatization remain unanswered. Numerous clinical trials are testing inhibitory molecules of the PI3K/AKT/mTOR pathway at different levels, focusing on the treatment of luminal B tumors [26].

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HER2 positive type of breast cancer

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Introduction

The most common type of cancer in women is breast cancer. Prevalence of this type of cancer is growing highly worldwide from 0.0193% women in region of Eastern Africa to 0.0897% women in the region of Western Europe (1). It is well known that there are a significantly higher percentage of black women that have this malignant disease in comparison to white women (2). It is estimated that HER2 is amplified at about 20-30% cases of breast cancers. Types of breast cancers which are HER2 positive include Ductual Carcinoma in situ (DCIS) and also at Inflammatory Breast Cancer (IBC) and Paget disease of the nipple (3). Survival rates are different worldwide ranging from 80% and above in Sweden, Japan and North America to 60% in middle-developed countries and below 40% in low-developed countries. Low survival rate in these countries is mainly consequence of poor chance for early detection, as well as lack of right diagnostic procedure and treatment conditions (1).

Molecular basis

The human epidermal growth factor receptor 2 oncogene (HER2) is protein that encodes the type 1 receptor tyrosine kinase (4). Activation of HER2 leads to proliferation, migration and invasion in breast cancer (5). HER2 is receptor-like tyrosine kinase, also a member of the membrane-spanning type I. This family of receptors dimerize after being stimulated by ligand which leads to subsequent autphosphorylation and recruitment of many different downstream signaling cascades. Hyper-activated signaling trough this pathway results with dysregulation of the cell cycle homeostatic system. This can lead to dysregulated growth, oncogenesis, metastasis and potential chemorestistance (3).

Mechanism that includes tumorigenic ability of HER2 is related to high basal autophosphorylation activity of this receptor-like tirosin kinase. In process of overexpression at the surface of the cell homodimers may be spontaneously formed, and may increase its availability for heterodmiers formation when a ligand binds to its direct receptor (3).
HER2 is significantly enhanced when it is co-expressed with ErbB1 and ErbB3. Overall overexpressed HER2 can promote carcinogenesis primarily in the context of a ligand driven heterodimer and also interacts with MAPK and PI3K pathways. MAPK signaling is being prolonged in situation of HER2 overexpression (3). One of the co-receptors that seem to have a pivotal role in HER2 signaling is ErbB1. Its overexpression correlates inversely with ER status and also correlates with poor prognosis of treatment (3). ErbB3 and ErbB4 have positive correlation with ER status and increased survival prognosis (3). Cyclin D plays a pivotal role in process of breast cancer where in process of HER2 overexpression results with upregulation of cyclin D protein expression and that upregulation involves SP1 and E2F transcription factors (3).

Diagnose

Right diagnostic procedure of human HER2 status is crucial for obtaining wanted breast cancer outcome. Tumors that have HER2 overexpression are labeled as HER2 positive while those with normal HER2 levels are labeled as HER2 normal or HER2 negative (4).
HER2 positive tumors have higher mortality prevalence in early-stage disease, reduced relapse time and increased indices of metastases (4). The most commonly used technique to detect HER2 status is immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). IHC assesses the expression of HER2 protein in cell membranes while FISH method assesses HER2 gene amplification (6). There

are many commercially available testing units approved by FDA. They included IHC tests, FISH assays and colorimetric ISH assays.
Interpretation of a immunohistochemistry test is based on a 0, 1+, 2+, 3+ scoring system. Results that demonstrate strong complete membrane staining in > 10% of tumor cells are classified as 3+. Specimens which show weak-to-moderate staining in >10% of tumor cells are scored 2+ and are labeled as equivocal and require confirmation of HER2 status by alternative method, usually ISH. Specificity is reported at level of 100%, sensitivity of 70% and accuracy of 85-89 %. (8)
HER2 FISH testing was approved based on the high rate of concordance reported between the IHC CTA and FISH assays of 82% to 92% (9). These two methods are considered as gold standard in this area of interest. FISH method is more accurate, reproducible and robust compared to IHC. Generally IHC is widely used as the primary test for HER2 status. IHC is comparatively quick while results can be viewed using a conventional microscope and strained tissue do not degrade over time. IHC also allows parallel viewing of tumor cell morphological features (4). CISH and silver-enhanced in situ hybridization (SISH) are new technologies that are being developed. CISH uses a peroxidase enzyme-labeled probe for chromogenic detection by diaminobenzidine, while SISH uses the same system with a silver-based detection system (4).

Therapy
Generally, woman diagnosed with breast cancer are being treated with adjuvant systemic therapies to reduce the risk of recurrence. (10) The main focus in the treatment of HER2-positive breast cancer in recent years has been based on developing therapeutic agents to either potentiate the effect of trastuzumab or target cells which have become resistant to trastuzumab. Preclinical evidence suggest that co-inhibition of HER2 as well as other members of the HER family and/or the downstream pathway by giving trastuzumab/lapatinib in combination with other targeted therapies might prevent or prolong time to resistance and treatment failure (11). Trastzumab is a recombinant, humanized monoclonal antibody which effects through binding to the extracellular domain IV of HER2. It is combined usually with lapatinib who is reversible dual HER1/EGFR and HER2 tyrosine kinase inhibitor (TKI). Trastazumab can also be combined with pertzumab. Pertzumab is fully humanized antibody binding to domain II of HER2 (12). Antibodies conjugated to toxins, radionucleotides and prodrugs are showing promising results in animal models (3). In the recent time, the outcome for patients with HER2- positive breast cancer has improved significantly. Clinical guidelines recommend HER2-directed therapies as backbone therapy for these patients. Otherwise, resistance to HER2-directed therapies remains a challenge.
Evidence suggests that combinations of HER2-directed agents may show additive or synergistic effect and lead to better outcome (12). However, target populations for specific HER2-directed therapies remain to be defined. Also the ongoing concern regarding side effects especially cardiotoxicity needs to be addressed. There is an urgent need for prospective biomarker-driven trials to identify patients for whom dual targeting is cost-effective (12).

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Basal-like type of breast cancer

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Introduction

Basal-like breast cancer (BLBC) is one of the five major breast cancer intrinsic subtypes based on gene expression profiling [1, 2]. It was named so because the cancer cells consistently express genes that are expressed in the normal basal/myoepithelial breast cells [3]. It is a particularly aggressive subtype and is associated with larger tumour size and more rapid tumour growth. Patients with these tumours often relapse rapidly [4]. Basal-like breast cancers account for 15-25% of all breast cancer cases. They are more frequent among premenopausal women. Patients with BLBC are in average younger than patients with other subtypes of breast cancer. It is also known that BLBC is more prevalent in African American women than non-African American women [5].

Molecular basis

Basal-like breast cancers have a specific genetic pattern. The majority of them is triple-negative, i.e. the tumours lack or show low expression of estrogen receptor (ER), progesterone receptor (PGR) and human epidermal growth factor receptor 2 (HER2) [4]. On the other hand, neoplastic basal-like cells express abnormally large amounts of genes that encode proteins with different functions (signal transduction, cell growth and division, angiogenesis, apoptosis, DNA replication and recombination) [4] such as cytokeratins (5/6, 14 and 17), epidermal growth factor receptor (EGFR), P-cadherin, caveolins 1 and 2, tyrosine kinase receptors, etc [6]. Some of them (cytokeratin5/6, EGFR) are associated with poor prognosis in breast cancer [4].

Furthermore, basal-like tumours are similar to the majority of tumours arising in BRCA1 mutation carriers regarding morphological phenotype, molecular genetic profile and clinical outcome (high proliferation, triple negativity, expression of basal cytokeratins, genome instability, poor prognosis, etc.) [7, 8]. Because of all these similarities and BRCA1 mutations that are very frequent within the basal subtype [1] scientists assume that there is a defect in the BRCA1 DNA-repair pathway in sporadic basal-like breast cancers (triple-negative), but it is not clear whether BRCA1 is a cause for or a consequence of basal phenotype [9]. BRCA1 is a gene that encodes a nuclear phosphoprotein which acts as a tumour suppressor and also plays a very important role in transcription, recombination and maintaining genomic stability by combining with other proteins to mend double-stranded breaks in DNA caused by different factors [10].

First step in repairing double-stranded breaks involves protein kinases ATM and ATR which sense and recognise the damaged DNA and transduce this signal onwards by phosphorylating particular proteins, including BRCA1 [11]. Phosphorylated BRCA1 can activate proteins BRCA2, RAD51, BARD1 and other components and form complex termed BRCA1-BRCA2-Containing Complex (BRCC) which has E3 ubiquitin ligase activity associated with DNA repair [12]. On the other hand, activated BRCA1 can be a part of BRCA1-Associated Genome Surveillance Complex (BASC) which includes tumour suppressors, signal transducers and DNA damage sensors. BASC is associated with DNA replication or DNA-replication repair [11, 12].

Because BRCA1 plays a significant role in sustaining genomic integrity of the cell, mutations, that prevent the production of BRCA1 protein or cause the production of abnormal and inactive protein, can be the reason for developing a cancer especially breast and ovarian cancer [11].

Diagnosis

Basal triple negative carcinoma is usually diagnosed by mammography or ultrasound where is visible ill-defined oval, round or lobulated mass lesion with partially indistinct margins. Ultrasound may show pseudocystic component due to necrosis in larger lesions. Biopsy of most basal carcinomas display distinctive morphologic features like circumscriptions with pushing borders, sheets of phleomorphic tumour cells with syncytial-like grow pattern, high nuclear rate and mitotic index [14]. Immunohistochemical testing is negative for estrogen receptors, progesteron receptors and epidermal growth factor receptor 2 [15]. Testing for BRCA1 mutation by genetic screening is also necessary [14].

Therapy

Among the subtypes basal triple negative tumours are particularly challenging. In contrast to ER-positive or HER2-positive tumours, no targeted therapy is currently available for these tumours [4]. The triple negativity of basal breast cancer tumours does not render them candidate to hormone therapy and anti-HER2 therapies (e.g. trastuzumab), and until now, surgery in combination with radiation therapy and chemotherapy was the sole available treatment [16]. Recent insights in the pathogenesis of these tumours are being translated into development of new strategies targeting molecular alterations. Some researchers have shown that hormone-receptor-negative breast cancers, actually respond better to chemotherapy than hormone-receptor-positive breast cancers [4]. This concept is supported by most neo-adjuvant anthracycline or taxane-based chemotherapy studies, which reported a higher rate of pathological complete response in the basal subtype than in others [17, 18]. However, despite relatively high rate of pathological complete response, basal tumours are associated with a relatively poor prognosis [19]. Most patients relapse within first and third year and the majority of deaths occur within five years following therapy [20]. This high relapse rate calls for the development of more effective chemotherapy and clinical trials are underway, which undoubtedly, will contribute to enlarge therapeutic possibilities in a near future [21, 22]. The first strategy utilizes the defect in double-strand DNA break repair mechanism, which should confer sensitivity to certain drugs (e.g. platinum compounds, anthracyclines or bleomycine) [23]. The other way to profit from the DNA repair defect is the use of poly (ADP-ribose) polymerase (PARP1) inhibitors. This enzyme is crucial in the base excision repair of single-strand DNA breaks. In its absence, single-strand breaks degenerate to double-strand breaks, which are not able to repair, because BRCA1 is deficient [24]. Several PARP1 inhibitors (e.g. iniparib, veliparib) are, alone or in combination with chemotherapy, in clinical development [25, 26].

Several other potential targets for triple negative tumours are connect with signal pathways and secondary messengers, including everolimus, a mammalian Target of Rapamycine (mTOR) inhibitor. mTOR activation is frequent in triple-negative breast cancers and has been associated with cisplatinum resistance [27]. Dasatinib, which inhibits ABL and SRC family kinases, is another possible drug [28].

Another study reports the phase I/II clinical trial of the drug IMMU-132, which was not long ago granted "breakthrough" status by the FDA, meant to speed the approval process of the most promising new drugs. IMMU-132 is an antibody-drug conjugate, which is composed of an irinotecan and an antibody that binds to the protein Trop2, which is overexpressed in about 80 percent of all triple-negative breast cancers. The approach is similar to the mechanics of the immune system in which an antibody recognizes the surface proteins of a bacterium or virus and then directs a T cell to target invaders.

Today no targeted or cytotoxic agent has yet been registered in triple-negative breast cancer, but many drugs are under development, and more trials will be soon activated in the metastatic, neo-adjuvant and adjuvant settings, which may improve patients' life.

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Gastric cancer (GC)

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Epidemiology

The stomach is divided into several anatomic subsites, including the cardia, fundus, body, pylorus, and the antrum. These areas are distinguished by anatomic demarcations, histological differences, or both. Most important is the difference between adenocarcinomas arising from the cardia (cardiac GC) and other parts of the stomach (noncardiac GC), as they have different epidemiologic patterns and causes.

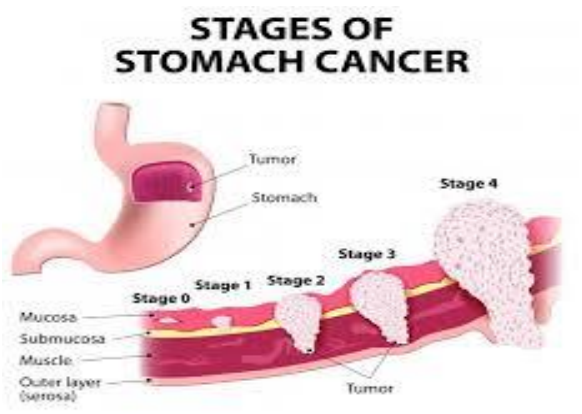

Figure 1: Stages of gastric cancer (9)

Risk factors for cancers arising from cardia and noncardia regions of the stomach may be different. Common risk factors for both are older age, male sex, tobacco smoking, radiation, and genetics. Intake of aspirin and statins may prevent against both of these cancers. While race is a risk factor for each, the direction differs by site. Factors associated with cardiac GC, but not noncardiac GC, include obesity and gastroesophageal reflux disease which may transform to Barrett's Esophagus. On the other hand, risk factors that are exclusive for noncardiac GC include *H. pylori* infection (especially in Western countries and Japan), low socioeconomic status, and perhaps diet factors such as low consumption of fruits and vegetables and high intake of salty and smoked food (1,2).

Molecular basis

Increased mutations in specific genes has impact on the expression of factors connected with the tumor etiology such as: epidermal growth factor (EGF), EGF receptor, K-sam, human epidermal growth factor treceptor (HER-2), interleukin (IL)-8, vascular endothelial growth factor (VEGF), cyclin E, p27, E-cadherin, CD44v6, matrix metalloproteinase-1 (MMP-1), and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) GC. Microsatellite instability status (MSI) is defined as the presence of replication errors in simple repetitive microsatellite sequences due to DNA mismatch repair deficiency. It is classified as high- frequency (MSI-H), low-frequency (MSI-L), or stable (MSS). All those pathological changes and pathways are often found in patients with the *H. pylori* infection (3,4,5).

Diagnostics

Nowadays, GC might be identified by various diagnostic biomarkers, for example: p53, adenomatous polyposis coli (APC – tumor suppressor protein), and CD44 have been used as markers for differential diagnosis. Epidermal growth factor receptor (EGFR), c-met, and c-erbB2 have been used to show the stage of malignancy, and MSI has been used as a marker for the screening of genetic instability.

A recent report showed that MSI and inactivation of hMLH1 by hypermethylation were more used in early gastric cancers than in gastric adenomas. Techniques of detecting MSI are very efficient because they can diagnose tumors before they become microscopically seen. (2)

Therapy

Chemotherapy

The goal of chemotherapy can be to destroy cancer remaining after surgery, slow the tumor's growth, or reduce cancer-related symptoms. It also may be combined with radiation therapy. Currently, there is no single standard chemotherapy treatment regimen that is used worldwide. However, most chemotherapy treatments for GC are based on the combination of at least 2 drugs, 5-fluorouracil(5-FU), and cisplatin. Newer drugs similar to 5-FU, such as capecitabine, and similar to cisplatin, such as oxaliplatin, appear to work equally well. In addition, the adjuvant therapy may be applied which consist of leucovorin: in these patients thymidylate synthetase inhibition rate (TSIR) was significantly higher.

Targeted therapy

This type of treatment blocks the growth and spread of cancer cells while limiting damage to healthy cells. Recent studies show that not all tumors have the same targets. Imatinib, as a tyrosin kinase inhibitor, is widely used due to its high selectivity on proliferation of maligant cells in GIST. However, there are patients that tend to have primar resistance to imatinib, develop secondary resistance or have introlerance on imatinibe therapy. Thus, in these cases, sunitinbe malate is used. Patients with later-stage cancer whose stomach tumor produce too much of the protein HER2, called HER2-positive cancer, may benefit from receiving trastuzumab with chemotherapy. For patients whose tumor has grown while receiving initial chemotherapy, the drug called ramucirumab was approved in 2014 as an additional treatment (6,7,8,9).

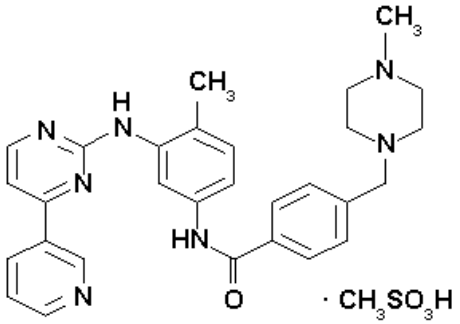

Figure 2: Chemical structure of imatinib mesylate (10)

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Colon cancer

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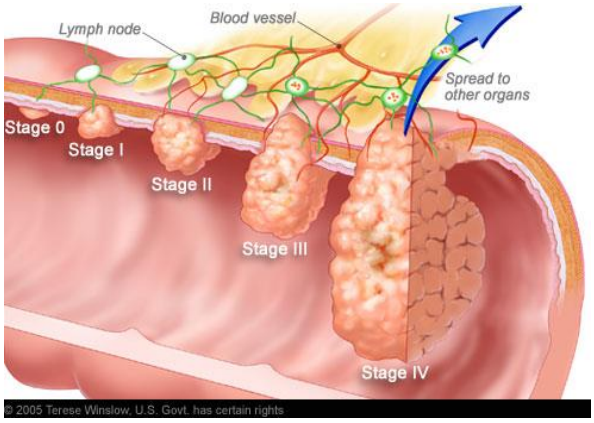
Introduction

Colon canceris a malignant tumor that starts in the colon, more specifically in the inner wall of the large intestine. Symptoms can be very different according to the specific location within the colon where the tumor is located or are even not present in early stages. Most common problems, which are not tending to be specific only for this condition and are therefore sometimes hardly recognizable, are:

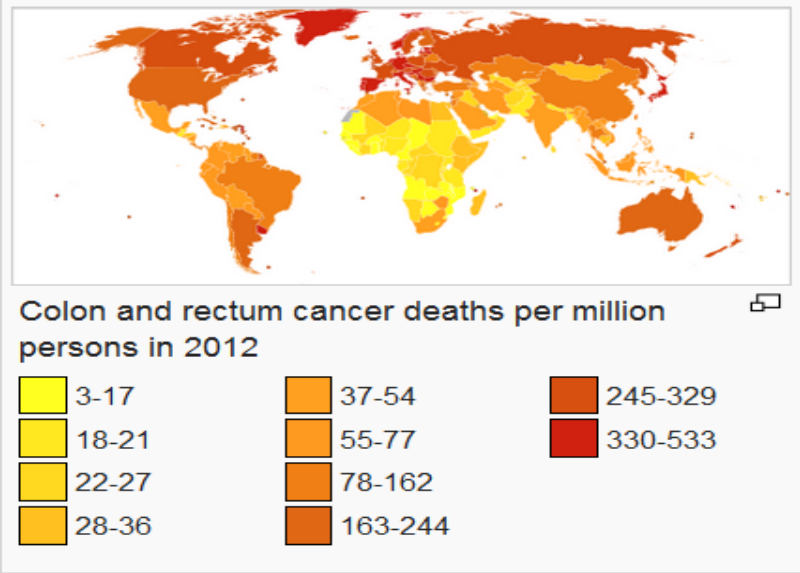
- Rectal bleeding or blood in the stool
- Dark-colored stool
- Change in bowel habits
- Change in stool consistency
- Constipation
- Diarrhea
- Narrow stools(1)
- Weight loss
- Feeling tired all the time(2)
- Anemia (3).



Picture 1

After Duke's Classification, colon cancer can be divided in 4 stages. In firstone, stage A, cancer is located in the inner lining of the intestine. In stage B cancerextend in the muscle layer.After the invasion of the lymph nodes, we talk about stage C and when it metastasized it is classifiedas stage D (2).

Epidemiology



Picture 2

Colon cancer isthe fourth leading cause of cancer death, the second most common cancer in women and the third most common in men. It occurs more often in developed countries. We found highest incidence of colon cancer in Australia, New Zealand, Europe and the US and lowest rates in Africa and South-Central Asia. Data shows that more than 1 million people are diagnosed with colon cancer every year (2).

Molecular basics/mechanism

It typically originates in the secretory cells lining the gut (4).The disease begins as a benign adenomatous polyp, which may form on the inner wall of the colon or rectum. Polyp can develop into an advanced adenoma with high-grade dysplasia and then progresses to an invasive cancer (5).In most cases this process is slow, taking at least 8 to 10 years to develop as clinically developed disease (1).

In a younger personit is often associated with hereditary syndromes like Peutz-Jegher's, hereditary nonpolyposis colorectal cancer or familial adenomatous polyposis (3).

Risk factors for developing colon cancer arefamily history of cancer of the colon or rectum,certain hereditaryconditions such as familial adenomatous polyposis and hereditary nonpolyposis colon cancer (HNPCC; Lynch Syndrome), history of ulcerative colitis or Crohn disease, personal history of cancers, personal history of polyps (1).

Chromosomal instability, DNA-repair enzymes defects and aberrant DNA methylation can lead to the development of colon cancer by facilitating the acquisition of multiple tumor-associated mutations. Cause of the colon cancer can also be a mutational inactivation of tumor-suppressor genes (APC, TP53, TGF-β) or activation of oncogene pathways (RAS and BRAF, Phosphatidylinositol 3-kinase) (5).

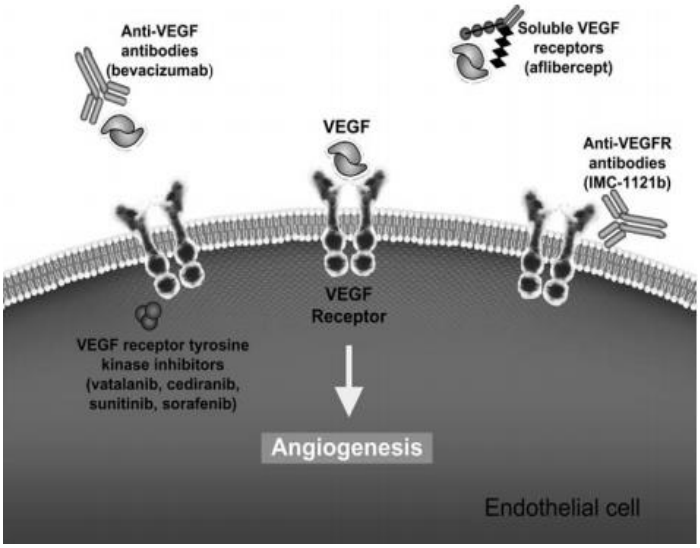
Diagnostic

In the early stages of colon cancer it can be detected because of the bleeding of a polyp, colloquial bowel pain or a bowel obstruction (3).The following tests and procedures can be used as a diagnostic approach:

1) Physical exam and history
2) Digital rectal exam
3) Fecal occult blood test
4) Barium x-rays
5) Sigmoidoscopy
6) Colonoscopy
7) Virtual colonoscopy/colonography or CT colonography
8) Biopsy (1)
9) Stool DNA screening test (2)

Therapy

Picture 3



Colorectal cancer has a comparatively good prognosis when detected early (3). Most colorectal cancers develop from polyps which can be removed surgically. With thatthe development of the colon cancer could be prevented(1).

As a therapy surgery and/or targeted therapy drugscan be used.One possibility is anti-angiogenic therapy which is a new standard of care in advanced colon cancer. This kind of therapy uses monoclonal antibodies that target either vascular epithelial growth factor(VEGF), vascular epithelial growth factor receptor(VEGFR) or inhibit VEGFR tyrosine kinase enzyme. There was a

breakthrough in this kind of therapy during some clinical trials with bevacizumab, a monoclonal antibody directed against VEGF (6).

Some colon cancers can be stromal tumors (5-10%). Most of gastrointestinal stromal cancers (GIST) have activating mutations in stem cell factor receptor (KIT). Approximately 5% of GIST's have activating mutations in the related gene encoding platelet-derived growth factor receptor alpha (PDGFRα). They are mutually exclusive. Drug imatinibmesylatecan be usedfor the treatment. It selectively blocks KIT and PDGFRα, and it acts as a receptor tyrosine kinase (RTK) inhibitor. Sunitimib malate can also be used after disease progression or when the patient is intolerant to imatinib. It is also an inhibitor of RTK (7).

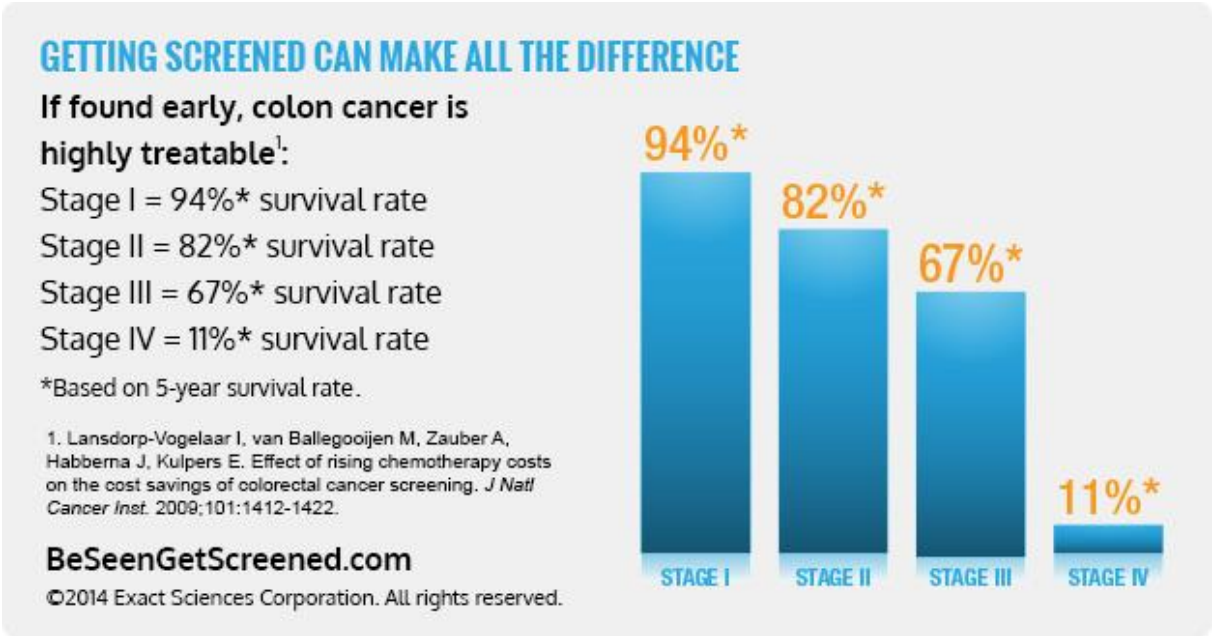
Anti-EGFR (endothelial growth factor) antibodies (cetuximab, panitumumab) can be used for treatment, except if the patient has the V-Ki-ras2 Kirstenratsarcomaviraloncogenhomolog (KRAS) mutation.

Recommendations

Lifestyle is really important. It is advisable that people exercise, quit smoking, drink less, eat properly and avoid stressful situations. Studies have shown that people who are physically active cope easier with the troubles of cancer therapy (8). People shouldfollow diets that contain a lot of vegetables, high-fiber foods and that have a low fat content which products can act as carcinogens (1).

Some medications and supplements, like aspirin, celecoxib, calcium and vitamin D, could be associated with reduced risk of colon cancer (2).

It is also important to have a regular check up at the doctor's office and taking screening test.



Picture 4

Picture references

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Liver cancer – hepatocellular carcinoma

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INTRODUCTION

Liver carcinoma is charakterized, such as odher carcinoma, by excessive proliferation of the mutated cells. Hepatocarcinogenesis is a complex process associated with accumulation of genetic and epigenetic changes that occur during initiation, promotion, and progression of the disease. Liver cancers can be divide into the primary carcinomas or secundary carcinomas – metastasis. In this abstract we want to focus on primary carcinoma. The most common tumor of liver is hepatocellular carcinoma (HCC). It is the third most common cause of cancer-related death.The pathophysiology of HCC is not understood clearly, but underlying liver dysfunction, especially cirrhosis, is a predisposing condition (Mittal at all 2013, Aravalli at all 2008).

EPIDEMIOLOGY

The distribution of HCC varies according to geographic location (Fig. 1). The disease burden is highest in areas with endemic Hepatitis B Virus (HBV) infection, such as in sub-Saharan Africa and Eastern Asia, with incidence rates of over 20 per 100,000 individuals. In regions of high incidence the most common cause is HBV transmitted at birth. In this areas is HCC diagnosed about one decade earlier as compared with North America and Europe with incidence rates of over 3 per 100,000 individuals (Mittal at all 2013).

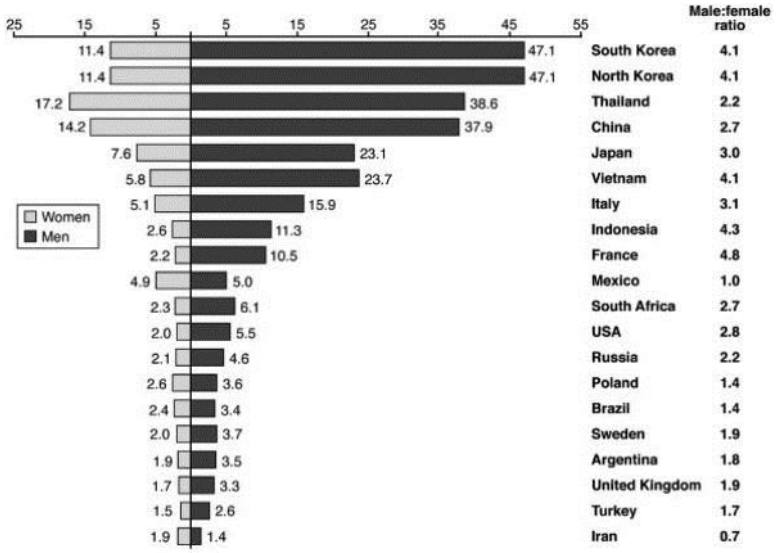

Fig. 1 Distribution of HCC in the world (taken from Mittal at all 2013)

A variety of risk factors have been associated with HCC. They include exposure to hepatitis viruses, vinyl chloride, tobacco, just food contaminated with aflatoxin B1, heavy alcohol intake, nonalcoholic fatty liver disease, diabetes, obesity, diet, coffee, oral contraceptives and hemochromatosis(Fig.2). This risk factors are dependent on geographical region (Aravalli at all 2008).

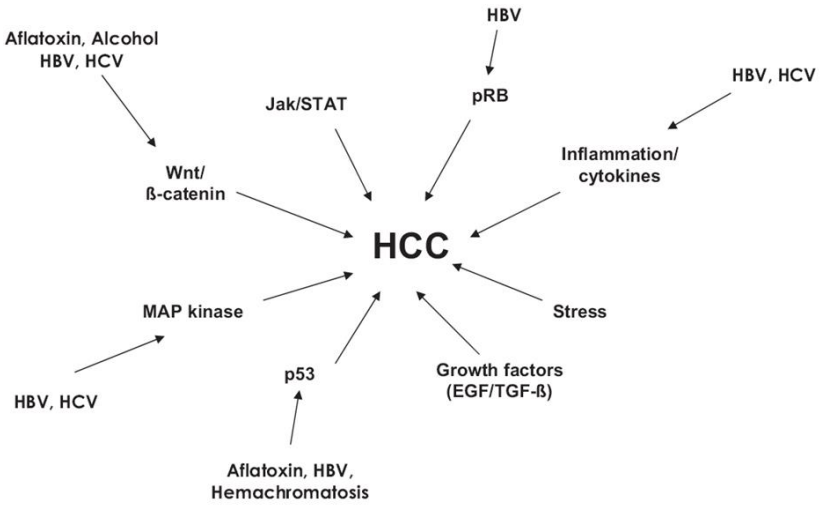

Fig. 2 Risc factor for development HCC (taken from Aravalli at all 2008)

MOLECULAR BASIC

Cellular action are often accompanied by increased expression of several factors that influence the survival of cancerous cells by suppressing apoptosis and regulating cell cycle. Some cell signaling pathway activated by risk carcinoma factor are known (Tab. 1). The overwiev of cell signaling pathways is shown on Fig. 3.

Risk Factor	Pathway Affected	Reference
Aflatoxin	Wnt/β-catenin	28
	p53	35-37
Alcohol	Wnt/β-catenin	24
HBV	Wnt/β-catenin	24
	p53	37
	pRb	43, 44
	MAP kinase	49
	Cytokine signaling	68
HCV	Wnt/β-catenin	24
	MAP kinase	49
Hemochromatosis	p53	38
Chemical carcinogen	Ras	52-58

Tab. 1Cell signaling pathway associated with risc factor (taken from Aravalli at all 2008)

Wnt/β-Catenin signaling pathway has a function as a key regulator in tumor development and differentaition. Members of Wnt protein family bind to cell surface receptor and their co-receptor and iniciate signaling pathway. Complicated cascade leads to increases expression of β-Catenin reaching the nucleus.

A variety of researchs summarize that the p53 tumor supressor gene has role in carcinogenesis. Tumor supressor gene is inactivated by a single point mutation, that leads to cell cycle arrest and subsequent apoptosis is defective.

Mitogen-Activated Protein Kinase Pathway (MAPK) affects most cellular process such as differenciation, proliferation or adhesion. Proteins of HBV, HCV, and hepatitis E virus modulate MAPK signaling by targeting multiple steps along the signaling pathway.

Rb protein is critical for the development of several cancer types. Rb protein binding nuclear transcription factors and prevent the cell from replicating damaged DNA. Cells stay in G1 phase.

Mutation of Rb leads to reduced control in cell cycle progression subsequently resulting in the development of cancer.

Ras signalling proteins are localized at the inner surface of the plasma membrane. They are molecular switches that transduce extracellular signals into the cytoplasm to protein signaling pathway,in order to perform an effect on cell growth, diferentiation and apoptosis.

Less common signaling pathways that are involved in carcinogenesis are: JAK/STAT Pathway, Epidermal Growth Factor Receptor and Transforming Growth Factor- Pathways, additional pathwaysof physiological processes such as alcohol metabolism,cellular transport, and ubiquitins, stress Response Signaling.

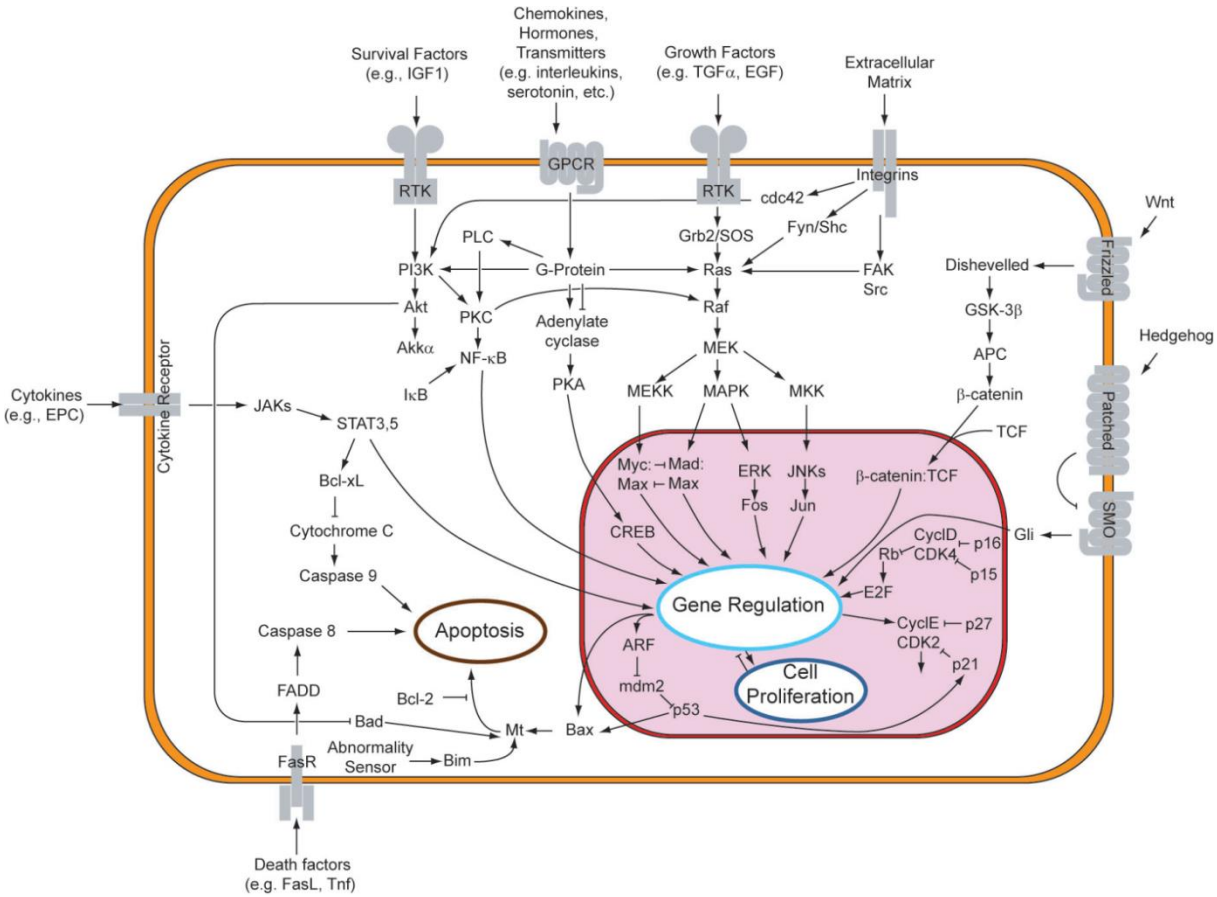

Fig. 3 Overwiev of cell signaling pathway (taken from wikimedia)

DIAGNOSTICS

When it comes to cancer, time of diagnosis is of utmost importance. The story is no different with HCC, because long-term survival requires detection of small tumors before the disease advances into its more advanced stages. Unfortunately HCC is usually diagnosed when clinical manifestations have already developed at which time survival is measured in months. Since early detection of HCC is so important, surveillance of high-risk individuals is performed by measuring serum alfa-fetoprotein (AFP) and ultrasonography. Diagnosis of HCC on the other hand requires more sophisticated imaging methods, among which CT and MRI are the most frequently used. Liver biopsy is also a viable option, but it is only used if the diagnosis of HCC remains unclear, because of its invasive nature. (4)

Surveillance of high-risk individuals. As already mentioned, surveillance of high-risk individuals is preformed through screening test by measuring serum alfa-fetoprotein (AFP), usually complemented

by ultrasonography, since it can, combined with AFP values, improve predicted positive value up to 94 %. Successful surveillance heavily depends on properly identifying which individuals have a high-risk of developing HCC. Among those are patients that are older, of male gender, have a family history of HCC, or have cirrhosis. (4)

Alpha-fetoprotein (AFP). AFP is a serum glycoprotein, which consists of 591 amino-acids and is produced in high concentrations in fetal yolk sac and fetal liver. The values fall to low levels within 300 days of birth, so elevated numbers are therefore a good indication of pathological changes, which could very well be malignant. AFP is unfortunately not an ideal marker, since other conditions can also result in elevated AFP concentrations and even in patients with HCC the values can range from normal to >100 000 ng/ml (reference values for non-pregnant subjects are < 6ng/mL). Attempts to improve the accuracy of AFP have been made through identifying different isoforms. (4)

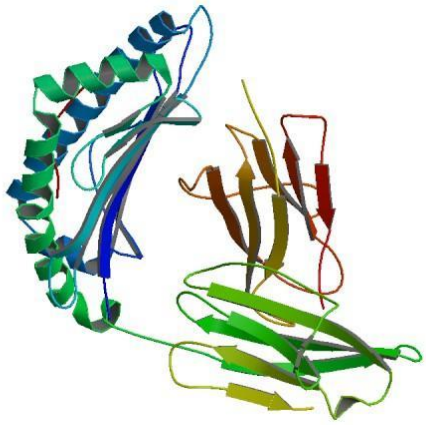

Fig. 4 The structure of human alpha-fetoprotein (taken from PhosphoSitePlus)

Ultrasonography. Ultrasound is widely used for detecting tumors that are smaller than 3 cm. It's commonly used because it is noninvasive, inexpensive and easily available. But just like all other methods and techniques, ultrasound also has its limitations. They are dependent on operator and imaging of obese and cirrhotic patients can be problematic. (4)

Diagnosis. The diagnosis of HCC is a multi-step procedure which usually requires different diagnostic tools, of which computer tomography (CT) and magnetic resonance imaging (MRI) are most commonly used. If a liver mass, which is greater than 2 cm in diameter, is discovered on ultrasound and it is known that the patient has pre-existing cirrhosis, there is a greater than 95% chance that the lesion is a HCC. The next step in line is measuring AFP, and if its values are raised the diagnosis is confirmed and further investigation is only required to establish the most appropriate therapy. On the other hand if AFP is normal, further radiological imaging is required. In these cases CT, MRI or lipoidol angiography with follow up CT will give us a reliable answer without the need for biopsy. Liver biopsy is only really required and recommended if doubts about the diagnosis still persists and should only be applied if truly necessary. (5)

THERAPY

A multidisciplinary setting in which patients are cooperatively managed by a wide number of different specialist is the most effective way of treating HCC. Specialist can choose from surgical or non-surgical therapies, depending on the patient's condition. (6)

Surgical therapy. Among surgical therapies surgical resection and liver transplantation are viable options. The definitive treatment for HCC is surgical resection. It is also the only therapy that offers

the prospect of cure or at least long-term survival, however most patients do not have resectable diseases and the recurrence rate is up to 80 % in 5 years. (8) Liver transplantation is actually the most efficient treatment in patients with small tumours, but unfortunately less than 30 % of patients are eligible because of restrictive criteria, graft unavailability and high-cost. (7)

Non-surgical therapy. Non-surgical therapies are used if patients are not candidates for resection or liver transplantation. (3) Transcatheter oily chemoembolization (TOCE) is the most commonly offered non-surgical therapy, in which an emulsion of a cytotoxic drug and lipiodol is slowly injected through a catheter that is placed in the appropriate tumour-feeding artery. As a result an embolization takes place. Other therapies such as percutaneous alcohol injection, thermal and laser ablation, radiotherapy and systemic chemotherapy can also be used. There are several chemotherapeutics that can be used such as doxorubicin-based regimens, tamoxifen, antiandrogens (eg, cyproterone, ketoconazole), interferon, interleukin (IL)-2, octreotide, gemcitabine, oxaliplatin, sorafenib and others. (6)

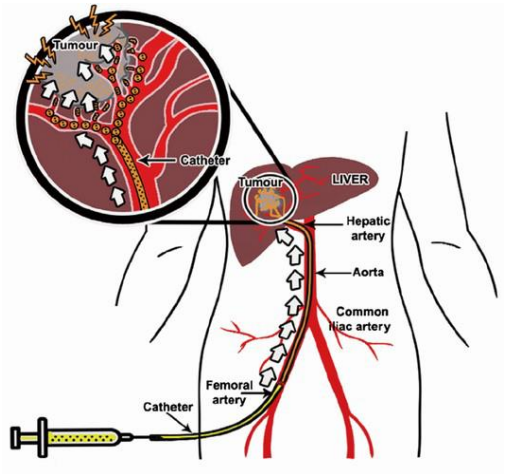

Fig. 5: Principle of conventional transarterial chemoembolization (taken from Wang YXJ et al)

CONCLUSSION

If we conclude, the pathophysiology of HCC, the most common tumor of liver and third most common cause of cancer-related death, is not completely understood at the present moment. Different risk factors have been associated with HCC, of which by far the most common one is infection with HBV. These risk factors activate or change different pathways, which usually lead to increased expression of several factors that influence the survival of cancerous cells by suppressing apoptosis and regulating cell cycle. Since long-term survival requires detection of small tumors before the disease advances into its more advanced stages, surveillance of high-risk individuals is performed by measuring serum alfa-fetoprotein (AFP) and ultrasonography. Diagnosis of HCC on the other hand is a multi-step process which requires different diagnostic tools through which we can come to a proper conclusion. When it comes to treating HCC choosing a multidisciplinary setting in which patients are cooperatively managed by a wide number of different specialist is the most effective way of treating HCC. Therapies that are available can be of a surgical or non-surgical nature. The definitive treatment for HCC is surgical resection also the only therapy that offers the prospect of cure or at least long-term survival, unfortunately most patients do not have resectable diseases.

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Tab. 1: Aravalli NR, Steer CJ. Molecular Mechanisms of Hepatocellular Carcinoma. HEPATOLOGY. 2008; Vol. 48, No. 6

Fig. 1: https://upload.wikimedia.org/wikipedia/commons/f/fb/Signal_transduction_pathways.png

Fig. 2: https://upload.wikimedia.org/wikipedia/commons/f/fb/Signal_transduction_pathways.png

Fig. 3: https://upload.wikimedia.org/wikipedia/commons/f/fb/Signal_transduction_pathways.png

Fig. 4: The structure of human alpha-fetoprotein. Available on world wide web: http://www.phosphosite.org/proteinAction?id=14609&showAllSites=true

Fig. 5: Wang YXJ et al. Transcatheter embolization therapy in liver cancer: an update of clinical evidences. Chinese journal of cancer research 2015; 27 (2)

Epidemiology and Molecular Basis of Lung Cancer

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Epidemiology

Lung cancer is one of the most common malignant diseases and leading cause of cancer death around the world. Each year, more people die of lung cancer than of colon, breast, and prostate cancers combined (1).

Lung cancer can be divided into two major forms: non–small cell lung cancer (NSCLC) that accounts for 85% of all lung cancers and small cell lung cancer (SCLC) that accounts for the remaining 15% of cases. NSCLC can be further divided into 3 subtypes: adenocarcinoma, squamous cell carcinoma and large cell carcinoma (1).

At the time of the diagnosis (average age is about 70 years) most of the lung cancer cases are inoperable. The majority of lung cancer patients also do not respond well to chemotherapeutics or radiation therapy and more than 85% of them die during the first 5 years. There has been some improvement in survival during the past years but in the last three decades the mortality rate has not changed significantly (2).

Lung cancer is the second most common cancer in both men and women. The probability that a man will develop lung cancer in his lifetime is about 7,1%, while for a woman, the risk is about 5,9%. This include both smokers and non-smokers. For smokers the risk is much higher. It has been estimated that active smoking is responsible for about 90% of all lung cancer cases (3).

There is also a significantly increased risk for lung cancer in people with a family history of early-onset lung cancer. Genetic predisposition include polymorphisms of the tumor suppressor genes and the allelic variants of the genes involved in detoxification. Some nonmalignant diseases have been associated with an increased risk for lung cancer, such as COPD and asthma. Enviromental pollution and carcinogenes also increase risk for lung cancer (asbestos, radon, arsenic, chromium, nickel,vinyl chloride etc.) (2).

Molecular Basis of Lung Cancer

Molecular basis of lung cancer is very complicated and complex. Many different factors (environment, genetics, hormonal factors, viral factors …) have been linked to carcinogenesis. Genetics and environment play a very important role in lung cancer development. An important process in tumor progression is also angiogenesis in which vascular endothelial growth factor (VEGF) is the major mediator (2, 4).

Genetics of lung cancer:

Genetic changes in lung cancer usually involve both oncogenes and tumor suppressor genes and they may or may not be rate-limiting events. Most often genetic alterations that occur in early phase of lung cancer involve loss of some genetic material on short arms of chromosomes 3 and 9, deletion of 5p and mutations of K-Ras and p53 (5).

Major tumor suppressor genes that are involved in lung cancer are TP53, RB1, CDKN2 and several genes located on 3p. Mutations of TP53 are associated with smoking and more aggressive tumors. Tumor suppressor genes are usually inactivated not only by one factor but a combination of genetic changes like point mutations, chromosomal rearrangements and mitotic recombination and non genetic changes (epigenetics). Methylation and acetylation (epigenetics processes) affect on expression of tumor suppression genes and oncogenes (5, 6).

Many proto-oncogenes are activated by chromosomal rearrangements (translocations, inversions etc.), amplifications of genetic material (MYC, EGFR, HER2 etc.) and genetic mutations (EGFR, KRAS etc.) (5).

Environment

Very important environmental factor for development of lung cancer is tobacco. Tobacco carcinogens are in human body firstly metabolised by cytochrome P-450 and then are the oxygenated intermediate metabolites further metabolised by different enzymes (glutathiones, sulfatases etc.) in subsequent transformations (detoxification and secretion). Some of metabolites that were generated then bind with the DNA and form so called DNA adducts. That process is called metabolic activation and it is necessary for carcinogenic effect of tobacco metabolites (polcyclic aromatic hydrocarbons etc.). Formation of DNA adducts can result in miscoding leading to permanent mutations such as K-Ras, p16, p53 or other mutations. Consequently, expression of tumor suppression genes and oncogenes is changed which can result in lung cancer. However our body can protect itself by activating repairing mechanisms or apoptosis of damaged cells.
Metabolic activation and detoxification of potential carcinogens both influence the development of lung cancer. Because of that not all smokers develop lung cancer but up to 20% do (7).

Angiogenesis and VEGF

Tumor needs to develop more blood vessels in order to spread (more than 2 mm^3) because of a need for oxygen and nutrients. Major mediator in angiogenesis is VEGF, which is normally expressed by endothelial cells that are generating blood vessels. VEGF needs a receptor to affect. The most important one is VEGFR 2. The binding of VEGF to its receptor causes cascade of many signaling pathways, i.e. dimerization of the receptor, leading to activation of the PLCɣ-PKC-Raf kinase-MEK-MAPK and cell growth.
VEGF expression in tumor cells is influenced by several different factors (hypoxia, low pH, growth factors, inflammatory cytokines (e.g., interleukin-6), sex hormones etc.) and genetic changes (activation of oncogenes, inactivation of tumor suppressor genes etc.).
Tumor cells do not express VEGFR (so, VEGF from tumor cells cannot initiate signaling pathways) however tumor angiogenesis is still possible because of VEGFR of the other normal host cells (4).

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Diagnosing lung cancer

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Lung cancer is often diagnosed in the advanced stage, after metastatic spread and thus has poor prognosis (1). New approaches are desired to achieve an early and accurate diagnosis, which is crucial for improvement of lung cancer survival rates (2).

Lung cancer is suspected in patients with risk factors (current/former/passive smokers, 55-80 years old with a smoking history of 30 or more years, individuals exposed to environmental carcinogens, individuals with a family history of lung cancer etc.) and/or symptoms such as a cough, bloody sputum, shortness of breath, wheezing, fatigue, weight loss, headache etc. (2, 3). These patients undergo physical examination and chest X-ray. If the chest radiograph is abnormal patients should proceed with diagnostic procedures. The course of diagnosis is dependent on the type (i.e., small cell or non-small cell lung cancer), the size and location of the primary tumor, and the presence of metastasis and overall clinical status of the patient. The least invasive approach with the minimum steps possible should be used. Nevertheless, bronchoscopy is often essential for a lung cancer diagnosis (3, 4).

In the case of an abnormal chest radiograph, the possibility of other medical conditions such as pneumonia, must be excluded. The next step is a Computed Tomography scan (CT scan) of the chest and abdomen which provides a more detailed picture of the tumor. Another option is a combination of a CT scan and a Positron Emission Tomography scan (PET CT scan) which uses radioactive sugar to identify the location of cancer cells which appear brighter on the PET CT scan. If we detect a tumor at its metastatic phase, an additional Magnetic Resonance Imaging (MRI) is required. In case of enlarged nodes, neck or endobronchial (EBUS), an ultrasound should be done. If all of these tests are positive, a lung biopsy is the next step. There are several different possibilities for collecting a tissue sample, depending on the location of the tumor, including a needle biopsy and bronchoscopy. Open surgery is sometimes the only option to collect suspicious tissue mass. If pleural effusion is present, thoracentesis can be used (5). If cancer cells are found in sputum, we can avoid invasive procedures by performing a sputum cytology. If this provides a negative result, a possibility of lung cancer must not be excluded, as the results of this test can often come up as false negative (4).

There are some downsides to the current diagnostic methods such as high radiation exposure when performing a chest X-ray and CT scans, even when lower doses of radiation are used (1, 6). Other problems include high cost, overdiagnosis and false positive results (1).

The future of early diagnosis and a reduction in mortality might be successful screening tests. These are tests designed to detect lung cancer in patients with risk factors but no obvious symptoms or history of that disease. This approach provides a diagnosis at its earliest stages when lung cancer is most treatable (1). Screening tests such as a chest X-ray, sputum cytology, Computed Tomography (CT), fluorescence endoscopy and low-dose spiral CT (LDCT) had little success improving survival rates over the course of the last fifty years. Today novel screening methods are being developed on the basis of serum biomarkers. Free circulating DNA and RNA, exosomal microRNA, circulating tumor cells and different lung cancer specific antigens are being extensively studied for this purpose (7, 8). Currently, there are no validated biomarkers available for early detection of lung cancer. Potentially useful lung cancer biomarkers are for example progastrin-releasing peptide (ProGRP), carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), cytokeratin 19 (CYFRA-21-1), carbohydrate antigen-125 (CA-125), carbohydrate antigen-19.9 (CA-19.9) and alpha-fetoprotein (6, 7,

8). For example CYFRA 21-1 proteins indicate an increased level of cytokeratin 19 fragments which implies the presence of lung cancer. ProGRP is produced by the nueroendocrine tissues of the gastrointestinal and respiratory tracts and is also an available lung cancer biomarker. NSE is a glycolytic enzyme produced in central and peripheral neurons that is also increased in case of lung cancer. These protein biomarkers usually lack their lung-cancer specificity which is why their use in clinical practice is limited (9). However, a better outcome was achieved using a combination of biomarkers. Different combinations have shown to have higher sensitivity for a certain type of lung cancer (10).

Developing new lung cancer screening tests and diagnostic methods is challenging and plays an important role in lung-cancer-mortality reduction. With further research, we can expect new screening tests that will allow for the early diagnosis and treatment of lung cancer both of which are vital for improving lung cancer survival rates.

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Therapy of lung cancer

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CHEMOTHERAPY OF LUNG CANCER

When choosing a therapy, many factors must be considered such as likelihood of treatment achieving a cure and fitness of the patient. Those two are also linked – a patient whose fitness is borderline may not be able to tolerate a more extensive resection needed to achieve the cure. Chemotherapy improves survival in small-cell carcinoma and can even cure a small fraction of patients with limited disease. Surgery is the treatment of choice in 25 to 30 percent of patients with NSCLC who are stage I or II. Another 25 to 30 percent of patients, who are stage IIIa or IIIb are usually treated with radiotherapy or, occasionally, surgery. For the remaining 40 to 50 percent of patients, who are stage IV non-small- cell lung cancer at the time of diagnosis, palliative symptomatic treatment, most often irradiation of the chest, brain or bone is standard care. With these approaches, survival rates are respectively 40 percent, 4-8 percent and < 1 percent in a three year period[1]. Though moderate improval in survival and increased rates of tumor regression are possible, there is no group of patients with non-small- cell lung cancer in which chemotherapy is unequivocally effective.

Drug therapy of small-cell lung cancer

Chemotherapy is the main treatment for small-cell lung cancer (SCLC). It's known that this type of cancer produce the metastases very soon, so when metastases are present, surgery is not right choice. SCLC responds very well to chemotherapy.

Single-Agent Chemotherapy

Chemotherapeutic agents, including cyclophosphamide, etoposide, doxorubicin, vincristine, carboplatin and methotrexate induce tumor regression in at least 30 percent of patients. Single-agent therapy may still be useful in certain groups of patients, especially elderly and those with cardiopulmonary or renal dysfunction. Patients with impaired ability to ambulate, and patients in whom the likelihood of severe toxicity from combination chemotherapy is high.

Combination Chemotherapy

Combination chemotherapy is superior to single-agent treatment. Myelosuppression is the common dose-limiting toxicity, therefore combination therapy usually consists of four or fewer agents.The most commonly used combinations are currently etoposide and cisplatin (CAVE); cyclophosphamide, doxorubicin and vincristine (CAV); cyclophosphamide, doxorubicin and etoposide (ACE)[1]. People who are not able to have CAV or ACE therapy because of their medical conditions, may have topotecan chemotherapy capsules.

Drug therapy of non-small-cell lung cancer

There are no survival advantages of chemotherapy in patients with non-small-cell lung cancer. Due to that and the toxicity of chemotherapy, only fully ambulatory patients should be offered treatment.

Chemotherapy is used to treat NSCLC after surgery for early stage cancer; before, after or alongside radiotherapy and for locally advanced lung cancer. Chemotherapy after surgery can reduce relapse of the disease. Most common used combinations include cisplatin or carboplatin with at least one other drug (vinorelbine, gemcitabine, paclitaxel, docetaxel, doxorubicin, etoposid, pemetrexed). Chemotherapy before or after radiotherapy can help eliminate early stage NSCLC in people who

cannot have surgery and prolong life of people with advanced NSCLC. Chemotherapy can help shrink or slow the growth of locally advanced or metastatic NSCLC and also help control the symptoms for some people.

Some cancers respond very well to biological therapy, mostly patients with EGFR or VEGFR positivity.

BIOLOGICAL THERAPY OF LUNG CANCER

Biological or immuno-therapies are derived from, or target substances that occur naturally in the body. Actually most biological therapies were performed by monoclonal antibody (immunotherapy of the cancer). They target and destroy particular types of cancer cells. Possible side effects of biological therapy are: tiredness, diarrhea, skin changes (rashes, discoloration), a sore mouth, weakness, feeling sick, loss of appetite, low blood counts, swelling of parts of the body, due to build-up of fluid.

Erlotinib (Tarceva) works by blocking epidermal growth factor receptors (EGFR) in the cancer cells. It is used for locally advanced or metastatic non-small-cell lung cancer that is EGFR positive, if a patient has not had drug treatment before (a first line treatment) or if a patient has already had chemotherapy, because there was a delay in finding out if the cancer is EGFR positive.

Gefitinib (Iressa) works by blocking epidermal growth factor receptors. It is used as a first treatment for NSCLC lung cancer that is locally advanced or has spread. A meta-analysis of trials comparing gefitinib with chemotherapy found that there was no difference in how long people lived, but there was a difference in the side effects: less tiredness, sickness and effects on the blood cells, more skin problems (rashes), diarrhoea, and irritation of the lung.

Crizotinib (Xalkori) can help to control advanced NSCLC in people whose cancer cells have an overactive version of anaplastic lymphoma kinase (ALK).

Afatinib (Giotrif) is a protein tyrosine kinase inhibitor (TKI). It blocks tyrosine kinases and also blocks the epidermal growth factor receptor proteins in cancer cells. It is used for people with advanced not-small-cell lung cancer who had not already had any other type of cancer growth blocker.

Nintedanib (Vargatef) is a treatment for adenocarcinoma. It is multi kinase inhibitor which works by blocking particular proteins called protein kinases on cancer cells. It also stops the cancer tissue neovascularisation. .

Cetuximab (Erbitux) is a monoclonal antibody. It blocks growth factor receptors on cells. It has been used, with chemotherapy, in trials for advanced non- small- cell lung cancer.

Bevacizumab (Avastin) is a monoclonalantibody (MAB) that stops cancer cells making the blood vessels (neovascularisation) they need so that they can grow.

RADIATION THERAPY

Radiotherapy commonly means external radiotherapy, conventionally delivered as one fraction per day, 5 days a week, for 5 to 7 weeks. Radiotherapy is suitable for treating a wide variety of NSCLC patients. This treatment should be the choice for patients with early stage lung cancer and co-morbiditety who present a high surgical risk or where patient makes an informed choice not to have surgery.

Radiotheraopy is indicated for patients with stage I, II or III NSCLC who have good performance status. All patients sould undergo pulmonary function test before having radical radiotherapy of

NSCLC. One meta-analysis showed 14% reduction in mortality rate in patients who were treated with radiotherapy compared to chemotherapy.

Brachytherapy

Lung brachytherapy can be used almost any time when the tumor can be seen in an airway on bronchoscopy. During bronchoscopy, a thin plastic tube is placed down the nose, and down into the airways of the lung, into the diseased bronchus. The bronchoscope is then removed, but the thin tube will stay comfortably in place for about 45 minutes. During this time, a brachytherapy treatment will be given through the segment of the tube which is lying against the cancer. This treats the cancer from the inside-out. Brachytherapy places radioactive sources inside the patient on a temporary or permanent basis to damage cancer cells DNA and destroy their ability to divide and grow. This treats cancer from the inside-out.

TARGETED THERAPY

With current treatment, the disease is rarely curable, and prognosis is poor, as patients are often diagnosed with metastatic disease. Current systemic therapies have limited effectiveness. Thus, novel strategies for the treatment of lung cancers are necessary. Knowing which genetically and epigenetically changed molecules activate signaling pathways important in carcinogenesis made it possible to develop molecularly targeted therapies which hold promise for improving lung cancer outcomes. Only two of these have been approved until now, for advanced NSCLC:

EGFR pathway inhibitors

The EGF pathway is frequently disregulated in human cancers, making it an attractive target for anticancer therapy. The EGFR family of receptors consists of transmembrane tyrosine kinase receptors and its ligands are frequently overexpressed in NSCLC tumors, but rarely expressed in SCLCs. Binding of ligand to the EGFR leads to proliferation, inhibition of apoptosis, angiogenesis and invasion, all resulting in tumor growth and spread. Agents targeting EGFR include the TK inhibitors, such as gefitinib and erlotinib, and the monoclonal antibodies.

Angiogenesis inhibitors

The monoclonal antibodies against VEGF and the VEGFR TKIs are among the agents aimed at targeting the VEGF/VEGFR signaling network. Bevacizumab is a monoclonal antibody that binds to all isoforms of VEGF-A. Its addition to the classic first-line treatment of patients with advanced nonsquamous NSCLC provides a significant survival benefit and has been approved for use in NSCLC. Pulmonary hemorrhage associated with bevacizumab treatment is an occasional but serious life-threatening side effect, highlighting the importance of patient selection and monitoring for this therapy. Patients with squamous cell histology are particularly at risk of bleeding. VEGFR TKIs are small molecules that bind to the ATP pocket of the TK residues of the intracellular domain of VEGFR, thus inhibiting downstream pathways.

One of the most promising lung cancer treatments that are still in clinical development are cancer stem cell-specific therapeutic approaches.

Cancer stem cell-specific therapeutic approaches

Most malignancies may arise from a rare subpopulation of stem-like tumor cells, i.e. ''cancer stem cells''. They may be inherently resistant to the common therapeutic protocols due to their low proliferation rate and drug transporter expression. Thus, the development of therapies speciffically targeting those cells represents a strategy to completely eradicate tumors and potentially lead to cure, even in advanced-stage disease. One of the targets is the telomerase, which is upregulated in cancer stem cells. Telomerase inhibitors can potentially target both cancer stem cells and more mature cancer cells. Human telomerase contains two essential components, a telomerase reverse transcriptase (hTERT) catalytic subunit and a functional telomerase RNA (hTR) component. Activation

of telomerase plays a role in the immortalization of cells as an early step in tumorigenesis. Detectable telomerase levels are found in approximately 80% of NSCLCs and 100% of SCLCs. Agents targeting telomerase include telomerase antagonists the RNA template region of hTR (whuch inhibit anchorage-independent growth of lung cancer cells), immunotherapy, gene therapy and hTERT inhibitors.

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Epidemiology and diagnostics of malignant melanoma

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Epidemiology

Melanomas are common cancers arising from the pigment cells of the skin. Local melanomas are curable by surgery, but advanced disease is hard to treat and could be lethal. [1] Approaches to control the disease are mainly focused on prevention and early detection. To apply these approaches, it is crucial that the epidemiology of melanoma is understood.

Melanoma mainly affects Caucasian populations (10-20-fold higher than dark-skinned people). The incidence varies greatly depending on geographic location of a certain population. In fact, melanoma incidence variation among populations is one of the greatest in any cancer. [2] This is the most convincing evidence regarding the role of environment (sunlight) as the cause of this cancer. International Agency for Research on Cancer (IARC) shows that the highest incidence rates for melanoma occur in Australia (39:100,000) and New Zealand. Next is USA, followed by Scandinavian countries (15:100,000). Non-Caucasian populations in Africa, Asia and Pacific have rates lower than 3:100,000 per year. People living at low latitudes experience higher rates of melanoma and the difference is clear even within Australia. [3] This latitude effect, however, is not observable in northern hemisphere. Mediterranean countries experience lower rates of melanoma than Scandinavian ones, presumably because of darker skin of Mediterranean people compared to Scandinavians. [4] With rising incidence rates, mortality also rises, but at a slower pace. [5] In all populations, melanoma is rare before the age of 40 and it peaks in the seventh or eighth decade of life, with incidence rising with age. [6, 7, 8, 9, 10] Historically, melanoma was more common in women, but recently the occurrence pattern has changed and rapid increases of melanoma in males changed that. [8, 11]

Risk factors

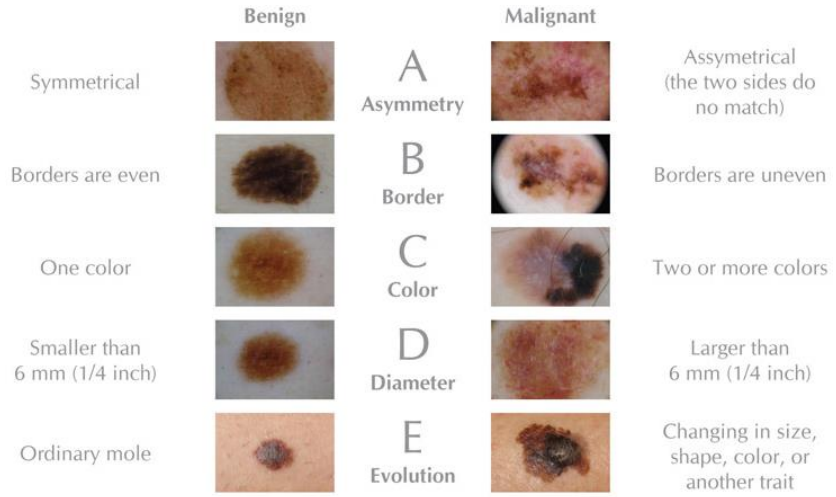
Sunlight and especially its UV spectrum is the leading cause of melanoma. Some people, however, believe that sunlight is not the main cause of melanoma. Most melanomas occur on the back and shoulders, which are normally protected by clothing, whereas face and hands rarely produce melanomas, yet are constantly exposed to sunlight. A comprehensive study is difficult to perform, because everyone has been subjected to different amounts of sunlight and other risk factors. There is no reliable and objective method for determining past exposures to the sun. Besides environmental risk factors, there are also genetic risk factors. Number of nevi each person has also plays a role in risk assessment. Persons with >100 nevi have up to sevenfold higher risk compared to <15 nevi. [13] Clinical observation that 10 % of people with melanoma had a family history of the disease hint at a genetic cause. Genetic epidemiologists identified a region on the short arm of chromosome 9, linked to melanoma. The deleted locus was a *CDKN2A* gene. Germ line mutations in this gene are “melanoma-prone”. Two other genes were since found within the same locus, *P14ARF* and *CDKN2B.* Proteins coded by these genes are all potential tumor-suppressors, playing a role in cell-cycle arrest. [14, 15, 16]

Previous studies were designed under the assumption that all melanomas are a single homogenous group. But recent findings show that this may not be the case and melanomas are actually heterogeneous and may arise through several different pathways.

Diagnosis

Melanoma is often discovered by routine health check-ups or by patients itself who come to see a doctor because of suspicious mark on their skin. It is essential to make diagnosis as early as possible to enable detection and removal of the abnormal growth before it can progress to more advanced stage of disease that is more difficult to treat and result in significant drop of survival rate. From almost 100 % if discovered early to 10-20 % if distant metastasis is presented. (1)

The most common diagnostic technique is visual inspection with good lighting and magnification. To improve the sensitivity (detection of melanomas) and specificity (percentage of non-melanomas correctly diagnosed as benign) the used technique is skin surface microscopy (dermatoscopy). First symptoms of melanoma are usually changes in the size, colour, shape or sensation of an existing mole or presence of dark area that can look like a new mole.



ABCDE system of diagnosis

These features have been used as the so-called ABCDE system of diagnosis. Each letter presents different characteristic described in picture below. Letter A stands for asymmetry which means that the two sides do not match compared to symmetrical benign lesion. B stands for border which is irregular meanwhile the benign mark has even borders. C stands for colour. Malignant lesion is two- or multi-coloured and benign is homogenously coloured. D stands for diameter. Moles that are wider than 6 mm are more likely to be malignant but not necessary. They can be even smaller. E means evolution. Malignant lesion is changing over time in its size, colour, shape or another trait. When the diagnosis is uncertain the excision biopsy is performed for histological examination which is golden standard in diagnosis of melanoma. The biopsy will provide details of the thickness of the tumor and any unfavourable features such as ulceration, regression, level of invasion, mitotic rate, vascular invasion. All this information is important to define the melanoma stage, to decide for proper

treatment and to estimate prognosis at five years after diagnosis. (2) To support a diagnosis of melanoma histochemical staining of the S100 protein family has been used. Especially one member so called S100B is used in staging malignant melanoma, in establishing prognosis, in evaluating treatment success and in predicting relapse. Survival rate of melanoma patients with normal S100B levels is significant longer compared to those with elevated level. (3)

Depending on the stage of melanoma additional tests are performed. Besides visual examination other various tests are ordered to confirm a diagnosis of melanoma and/or determine if or where the disease has spread. All patients with a primary melanoma greater than 1 mm in depth should be offered lymph biopsy. Other tests include blood exam, liver tests, lactate dehydrogenase test (high levels often indicates metastatic spread to the liver), x-ray and special scanning tests such as ultrasound, CT (computed tomography), MRI (magnetic resonance imaging) and PET (positron emission tomography). (4)

Poor prognosis for individuals with metastatic or advanced melanoma and poor treatment results, either due to imprecise determination of melanoma stage at time of diagnosis or inappropriate therapy, or both, are leading towards increasing importance to targeted therapies. In order to sufficiently target specific parts of malignant cell mechanism, adjusted cell signaling pathways have to be well known. [21, 23]

The most important molecular pathways in melanoma are: [21, 23]

- Tyrosine kinase receptors (TRK),
- Ras/Raf (BRAF)/MEK/MAPK/ERK/Mitf pathway
- Ras/ /Phosphotidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapcamycin (mTOR) pathway with phosphatase and tensin homolog (PTEN) affecting PI3K and negatively regulating the pathway mentioned above
- Melanin synthesis pathway (MC1R pathway)
- Pathways of cell cycle regulation such as p53/Rb/p14ARF/p16INKA as a consequence of CDKN2A mutation (very common in inherited melanomas)
- Gene expression regulation (histone acetylation, DNA methylation and RNA interference)
- Apoptosis pathways and apoptosis effectors

High number of somatic mutations and deviations is observed in melanoma.http://cco.amegroups.com/article/view/6721/7550 - B26 Most well- known and frequent among them are BRAF (occurrence in 35-45% cases), NRAS (occurrence in 15-25% cases) and KIT (occurrence in 3-4% of western population cases). Firstly, BRAF mutation is present on the gene coding the serine–threonine protein kinase and is responsible for most melanomas arising from skin without chronic sun damage. This mutation, which most common mutated form is BRAF V600E, is also present in melanomas arising from acral surfaces and melanomas deriving from skin chronically exposed to sunlight. Secondly, NRAS mutations are present in 20% of melanomas with higher prevalence in superficial and spreading melanomas deriving from chronic sun exposure cases melanomas. Nonetheless, NRAS seemed to be involved in melanomas arising from giant congenital nevi and mucosal surfaces. KIT (receptor protein tyrosine kinase) mutation is most commonly present in mucosal and acral melanomas. [24, 25]

Apart from targeted therapies, knowledge of these pathways is giving us a wider range in possible assays in field of diagnosis and prognosis. Several techniques are being used nowadays to specify the features of pathology in order to define the characteristic of individual melanomas, to diagnose and set prognosis, to introduce the appropriate therapy and to monitor the therapy.

Aside from histologic examination, which is widely available and inexpensive, functional proteomic analysis and gene expression arrays are increasing the specificity and sensitivity of the melanoma's diagnosis. These techniques comprise of immunohistochemistry, comparative genomic hybridization (CGH), chromogenic (CISH) and fluorescent (FISH) in situ hybridization, multiplex ligation-dependent probe amplification (MLPA), ligase detection reaction and reverse transcriptase in situ polymerase chain reaction (RT in situ PCR). [22, 23]

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Treatment of malignant melanoma

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Treatment

Treatment of malignant melanoma depends on stage. In stages II and less, the recommended therapy is surgical excision. In stage III, the best treatment is a complete lymphadenectomy, and in stages IIIB and IIIC also a local therapy applying imiquimod, an immune response modifier, can be considered. [1] Stage IV demands systemic chemotherapy.

Pharmacotherapy in malignant melanoma includes targeted drugs, dabrafenib and vemurafenib, for patients with inoperable or metastatic BRAF V600 mutation positive melanoma. According to the fact that malignant melanoma is the most immunogenic cancer, targeted immunotherapy drugs such as ipilimumab are also recommended for previously treated advanced melanoma. Classic cytotoxic chemotherapy with dacarbazine should be considered only if immunotherapy and targeted therapy are not suitable (NICE guidelines – Melanoma: assessment and management). Other drugs that may be considered are also checkpoint therapy monoclonal antibodies such as pembrolizumab or nivolumab, and a MEK inhibitor trametinib.

Ipilimumab

Ipilimumab (trade name Yervoy) is a new agent in melanoma therapy which enhances the immune response by blocking negative signaling from cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4). CTLA-4 is an immune checkpoint molecule that down-regulates pathways of T-cell activation. Generation of an immune signal requires presentation of tumor antigen by major histocompatibility complex (MHC) class I or II molecules on an antigen presenting cell (APC). However, T cell activation and proliferation requires a second signal, typically generated when CD28, a receptor on the T cell surface simultaneously binds to a costimulatory B7 molecules on the APC. Following activation, T cells upregulate and translocate CTLA-4 receptor molecules to the surface, which bind B7 with a higher avidity than CD28. CTLA-4 successfully outcompetes with CD28 to generate an opposing signal that inhibits T cell proliferation and IL-2 secretion. CTLA-4 is therefore a key negative regulator of endogenous T cell-mediated responses, serving as a natural braking mechanism and allowing for a return to homeostasis following an immune response. [3] Ipilimumab, a fully human monoclonal antibody (IgG1) blocks CTLA-4 expressed by activated T-cells to promote antitumor immunity (Figure 1).

Pembrolizumab

Pembrolizumab (trade name Keytruda) is a potent, highly selective humanized monoclonal antibody (IgG), designed to directly block the interaction between PD-1 (programmed cell death 1) receptor, expressed on T cells and its ligands, PD-L1 and PD-L2, without antibody-dependent cell mediated cytotoxicity (Figure 1). The PD-1 receptor is a cell surface receptor, belonging to the CD28 family of T cell regulators, within the immunoglobulin superfamily of receptors. It is expressed upon activation in mature hematopoietic cells, such as T and B cells, natural killer cells and monocytes, after prolonged antigen exposure. Through its binding to PD-L1 and PD-L2. PD-1 receptor downregulates T-cell activation and proliferation, along with downregulation of the expression of the anti-apoptotic molecule Bcl-xL, cytokine expression, and the mTOR pathway. PD-1 receptor is highly expressed on tumor-infiltrating lymphocytes (TIL) in response to tumor antigen expression, and its binding to PD-L1 on either tumor cells or APCs, functions as an immune checkpoint to curb persistent immune response. [4]

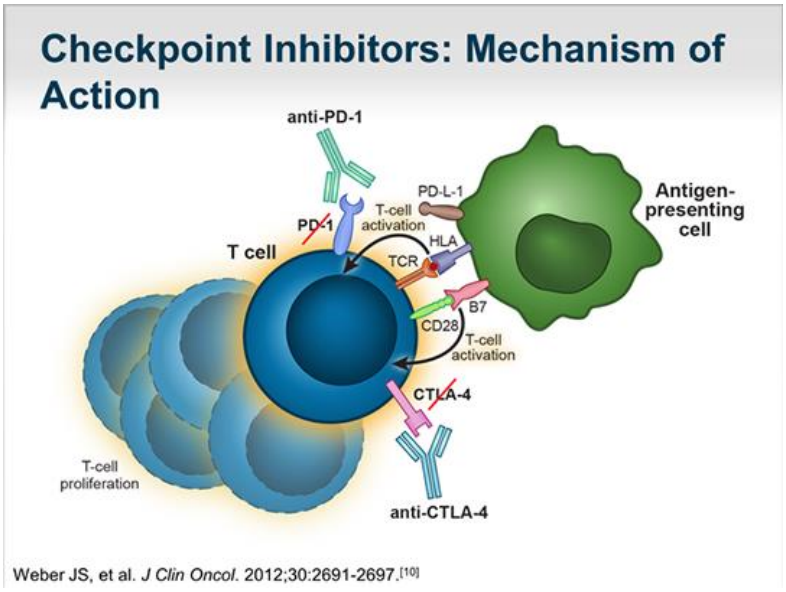

Figure 1. Mechanisms of action - ipilimumab (anti CTLA-4) and pembrolizumab (anti-PD-1)

Target therapy

Target drugs act in a way that they detect and react with the part of melanoma cells that differ them from normal cells. They can be used when chemotherapy doesn't work and they usually have less side effects. (NICE guidelines – Melanoma)

BRAF inhibitors

Vemurafenib, Dabrafenib

About half of all melanomas have changes in BRAF gene on the position 600, Valin being exchanged with Glutaminic acid. These changes result in altered BRAF protein which helps these cells grow. Therefore, in order for therapy to be effective, biopsy samples should be tested to see if cancer cells have previously mentioned BRAF mutation. [8]

Drug is taken two times daily in amount of 940 mg per day. Adjustment of doses is required in case of liver or kidney damage as well as in case of geriatric and paediatric population. Common side effects include sensitivity to the sun, skin tightening, fatigue, hair loss and nausea. Less common but more dangerous ones include severe allergic reactions and heart rhythm problems. [7]

MEK inhibitors

Trametinid, Cobimetinib

These drugs are reversible inhibitors of activation and activity of Mitogen-activated Extracellular Signal-regulated kinase 1 (MEK1) and MEK2. MEK enzymes furthermore regulate extracellular signal-related kinase (ERK) which promotes cellular proliferation. These drugs are commonly combined with BRAF inhibitors and are taken once per day. Common side effects of these drugs are rash, nausea, swelling and sensitivity to sunlight. Even though, heart damage, excessive bleeding and lung problems also may occur. [9]

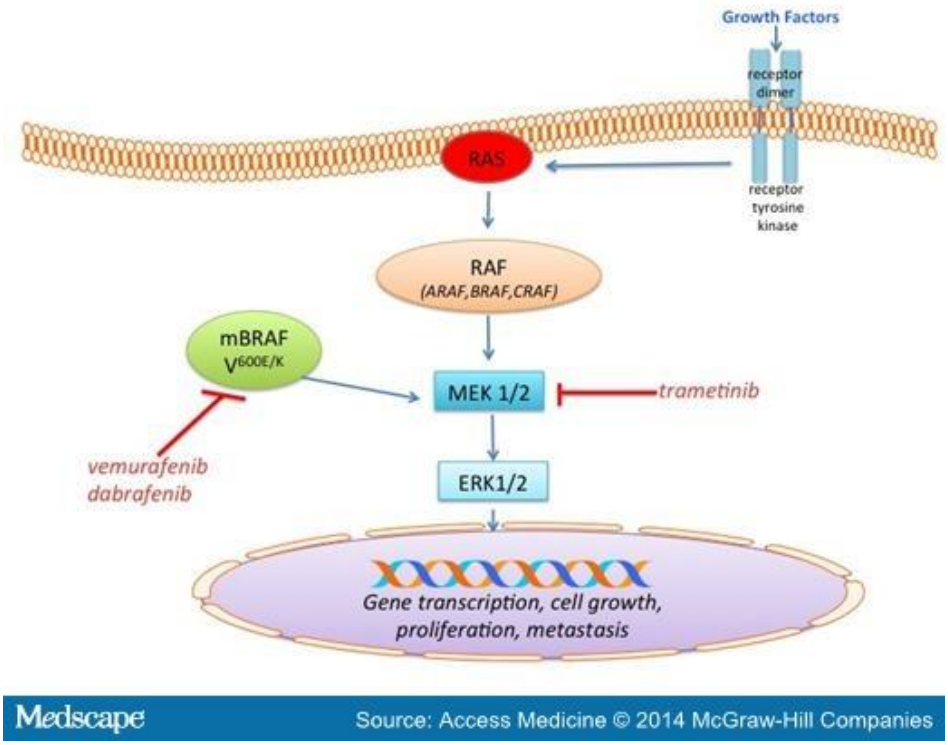

Figure 2. Mechanism of action of vemurafenib and trametinib

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Melanomas: resistance to RAF inhibition; paradoxical activation of MAPK pathway; adverse effect of BRAF inhibition

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Resistance to RAF inhibition: paradoxical activation of MAPK pathway

BRAF mutations, V600E, alternatively V600K/D, are the major driver mutations that lead to malignant melanoma development (Chapman, 2013). Selective RAF inhibitors vemurafenib (formerly designated as PLX4032) and dabrafenib are effective therapy in patients with BRAF ^{V600}-driven melanoma. Those two are one of the most compelling examples of targeted therapy knowing that BRAF gene mutations are responsible for tumorigenic behaviour of over 50% of all cutaneous melanomas. However, clinical responses to targeted anticancer therapeutics are frequently confounded by *de novo* or acquired resistance and patients show signs of disease progression after 6-7 months after the initialisation of therapy with RAF inhibitors (Poulikos et al., 2014; Gibney et al., 2013). Identification of resistance mechanisms is important for generating alternative 'druggable' targets and better treatment for patients with melanoma. (Gibney et al., 2013) In order to do that, understanding of signalling pathways that are implicated in melanoma is substantial.

There have been eight pathways identified in malignant melanoma development: MAPK, AKT/PI3K, c-KIT, CDK, GNAQ/GNA11, MITF, NRAS and P53/BCL* **(**Vidwans et al., 2011**).** There is a lot of cross-talk between these pathways and their downstream effectors. However, two signaling pathways implicated in melanoma are major: the MAPK pathway and the AKT/PI3K pathway which regulate cell growth, proliferation and cell death **(**Vidwans et al., 2011**).**

Several mechanisms mediating resistance to BRAF inhibitors have been described and they can be divided into paradoxical MAPK reactivation and MAPK-independent mechanisms. Paradoxical activation is a rapid recovering of MAPK signalling after BRAF inhibition as a result of depressed feedback inhibition. It includes the up-regulation of bypass pathways mediated by cancer Osaka thyroid kinase (COT), development of de novo NRAS or MEK mutations and variant splicing and/or dimerization of mutant BRAFV600 etc. MAPK-independent signaling that has been associated with BRAF resistance comprises receptor tyrosine kinases (TRK) such as platelet derived growth factor receptor β (PDGFβ), insulin-like growth factor 1 receptor (ILGF-1R), and hepatocyte growth factor receptor (HGFR) (Flaherty et al., 2012). Mechanisms of resistance seem to be BRAF-inhibitor-specific. NRAS mutations and splice variants of BRAF^{V600} mRNA are the two most common mechanisms identified so far. (Chapman et al., 2013)

Expression of BRAF^{V600E} splicing variants is the first resistance mechanism identified. It involves a structural change in BRAF. Different splicing variants of mutated BRAF were identified in cell lines resistant to vemurafenib. The 1.7kb cDNA band that was not present at the parent-cells lacks exons 4-8, compared to full length cDNA of BRAF^{V600E} and codes 61kD protein, called p61BRAF^{V600E}. Exons 4-8 include domains critical for RAF activation-RAS-binding domain (RBD) and cysteine rich domain (CRD). Dimerization of p61BRAF^{V600E} is significantly elevated compared to full- length BRAF^{V600E} which implies that deletion of exons 4-8 promotes dimerization even if the RAS is inactive (Poulikos et al., 2011).

These findings have been confirmed in tumours of patients which were treated with vemurafenib as proven by analyzing tumours from 19 melanoma patients with acquired resistance to vemurafenib.

$BRAF^{V600E}$ transcripts from resistant tumours lacked exons 4-10, 4-8, 2-8 or 2-10 compared to the vemurafenib- naïve tumours. (Poulikos et al., 2011).

Another mechanism of resistance in $B\text{-}RAF^{V600E}$-positive melanomas involves activation of RTK (PDGFRβ)-dependent survival pathway in addition to MAPK or reactivating the MAPK pathway via N-RAS upregulation. These two mechanisms accounted for acquired vemurafenib resistance in 5 of 12 patients in study cohort of Nazarian et al., 2010 and in 4 of 19 patients in the study of Poulikos et al., 2011.

MAPK3K8 (COT/TPL2) also drives resistance to RAF inhibition in BRAF V600E cell lines. Cancer Osaka thyroid kinase (COT) expression is sufficient to activate ERK primarily through MEK-dependent mechanisms that do not require RAF signaling. Cell lines expressing elevated COT exhibit de novo resistance to PLX4720 treatment (Johannessen et al., 2010).

Adverse effects of BRAF inhibition

Besides the resistance, as a complication that interferes with positive outcomes in BRAF-inhibition therapy of patients with malignant melanoma, significant toxicity as well as development of secondary malignancies stands out.

Toxicity of dabrafenib and vemurafenib is relatively mild in majority of patients as compared to classical chemotherapy (Gibney et al. 2013). Common adverse effects include skin events, gastrointestinal symptoms, headache, fatigue, pyrexia (Gibney et al. 2013:4). These adverse effects in most cases lead to dose interruptions or modifications, but the patients usually continue the therapy (Gibney et al. 2013). In only 3% of patient adverse effects are the cause of drug discontinuing (Gibney et al. 2013).

Cutaneous events are more serious and include hyperkeratosis, keratoacanthomas and squamous cell carcinomas (Gibney et al. 2013).

Squamous cell carcinoma (SCC) is the second most common skin cancer among Caucasians (Alam et Ratner, 2001). Risk factors for developing SCC are numerous: the most common is exposure to UV-radiation which causes DNA damage and dysfunction of p53 tumour-suppressor gene (Alam et Ratner, 2001). The SCCs related to the treatment with RAF inhibitors are more differentiated than those developed as a consequence of sun damage (Gibney et al. 2013). In the most cases, SCC develop rapidly after the initiation of the therapy (median 8 weeks) (Gibney et al. 2013). Metastases are not reported and in most cases, the therapy is continued and lesions are managed by excision (Gibney et al. 2013).

The key relation between development of SCC and BRAF inhibitor therapy is a paradoxical activation of MAPK pathway which occurs through the BRAF-inhibitor-mediated formation of RAF dimers (Gibney et al. 2013). This theory is supported by the results of many preclinical studies (Gibney et al. 2013). Also, studies results indicated that MAPK signaling could be a SCC initiator, but it is not required for tumour maintenance (Gibney et al. 2013). This is demonstrated by the ability of MEK inhibitor to suppress SCC development following the BRAF inhibitor therapy, but lack of its effectiveness against established SCC (Gibney et al. 2013). In the cases where paradoxical activation of MAPK pathway occurs, an oncogenic event might arise in cells with pre-existing mutations (Gibney et al. 2013).

Rarely, adverse effects of BRAF inhibitor therapy include the development of secondary melanomas, colonic adenomas and gastric polyps (Gibney et al. 2013).

Considering aforementioned adverse effects, it is a challenge to develop the strategy for prevention of secondary malignancies. Several approaches are proposed:

- Concurrent downstream inhibition (MEK inhibitors combined with BRAF inhibitors) (Gibney et al. 2013).
- Usage of a new class of pan-RAF inhibitors, so-called paradox breakers. This class of drugs blocks the signaling without paradoxical MAPK pathway activation (Gibney et al. 2013; Zhang et al. 2015).
- Usage of synthetic retinoids: these drugs promote cell maturation and differentiation and decrease cell growth and malignant transformation (Gibney et al. 2013).
- Usage of cyclooxygenase-2 (COX-2) inhibitors: investigations of SCC carcinogenesis revealed that the COX-2 inhibitors therapy abrogated increased COX-2 expression and prostaglandin production and mitigates SCC development (Gibney et al. 2013).

Despite the paradoxical activation of MAPK pathway, which is leading to secondary malignicies, the development of BRAF inhibitors represents a major milestone in the therapeutic managemant of diseminated melanoma. With rationally designed drug combinations, the future of patients with BRAF ^{V600E}-driven melanoma continues to look increasingly optimistic (Gibney et al., 2013).

MAPK = Mitogen-activated Protein Kinase,
AKT/PI3K = Protein Kinase B/Phosphoinositide 3-kinase,
c-KIT = CD11,
CDK = Cyclin Dependant Kinase,
GNAQ/GNA11 = Guanine nucleotide-binding protein G(q) subunit alpha/ Guanin nucleotide-binding protein alfa 11,
MITF = Microphthalmia-associated transcription factor,
NRAS = Neuroblastoma RAS,
BCL = B-cell Lymphoma.

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Bone metastatic disease – Epidemiology and molecular mechanisms

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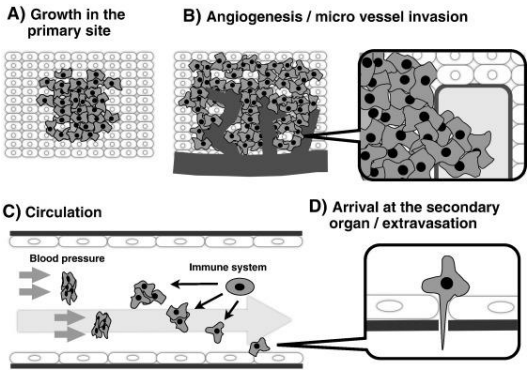
EPIDEMIOLOGY

Bone metastatic disease (BMD) is a type of metastatic cancer in which primary cancer cells have disseminated from the original location and start to interact with bone cells. The consequence is development of bone metastases that cause skeletal-related events (SREs) or skeletal complications like bone pain, hypercalcemia, pathologic fractures and spinal cord or nerve root compression. The pain generally appears at the bottom of the skull, in the neck, lower back, legs and pelvis. (1) When patients are diagnosed with bone metastasis, 22 % have a SRE at that time and almost half of them develop it later in progress of the disease. (1, 2)



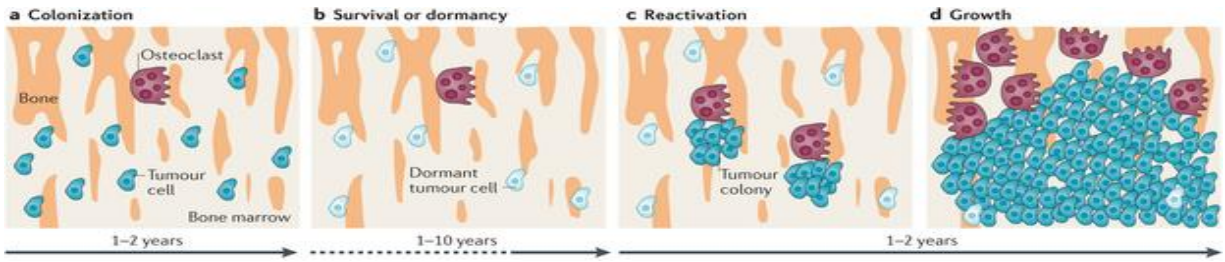
Picture 1: Tumor metastasis in bones (3)

Almost all types of cancers can develop bone metastasis. Only in renal cancer bone metastases occur in only one location, whereas in other types of cancer multiple bone metastases are present. (2) Patients with breast or prostate cancer are most prone to bone metastases with the incidence of about 70 %. These two cancers also have quite high prevalence worldwide and since the patients can now live longer because of better disease management, the prevalence of BMD in these cancers is increasing. (2, 4) Lung cancer is the third most prevalent cancer for BMD. It causes bone metastases in approximately 35 % of the cases. Patients with bladder cancer and renal cell carcinoma have 40 % and around 20 – 25 % chance to develop bone metastases, respectively. Although thyroid cancer has a 60 % incidence for BMD, the prevalence is not as high as in former mentioned cancers. The widest range of incidence for bone metastasis has melanoma, with the stretch of 14 – 45 %. Incidence of BMD caused by gastrointestinal cancer is less than 10 %. (1, 4) In the USA with a population of over 300 million people, around 1.4 million people are diagnosed with cancer every year; half of them have a type of cancer that commonly spreads to bones. Consequently, more than 400.000 patients per year develop BMD. Metastases generally appear in people older than 50 years, which corresponds to the class of primary cancer. (2, 5) The prognosis of the disease is mostly dependant on the pathology of the primary cancer and the presence of visceral metastasis. Due to this reason, the life expectancy of cancer patients at the time of bone metastasis diagnosis varies from only months to a few years. (1) The most variable is prostate cancer (patients live from 1 to 4.5 years); breast cancer has a survival rate of around 2 years after diagnosis. Higher mortality rates have

melanoma, renal cell carcinoma, bladder and lung cancer, visceral metastases and bone metastases of unknown origin with patients surviving 1 year or less. (2, 4)

MOLECULAR MECHANISMS

Metastases are a selective process in which cancer cells from primary site undergo the process of invasion, embolization, survival in the circulation, arrest in a distant capillary bed, extravasation, and re-growth in the microenvironment of the secondary organ, so that only a few phenotypically changed cells survive to form metastases in other organs and tissues in the body. Four stages of metastases development in bone have been described: colonization, survival or dormancy, reactivation and growth. (6, 3) Disruptions in signalling pathways in bone cells have the most important role in etiology of the BMD. The balance between two functions of the bone cells – lysis and genesis, is disturbed, causing different patterns in the metastatic bone lesion, ranging from mostly destructing (osteolytic) to mostly bone forming (osteoblastic) activity. (3) Mechanisms are explained with vicious cycle hypothesis, explaining reciprocal interactions between cancer cells, osteoblasts, osteoclasts and the mineralized bone matrix, that contribute to progression. There is no data whether cancer cells colonize haematopoetic stem cells (HSCs) and/or progenitor niches. (6) Premetastatic niche expresses cell surface ligands and receptors that provide a permissive environment for the migrating cancer cells, supported by growth factors, cytokines and chemotactic proteins presented in the bone marrow. (7)



Picture 2: Process of bone metastasis development (6)

Osteolytic metastasis:

Secondary cancer cells can secrete factors for direct and indirect stimulation of osteoclasts activity – differentiation, proliferation and activation from pluripotent osteoclast precursor cells, causing increased osteolysis. Indirect stimulation is based on the OPG/RANK/RANKL pathway, which regulates osteoclast formation and survival. RANKL (receptor activator of nuclear factor kappa B ligand) is a member of tumor necrosis factor ligand superfamily and is expressed on different types of cells, including osteoblasts, and exists in two isoforms – bound and soluble. RANK is a receptor that is expressed on osteoclasts and dendritic cells that after activation with RANKL activates multiple intracellular signalling processes in osteoclasts to stimulate their proliferation and survival. OPG (osteoprotegerin) is a soluble TNF receptor and is a negative regulator of RANK/RANKL pathway – it binds RANKL and decreases osteoclastogenesis and promotes osteoclast apoptosis. In BMD, tumor cells secrete numerous cytokines and growth factors which stimulate OPG/RANK/RANKL pathway, for example parathyroid hormone-related peptide (PTHrP), interleukins (1, 3, 8, 11) and TNF-α.

Two possible mechanisms exist for bone degradation: up regulation of RANKL expression or down regulation of OPG secretion and consequential increase of osteoclasts activity and bone degradation. Certain cytokines (IL-6, TNF- α, IL-8, etc.) can stimulate osteoclastogenesis independently of RANKL, but the pathways involved are not clear. (7, 10, 11) In response to factors described, osteolytic bone can secrete factors that can attract cancer cells to the bone surface and facilitate their growth and proliferation. (8) Cancer cells invade surrounding tissues to enlarge the tumor mass by secreting proteolytic enzymes such as aspartic, cysteine and serine proteases and matrix degrading metalloproteinases (MMPs). (9) Breast cancer is the main representative of cancer with osteolytic

bone metastases. Breast cancer cells have the ability to secrete PTHrP, a powerful stimulator of bone resorption. Due to TGF stimulation, tumor cells excrete PTHrP. (9)

Osetoblastic metastases:

Tumor cells secrete various pro-osteoblastic factors – cytokines, transcription factors and growth factors that transform normal bone remodelling into predominantly bone forming state. These factors play a role in differentiation, proliferation and maturation of osteoblasts and can also inhibit the activity of osteoclasts. Osteoblasts also secrete growth factors (TGF- β, BMP, VEGF). These factors interact with cancer cells and potentiate their survival and growth, so that the metastatic growth is amplified in a circle. There are three important pathways for osteoblastic metastasis: Wnt signal transduction cascade, the ET axis and the BMP pathway. Wnt glycoproteins promote embryonic and postnatal bone formation and can, when bound to the receptor complex, initiate a number of intracellular signalling cascades with effects on differentiation, survival and activity of osteoblasts. The ET-1 protein (endothelin) is formed by primary cancer cells and, when bound to its receptor, stimulates proliferation of osteoblasts, promotes mineralization, inhibits osteoclast motility and potentiates pro-osteogenic effects of other growth factors. Bone morphogenetic proteins (BMP) are growth factors in skeletal development and postnatal bone repair which stimulate osteoblasts proliferation, activity and survival in BMD. (7, 12) The prostate cancer produces factors like TGF-β, BMPs, thymosin 15 and ET-1, which stimulate osteoblastic bone formation. Most cancers have a mixed activity on the bone. Prostate cancer metastases are also osteoclastic in the beginning, in order to initiate osteolysis and subsequently communicate to osteoblasts to develop osteosclerosis. (9)

CONCLUSION

Bone is the most common site for breast and prostate cancer metastases, which probably means that the bones have a suitable environment and provide conditions that favor colonization and growth of disseminated cancer cells.

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Bone metastatic disease – Diagnosis and treatment

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INTRODUCTION

Bone metastases are a common consequence of advanced-stage cancer originating from various organs, most commonly from breast and prostate cancer in females and males, respectively (1). Furthermore, occurrence of bone metastases represents a negative prognostic factor regarding survival and quality of life (2, 3).

DIAGNOSIS

Bone metastases are classified according to their pathogenesis and growth pattern. Characteristically, metastases are either termed osteolytic, hence the cancer cells destroy and reduce the bone structure, or osteoblastic/sclerotic, if the tumor results in gain of bone mass (4, 5). Correct diagnosis of cancer as early as possible is essential for treatment and prognosis. Therefore, aims of diagnosing bone metastases include early detection, preventing pathological fractures and neurologic problems as a result of spinal impairment, and stopping the tumor progress (4, 5). Diagnostic methods like imaging techniques (radiography, computer tomography (CT), magnetic resonance imaging (MRI), nuclear imaging and angiography), biochemical markers of bone turnover in blood and urine, and histological analysis after biopsy are commonly used (5, 6, 7).

Radiography (electromagnetic radiation) is commonly used when the symptoms, such as bone pain (ostalgia), have already appeared (4). The method results in 2D images and provides information about the tumor site and localization of tumor-associated fragile bone structure (8, 9). Unfortunately, sometimes bone lesions are not visible by using this method and also lytic bone metastases can sometimes be misdiagnosed as osteoarthritis (5). *CT* scanning is more accurate and sensitive than radiography for diagnosing lytic metastatic lesions (5, 7). It is used for visualization of bone structure changes due to metastases of certain tumors (4). Results are shown as 3D images that are useful for assessment of tumor size, shape and stability. However, even advanced lesions of the impaired bone may sometimes not be detected by this method (5, 7). *MRI* is a commonly used method for the detection of metastases in the bone marrow and extra osseous soft tissues (4). In contrast to conventional radiography and CT, MRI does not involve radiation, but rather magnetic field for visualization of bones and other tissues (5). It is the method of choice for the detection of metastatic lesions, and also for evaluating the stage of disease (6, 10). Moreover, it provides information about the tumor mass and eventual bone impairment caused by infection, not by a malignancy. For better detection and illustration of the tumor, MRI as well as CT is commonly combined with conventional radiography (7). *^{99m} Tc skeletal scintigraphy, positron emission tomography (PET) and single photon emission computed tomography (SPECT)* are nuclear imaging methods for evaluation of local bone metabolism in early phases of different types of cancer. ^{99m} Tc skeletal scintigraphy enables detection of metabolic deposits formed by enhanced osteoblastic activity (5). Therefore, it is convenient for detecting asymptomatic osteoblastic bone metastases, as well as for tracking the response to treatment (4, 7). This cost-efficient high sensitivity method allows whole-body examination (5). ^{99m}Tc skeletal scintigraphy in combination with PET and CT is suitable for diagnosing metabolically active bone and soft tissues tumors and small lesions (4, 5). PET with ^{18}F-fluorodeoxyglucose, combined with CT (^{18}F-FDG-PET-CT), allows the visualization of increased glucose metabolism in cancer cells. SPECT is usually combined with CT in order to increase sensitivity and specificity of the test (4, 5).

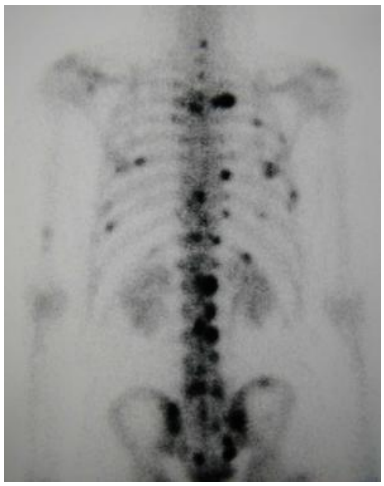

Figure 3: A ^{99m} Tc bone scientoscan of a patient with bone metastasis. (5)

Bone metastases often result in hypercalciemia and increased ALP. There are some *blood and urine tests* that point to possible bone lesions, such as calcium or ALP in blood and N – telopeptide in urine. However, these tests alone are insufficient to prove the presence of metastasis and require additional methods for clarification (7). *Biopsy* is a method used only if the non-invasive detection methods do not give a clear image in diagnosing bone metastases (7).

THERAPY AND TREATMENT

Bone-resident metastatic cancer cells are considered as highly malignant cells and have the ability to interfere with the tightly controlled intercellular signaling network, mainly composed of interactions between osteoblasts (bone-forming cells), osteoclasts (bone-resorbing cells) and other stromal cells. Therefore, bone metastases can either cause osteogenesis or osteolysis (2). Interestingly, much research is conducted based on the so called 'vicious cycle' theory, mainly dealing with the pathogenesis of lytic bone metastases. Thus, osteolysis induces liberation of a variety of growth factors including TGF-β, a promoter of tumor cell proliferation. In turn, tumor cells induce osteolysis, again resulting in growth factor release (1). Currently, bisphosphonates represent the gold standard therapy for lytic bone metastases, while radiopharmaceuticals are a better choice for patients with osteoblastic bone metastases (1,3,10).

Bisphosphonates are synthetic analogs of pyrophosphate (2) that inhibit osteoclast activity and decrease bone turnover caused by bone metastases (1,3,10) (Fig.2). The agents significantly decrease skeletal-related events (SRE) including bone pain (ostalgia), pathological fractures, spinal cord compression and hypercalcemia. Avoiding SRE is one of the main goals in bone metastases management (1). *Zoledronic acid* (ZA) is a second-generation bisphosphonate agent with direct antitumor effects in addition to its antiresorptive functions. With respect to postmenopausal lytic breast cancers, ZA is considered to lower the recurrence of bone metastasis as well as to increase patients' survival (2). Currently, there are several ongoing clinical trials dealing with the combination of bisphosphonates of different potencies and administration routes (2).

Recent gene expression studies revealed several other target sites for bone metastases therapy. Drugs targeting these sites include receptor activator of nuclear factor κB ligand (RANKL) inhibitor Denosumab and Cathepsin K inhibitor Odanacatib, as well as chemokine receptor 4 (CXCR4) inhibitors and TGF-β inhibitors targeting the 'vicious cycle' (1,2). RANKL is a crucial factor for osteoclasts differentiation and activation. Furthermore, RANKL affects the maturation and activation of various immune cells including dendritic cells (DC) (Fig.2). Denosumab is a fully human *monoclonal antibody against RANKL* and is approved for the treatment of osteoporosis, rheumatoid arthritis and cancer, especially osteolytic cancer. Denosumab is discussed to interfere with the biological

functionality of DC, thus to negatively affect the patients' immunity (2). The *Cathepsin K inhibitor* Odanacatib was developed for the treatment of osteoporosis, but seems to be beneficial for reduction of tumor burden in bone metastases too. The drug specifically targets bone resorption while maintaining the number of osteclasts and therefore the bone formation (1,2). With respect to breast and prostate cancer, representing the most common origins of bone-metastases, inhibition of the hormones driving the tumors is a novel therapy strategy. Inhibition of androgen synthesis like estrogen and testosterone for estrogen receptor positive breast and prostate cancer, respectively, are important issues in the management of these diseases. Beneficial response to this therapy is limited to patients with local tumors in the primary site; however, use of hormone inhibitors to treat advanced cancers with bone metastases doesn't seem to be sufficient (2).

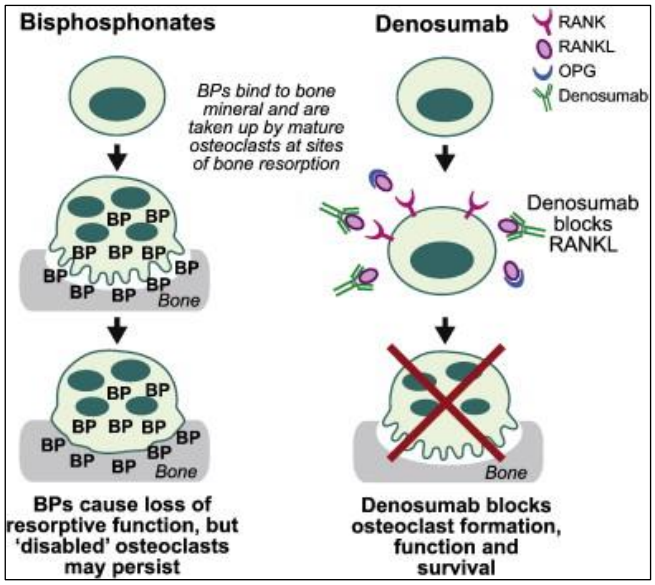

Figure 4: Bisphosphonate and Denosumab target osteolytic bone metastases. They decrease the survival of osteoclasts. Bisphosphonates cause loss of resorptive function in osteoclasts (left), whereas Denosumab specifically antagonizes RANKL, thus blocks osteoclast formation and function (right). Osteoprotegerin (OPG) competes with Denosumab for binding to RANKL and must be considered as negative predictive biomarker for Denosumab efficiency. (12)

Besides the management of bone metastases, targeting the underlying mechanisms and curing of the disease, prevention and treatment of ostalgia, the most common symptom of this disease, is a main goal in order to positively influence the patients' quality of life (10). *Maximal surgical resection and radiation* are included in the general palliate pain treatment. In cases with multiple ostoeblastic bone metastases, radiation therapy can be combined with other treatment options like *radiopharmaceuticals*, chemical molecules coupled to radioactive isotopes. This therapy mainly targets those sites in the bone with highly active turnover, hence the tumor sites, resulting in reduction of ostalgia and shrinkage of tumor size and mass (11). Bones weakened from malignancies tend to break, resulting in pathological fractures. Artificial *cementation* of the bones in combination with mechanical bone fixation and radiotherapy is a commonly used technique to prevent or fix pathological fractures. Amongst all analgesic drugs, *opioids* count as the most effective ones in alleviation of ostalgia. However, the side effects include drug addiction, thus patients must be supervised very precisely (10).

CONCLUSION

To conclude, all diagnostic techniques have their flaws. Radiograpy and CT are relatively insensitive in the detection of early or small metastatic lesions; CT also covers only a small area of the bone. On the other hand, bone scintiscan findings are sensitive but unspecific. Whole-body MRI and PET

scanning are accurate, but expensive. Therefore, combination of different methods is the best option in order to diagnose correctly and efficiently (5). The therapy solely targeting the underlying mechanisms of the tumor is not sufficient in the management of bone-metastases, but treatment of the symptoms is also necessary to achieve better life quality for the patients. (10)

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Leukemias

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INTRODUCTION

Leukemias belong to a broader group of tumors that affect the blood, bone marrow, and lymphoid system, known as tumors of the hematopoietic and lymphoid tissues (1).
The type of leukemia depends on the blood cell type that has become cancerous. Any of the blood-forming myeloid or lymphoid cells from bone marrow can turn into a leukemia cell (2).

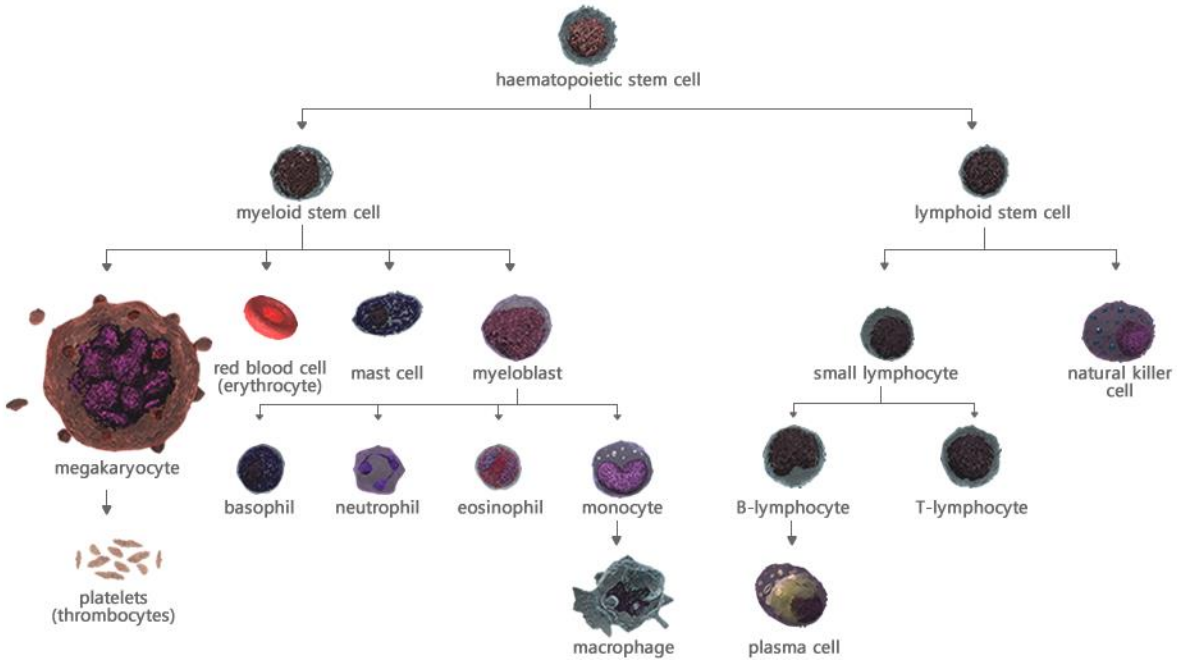

Fig. 1. Hematopoiesis
Source: https://www.hmrn.org/about/classification

Leukemia is either acute or chronic. Acute leukemia is a fast-growing cancer that usually gets worse quickly. Chronic leukemia is a slower-growing cancer that gets worse slowly over time. The treatment and prognosis for leukemia depend on the type of blood cell affected and whether the leukemia is acute or chronic.
Therefore, there are four main types of leukemia — acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) — as well as a number of less common types (3).

EPIDEMIOLOGY

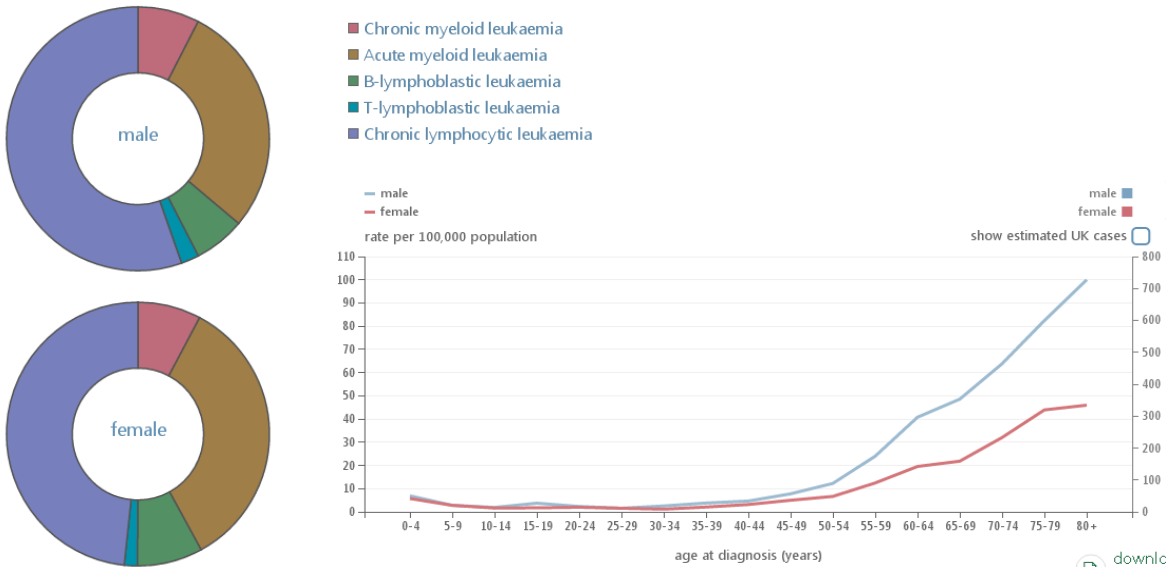

Fig. 2: Distribution of main types of leukemia by men and women and increase of incidence by age
Source: https://www.hmrn.org/statistics/incidence

Leukemia occurs most often in adults older than 55 years, and it is the most common cancer in children younger than 15 years. Incidence for almost all leukemias increases with age.

Acute myeloid leukemia (AML) is a relatively rare cancer, the median age at diagnosis is 63 years. AML accounts for about 90% of all acute leukemias in adults, but is rare in children.

Acute lymphoblastic leukemia (ALL) hits both children and adults, but its incidence peaks between ages 2 and 5 years.

The median age at presentation of chronic myeloid leukemia is 45 to 55 years, although some series report a median age of up to 67 years.

Chronic lymphocytic leukemia is primarily a disease of older adults, with a median age of 70 years at the time of diagnosis. The incidence of CLL increases very quickly with increasing age (3).

MOLECULAR BASIS

The core of the pathogenesis of leukemia, like in the other types of cancers, is genetic disorders. Genetic changes can be introduced by two distinctive mechanisms:

1. The first involves the structural alteration of a normal gene (a proto-oncogene) to generate a novel gene (an oncogene) whose protein product is involved in cellular proliferation, differentiation or survival.
2. The second mechanism involves the loss or inactivation of genes, known as tumor-suppressor genes or anti-oncogenes, whose proteins suppress cancer.

Different types of leukemias are differentiated by the alterations in members of specific genes. In general, certain oncogenes are activated in a wide variety of cancers, whereas others are restricted to certain tissues and tumor types. The genes potentially leukemogenic in hematopoietic cells can be grouped into five families:

1. Genes that convey growth-stimulating signals from cell membrane to the nucleus;
2. Genes that activate transcription (the protein products of these genes bind to specific DNA sequences near target genes and enhance the synthesis of messenger RNA);
3. Genes involved in tissue differentiation;
4. Genes involved in programmed cell death;
5. Anti-oncogenes that may normally function to suppress tumor development (4).

Myelogenous leukemias

The genetic basis for myelogenous leukemias is chromosomal translocation. Approximately 90% of patients with chronic myelogenous leukemia have an acquired genetic abnormality, the Philadelphia chromosome (Ph).

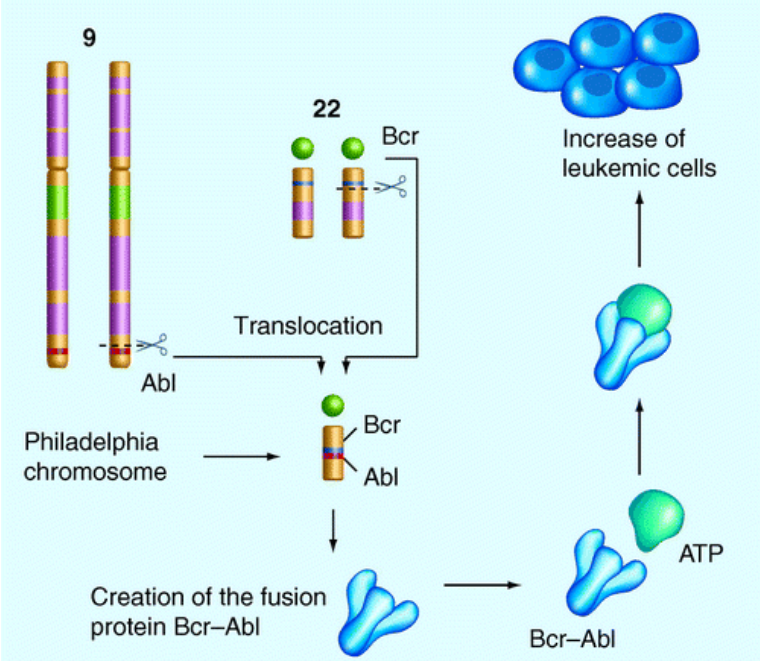

Fig. 3: Philadelphia chromosome
Source: Abl tyrosine kinase inhibitors for overriding Bcr-Abl/T315I: from the second to third generation.

The Ph is a shortened chromosome 22 resulting from a reciprocal translocation between the long arms of chromosome 9 and 22 t(9;22q34;q11). The proto-oncogene c-ABL is transposed from its normal position on chromosome 9 to M-BCR region on chromosome 22. By this translocation the new, fusion gene BCR-ABL is formed. The new gene encodes $p210^{BCR/ABL}$, an oncoprotein that has increased tyrosine kinase (TK) activity and increased binding to the actin cytoskeleton compared with the p145 Abelson protein, both of which contribute to transformation. The presence of $p210^{BCR/ABL}$ causes growth factor independence and leukemic cell growth in hematopoietic cell lines. Increased $p210^{BCR-ABL}$ protein levels in advanced disease further increases the resistance of the leukemic clone to apoptosis, resulting in tolerance to the genetic errors accumulating in the malignant clone. Subsequent dominance of one or more clones finally leads to culmination in fatal blast crisis (5).

Specific M3 subtype of acute myelogenous leukemias is ***acute promyelocytic leukemia*** (APML, APL). In APL there is an abnormal accumulation of immature granulocytes called promyelocytes. Over 95% of cases are characterized by a balanced translocation between chromosome 17q21, which encodes the retinoic acid alpha receptor gene (*RARA*) and chromosome 15q22. This leads to an abnormal fusion protein called PML-RARA. The fusion protein binds with enhanced affinity to sites on the cell's DNA, blocking transcription and differentiation of granulocytes. It does so by enhancing interaction of nuclear co-repressor (NCOR) molecule and histone deacetylase (HDAC) (6).
The retinoic acid alpha receptor gene (*RARA*) is mainly expressed in hematopoietic cells and has an important role in regulating gene expression. In the absence of retinoid acid, *RARA* is bound by nuclear corepressor factor, and this causes transcriptional repression. In the presence of retinoic acid, *RARA* is activated and terminal differentiation of promyelocytes occurs (7).

Lymphocytic leukemias
Lymphocytic leukemias have different genetic pathogenesis comparing to the myeloid leukemias. Chromosomal translocations are rare and no unifying mutations have been identified.

However, *mutation status* of V genes is used for the categorization of lymphocytic leukemias, as well as for the prognosis of the disease. Unfavorable prognosis is associated with the expression of non-mutated immunoglobulin heavy variable genes (UM-IgH-VH) and high level of 70 kD zeta-associated protein (ZAP-70). Two different cell profiles of B lymphocytes are possible: with mutated and non-mutated V-genes. In both cases, interactions between antigens and B-cell receptors of adequate affinity induce clonal amplification. The initial inducing lesion provides the marked cell with a growth advantage over other clones stimulated by the same or other antigens. Additional DNA mutations cause the cells to cross the boundary from “normality” to “leukemia”. Continued cycling leads to other genetic changes (*e.g.*, deletions at 13q, 11q, and 17p or duplication of chromosome 12) that determine the course of the disease. These changes appear to occur more frequently in patients with non-mutated CLL (8).

Chromosomal alterations are detected in >80% of cases and can discriminate patients with different outcomes:

- low risk, normal karyotype or 13q deletion;
- intermediate risk, 11q deletion or trisomy 12 and
- high risk, 17p deletion or complex karyotype.

Recent studies demonstrated that microRNAs are involved in an intricate interplay with B-cell receptors (BCR) signaling and microenvironmental stimuli. BCR signaling and immunoglobulin production can be regulated by microRNAs while the expression of certain microRNAs can be altered via BCR stimulation. Researchers have focused on the molecular impact of deregulation of microRNA expression in CLL. miR-15/16 cluster, miR-34b/c, miR-29, miR-181b, miR-17/92, miR-150, and miR-155 family members, the most deregulated microRNAs in CLL, were found to regulate important genes, helping to clarify molecular steps of disease onset/progression (9).

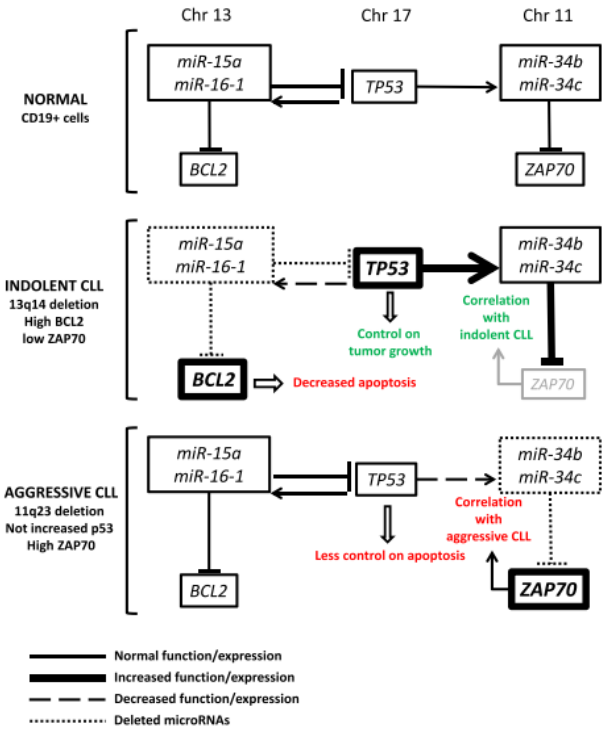

Fig. 4: Interplay between microRNA expression and chromosomal aberration in CLL
Source: Balatti V, Pekarky Y, Croce CM. Role of microRNA in chronic lymphocytic leukemia onset and progression. J Hematol Oncol 2015; 8:12

DIAGNOSIS

Due to many types of leukemias, diagnosis of this disease is not easy at all. Right diagnosis is the most important for the successful treatment of each type of leukemia. There are many different methods which help in diagnosing leukemia such as blood count (where we expect access of abnormal white blood cells, also sometimes leukemic blast are seen, and there is decreases in platelets and red blood cells), marrow aspiration and biopsy (enables measurement of percent of blast and basophils), cytogenetics or fluorescent *in situ* hybridization (FISH). FISH is a test that "maps" the genetic material in human cells, including specific genes or parts of genes which helps us to propose a diagnosis CML (1).

The most used techniques are: flow cytometry and microscopy of bone marrow or blood. Light microscopy is used for examinations of either marrow or blood. Flow cytometry, or one of its modification called fluorescence-activated cell sorting (FACS) is sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. The cell suspension enters in the center of a narrow, rapidly flowing stream of liquid. The flow is arranged so that there is a large separation between cells relative to their diameter, difference in their granulation and fluorescence of the labeled antibodies bound to the specific antigenes. An advantage of this method is that various antigens can be marked by fluorescence labeled antibodies (10).

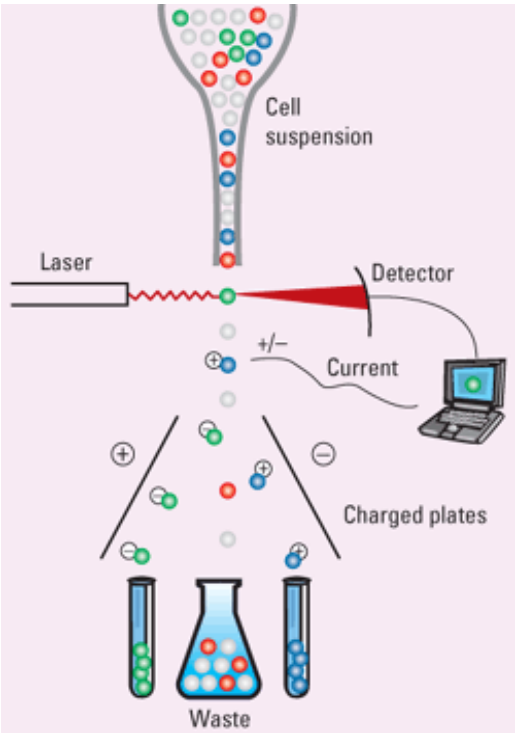

Fig.5: Scheme of mechanism of flow cytometry.
Source:
http://pubs.acs.org/subscribe/archive/mdd/v07/i11/html/1104feature_willis.html

TREATMENT

Therapy of leukemias combines more types of treatment: conventional chemotherapy, immunotherapy, allogeneic stem-cell transplantation, radiation *etc*. Different drugs can be combined in therapy such as antracycline, glucocorticoids, vinca alkaloids, hydroxyurea and tyrosine-kinase inhibitor (imatinib). The most used ones are antimetabolites (cytarabine). Anti-metabolites masquerade as a purine (azathioprine, mercaptopurine) or a pyrimidine, chemicals that become the building-blocks of DNA. They prevent endogenous substances to incorporate into DNA during the S phase (of the cell cycle), stopping normal development and division (11). There is a special therapy approach is for acute promyelocytic leukemia M3. Reduced affinity of the retinoic acid receptor alpha (RARα or RARA) for retinoic acid binding can be overcome with increased intake of retinoic acid, thus the retinoic acid is used as a drug.

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Lymphomas

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Introduction

Lymphomas are a group of malignant tumors of lymphoid tissue. There are two main types of lymphomas: Hodgkin and non-Hodgkin. Hodgkin lymphoma is a less common type and is characterized by the presence of Reed-Sternberg cells. Every other type is classified as non-Hodgkin lymphoma, which is further divided into more than 60 subtypes.(1) Both Hodgkin and non-Hodgkin lymphomas can occur in children and adults, and prognosis and treatment depend on the stage and type of cancer.

Signs and symptoms

The primary manifestation of lymphomas is lymphadenopathy, or swollen lymph nodes. Swelling of lymph nodes is one of the differences between lymphomas and leukaemias, and it is also important to note that the lymph nodes may be painless, while pain is usually present in infections. Systemic symptoms (or B symptoms) are common for both Hodgkin and non-Hodgkin lymphoma. Those are fever, night sweats and weight loss. Other possible symptoms include tiredness, loss of appetite, itchiness of skin and cough and shortness of breath.(2) Symptoms of lymphoma are usually non-specific and resemble greatly the symptoms of other illnesses, especially viral infections. The main difference is persistence.

Epidemiology

International Agency for Research on Cancer has compiled the data about the incidence and mortality of lymphomas. According to their research from 2012, in Europe age standardized rate of incidence per 100,000 people for Hodgkin lymphoma (HL) is 2.3, while for non-Hodgkin (NHL) it's 9.8. European mortality rate for Hodgkin lymphoma is 0.5, and for non-Hodgkin 3.5. Out of the European countries, Croatia has the highest incidence rate of Hodgkin lymphoma (3.3 out of 100,000 people), but Greece has the highest mortality rate (1.1 out of 100,000 people). For non-Hodgkin lymphoma, the incidence rate is the highest in Finland (15.6) and the mortality rate is the highest in Malta (5.3). The incidence rate for HL is roughly the same for men and women (2.5 and 2.1 respectively), while NHL is more common in men (incidence rate of 11.9, whereas for women it's 8.0).(3) Hodgkin lymphoma affects both children and adults, but it is very rare in children under the age of 5. It is most common in two age groups: ages 15 to 40 (particularly young adults in their 20s) and after the age of 55.(4) NHL is far more common in adults than in children. It is the sixth most common cancer in both men in women. The average age of the diagnosis of NHL is 65 years.(5)

Diagnosis and staging

The diagnosis of lymphomas is usually made by a lymph node biopsy. Most commonly, the biopsy will be executed by removal or excision of a lymph node in the neck, under the arm or in the groin.(6) When performing a biopsy, it is important to remove a large sample of tissue, preferably an entire lymph node, to ensure that the malignant cells are captured and correctly identified. Immunophenotypization of the excised tissue is performed using a panel of antibodies. Flow cytometry is used in the assessment of cell type and lineage. In most lymphoid proliferations, morphological assessment and immunophenotyping are sufficient to establish a diagnosis. In a minority of difficult cases molecular investigation may be required. The techniques used are polymerase chain reaction (PCR), real-time quantitative PCR (RQ-PCR), conventional cytogenetics and fluorescence *in situ* hybridisation (FISH).(7)

Various scans are used as secondary diagnostic tools, such as computed tomography (CT or CAT), magnetic resonance imaging (MRI), positron emission tomography (PET) or integrated PET-CT scan.(8) Because lymphomas often spread to the bone marrow, bone marrow aspiration and biopsy may be important in diagnosis and staging. Blood tests are often performed as additional tests. They may include a complete blood count (CBC), erythrocyte sedimentation rate (ESR or "sed rate") and liver and kidney function tests.

Staging helps us to identify the location of the tumor and if the disease has spread from original site to other parts of the body. Stage 1 means that lymphoma is in only one group of lymph nodes or in one body organ. Stage 2 means there is lymphoma in 2 or more groups of lymph nodes or an organ and 1 or more groups of lymph nodes. But all lymphomas must be on the same side of the diaphragm. Stage 3 means that lymphoma is on both sides of the diaphragm. Stage 4 means that many groups of lymph nodes contain lymphoma and it has spread to other body organs (*e.g*. liver, kidney, lungs). If patient has B-symptoms (night sweats, fever and loss of weight), the letter B is put after the stage. If the patient does not have these symptoms, the letter A is put after the stage.(9) Staging is usually performed by PET-CT scanning.

Hodgkin lymphoma

HLs are clonal B cell neoplasms. We recognize two types of HL: classical HL, which is further divided into 4 subtypes based on the appearance of the cells and the composition of background cellular infiltrate, and nodular lymphocyte-predominant HL (NLPHL). It is important to emphasize that the malignant cells are rare in the involved tissue, accounting for only around 1% of the tumor mass. The malignant cells are embedded in an infiltrate of reactive hematopoietic cells, which are considered to be non-malignant. But the clinical features, cell of origin and molecular pathogenesis of NLPHL and cHL are different.

Classical HL (cHL) is the most common type of Hodgkin lymphoma. It occurs in about 95 % of cases. cHL is diagnosed when characteristic abnormal lymphocytes, known as Reed-Sternberg cells, are found. Reed-Sternberg cells are giant multinucleated tumor cells with unusual immunophenotype that do not resemble any other normal cell in the body. If cell is mononucleated, they are called Hodgkin cells.(10) The most common type of cHL is nodular sclerosis HL. It affects up to 80 % of people diagnosed with cHL. In addition to Reed-Sternberg cells, there are also bands of connective tissue in the lymph nodes. About 6% of people diagnosed with cHL have lymphocyte-rich cHL. The lymph nodes contain Reed-Sternberg cells and many normal lymphocytes. Mixed cellularity HL usually develops in the abdomen and consists of many different cell types including large numbers of Reed-Sternberg cells. Only about 1% of people with cHL have lymphocyte-depleted Hodgkin lymphoma subtype. The lymph nodes contain almost all Reed-Sternberg cells.

The second type of HL is nodular lymphocyte-predominant HL which is present in about 5% of patients with HL. Nodular lymphocyte-predominant Hodgkin lymphoma is more similar at the protein and genetic level to B-cell non-Hodgkin lymphoma because cells have marker CD20 on the surface of the lymphoma cells. CD20 is protein usually found in people diagnosed with B-cell NHL. The malignant cell is the lymphocyte-predominant cell or ‘popcorn’ cell, so-named because of its characteristic multilobated morphology.(11)

Different from B-non-Hodgkin lymphomas, in Hodgkin lymphomas single cytogenetic abnormalities have not been found. There are many chromosomal imbalances, including recurrent gains of 2p, 9p, 16p, 17p, 17q and 22q and loss of 13q. Wealso know other minimally gained and lost regions (*e.g.* genes involved in NF-κB signaling). Patients who have gains of 16p11.2-13.3, which contain the multidrug resistance gene ABBC1, have poorer disease survival.

Approximately one third of HL is associated with EBV, so the virus is believed to play an important role in disease pathogenesis. In EBV-associated tumors, EBV is detected in all the Reed-Sternberg cells. The proof that infection has occurred prior to the transformation is that viral infection is clonal. Reed-Sternberg cells also express EBV proteins (EBNA-1, LMP-1, LMP-2 antigens) and EBV-encoded RNAs. EBNA-1 and LMP-1 are essential for transformation of B cells by EBV. EBV gene products contribute to Reed-Sternberg cell survival, proliferation and reprogramming.(12) A lot of signaling pathways are deregulated in the Reed-Sternberg cell. The most known is the NF-κB pathway which plays essential role in Reed-Sternberg cell survival. Infection with EBV probably leads to NF-κB activation.(13)

Non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of malignant tumors which includes many subtypes, each with distinctive features. The WHO classification from 2008 has divided NHL into two groups based on morphology and cell lineage: those of B-cell origin and those of T-cell/natural killer (NK)–cell origin. Within those categories, lymphomas are subdivided according to the stages of differentiation.(14)

NHLs may result from chromosomal translocations, infections, environmental factors, immunodeficiency states and chronic inflammation. The accumulation of mutations affecting proto-oncogens and tumor suppressor genes results in formation of immortal rapidly proliferating cells. Proto-oncogens are transformed into oncogens by chromosomal translocations, and tumor suppressors are inactivated by chromosomal deletion or mutation. In addition, certain viruses can introduce oncogenic genes into the genome of the cell. Some genetic modifications are associated with specific NHL subtypes, and are reflected in the presence of specific markers used for classification.

The most common chromosomal abnormality is the t(14;18)(q32;q21) translocation, in which the bcl-2 apoptotic inhibitor oncogene at chromosome band 18q21 is joined with heavy chain region of the immunoglobulin (Ig) locus within chromosome band 14q32. This translocation occurs in 85% of follicular lymphomas. The t(11;14)(q13;q32) translocation results in the overexpression of bcl-1 (cyclin D1/PRAD 1), a cell-cycle regulator on chromosome band 11q13, which is associated with mantle cell lymphoma. The 8q24 translocations that lead to c-myc deregulation occur in Burkitt's lymphoma and other types caused by HIV infection. Mucosa-associated lymphoid tissue (MALT) lymphomas are commonly caused by two chromosomal translocations. T[11;18][q21;q21]) translocates the apoptosis inhibitor AP12 gene with the MALT1 gene, resulting in the creation of an aberrant fusion protein. T(1;14)(p22;132) involves the translocation of the bcl-10 gene to the immunoglobulin gene enhancer region.

Viruses can cause uncontrolled B- or T-cell stimulation because of their ability to induce chronic antigenic stimulation and cytokine deregulation. Epstein-Barr virus (EBV) is associated with Burkitt's lymphoma, Hodgkin disease, lymphomas in immunocompromised patients and sinonasal lymphoma. Other viruses associated with various subtypes of NHL are Human T-cell leukemia virus type 1 (HTLV-1), Hepatitis C virus (HCV) and Kaposi sarcoma–associated herpesvirus (KSHV). The only bacteria known to cause lymphomas is Helicobacter pylori, which is associated with gastric mucosa-associated lymphoid tissue (MALT) lymphomas.

Other factors in the development of NHL include exposure to chemicals (*e.g.*, pesticides, herbicides, solvents, organic chemicals, wood preservatives, dust and hair dye), chemotherapy, radiation, immunodeficiency states, Celiac disease and autoimmune disorders.(15)

NHL can be divided into two prognostic groups according to growth rate. Slow-growing types of lymphoma are called indolent or low-grade. This group includes follicular lymphoma (the most

common kind), mantle cell lymphoma, marginal zone lymphomas (such as MALT lymphoma) and various others. Quickly growing NHL are referred to as aggressive or high grade. They include diffuse large B cell lymphoma (DLBCL), Burkitt's lymphomas, peripheral T cell lymphoma, lymphoblastic lymphoma *etc*. Over time, low grade lymphomas may change into a high grade type lymphoma.(16)

Treatment

Treatment of lymphoma in most cases consists of either chemotherapy, radiotherapy or the combination of the two. Oncologist (after discussion with the patient) chooses the therapy based on the stage of the disease, the patient´s age, location of lymphoma, general health, other possible diseases and the blood level of lactate dehydrogenase (LDH), which increases with amount of lymphoma. In chemotherapy cytotoxic drugs are used to destroy cancer cells or prevent their proliferation. Normally several chemotherapeutic drugs are used together and the combination usually includes antimetabolites, because of their success in inhibiting proliferation of cells. Radiotherapy is a local treatment in which diseased areas are exposed to ionizing rays. Another way of treatment is immunotherapy with monoclonal antibodies. They bind to the specific antigen on the surface of lymphocytes and cause their death through immune mechanisms. Unlike chemotherapy, this type of treatment is more specific and consequently, we can avoid the side effects.

For most patients with HL the treatment is very successful. Patients with early favorable HL (stage 1 or 2 and no adverse risk factors such as B symptoms, extranodal disease or bulky disease) are treated with radiation therapy alone. Radiation may also be used in the early stages of the HL type of nodular lymphocyte predominance, in order to achieve recovery. Patients with early unfavorable HL are prone to relapses and are treated with a combination of radiotherapy and chemotherapy. The most commonly used drug combinations are ABVD: doxorubicin, bleomycin, vinblastine, and dacarbazine and BEACOPP: bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone. Advanced (stage 2 and 3) HL is treated with ABVD and MOPP: mechlorethamine, vincristine, procarbazine and prednisone, but the treatment is more aggressive, higher doses are used and the dosing intervals are shorter. Stem cell transplantation may be used in cases when lymphoma relapses.(17)

Treatment of non-Hodgkin lymphoma depends on the histologic type and stage. Asymptomatic patients with indolent NHL are usually not treated but continually monitored, and the treatment begins when they develop symptoms or problems associated with the disease. Radiation therapy is usually the first choice. Early stage (stage 1 and 2) indolent NHL can be treated effectively with radiation therapy alone. When radiation treatment is not preferred, patients can be treated with rituximab (an anti-CD20 monoclonal antibody), alone or in combination with standard chemotherapy.

Optimal treatment of advanced stages of indolent NHL is controversial because of low cure rates with the current therapeutic options. Patients in advanced stages show a continuous rate of relapse, but they usually respond well to treatment and achieve long term remissions. The aim of the treatment is to prolong, as much as possible, periods of life which can be lived fully, without medications. Therapy options include rituximab, obinutuzumab, idelalisib, bendamustine, purine nucleoside analogs, alkylating agents, combination chemotherapy or radiolabeled monoclonal antibodies (yttrium-90–labeled ibritumomab tiuxetan). Numerous clinical trials are in progress to settle treatment issues. The approaches currently under trial are intensive therapy with chemotherapy and total-body irradiation (TBI) followed by autologous or allogenic bone marrow transplantation (BMT) or peripheral stem cell transplantation (PSCT), and the use of idiotype vaccines and radiolabeled monoclonal antibodies.

Aggressive lymphomas have to be treated immediately. The aim of such treatment is recovery and it depends on the stage of the disease. Standard treatment for aggressive stage 1 and 2 NHL is R-CHOP

(rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) with or without radiotherapy. R-CHOP is also standard for advanced stages of aggressive NHL, but other combination chemotherapy is also used. Bone marrow transplantation (BMT) is the treatment of choice for patients whose lymphoma has relapsed.(18)

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What you need to know about prostate cancer and current ways of its diagnostics

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INTRODUCTION

The most widely diagnosed male cancer in the Western world and the second leading cause of cancer-related deaths is prostate cancer. There are many risk factors involved in its development that are both environmental and genetic. Prostate cancer is highly prevalent and clinically heterogeneous, therefore clinicians must differentiate between indolent and more aggressive forms. Risk classification system characterizes seriousness of the disease and help guide appropriate treatment recommendations. Different diagnostic approaches will be mentioned, from screening methods to various biomarkers and genomic-based tests that are currently being researched.

EPIDEMIOLOGY

Prostate cancer (PCa) is the fifth most common cancer worldwide, the second most common cancer among men in the United States and most common non-skin cancer diagnosed among American males, affecting one in six men over the course of their lifetime. More than 200 000 patients are diagnosed annually and it is the second leading cause of cancer-related deaths in American men. The incidence spiked in the early 1990s because of the prostate-specific antigen (PSA) screening with which they have successfully diagnosed most of the previously undetected prostate cancer patients in the population. At the same time they have become able to diagnose PCa earlier-that is at local stages and incidence of metastatic diseases has decreased [1]. Mortality rates have been declining because of earlier detection, more aggressive treatment [2] and screening overdiagnosis of preclinical prostate cancers which may never progress clinically.

The major risk factors for the development of PCa are race/ethnicity and geographical location, advanced age, inherited susceptibility, hormonal factors and environmental factors such as diet. Incidence is highest in Scandinavia and North America (236.0 per 100000 men) and lowest in Asia (1.9 cases per 100000 annually) [3,4]. The highest mortality rates are found in the Caribbean and the lowest in Asia. Race is a major risk factor with respect to both incidence and mortality, and is for unknown reasons the highest in African-Americans.

90% of diagnoses occur at the age of 55 years and above (around 65 on average) with life expectancy of about 17 years. Older men are more likely to be diagnosed with high-risk PCa. Risk of PCa doubles for a male with one affected first-degree relative and is further increased with more than one affected relative [5-9]. In terms of diet, vitamin E, lycopene (a carotenoid with potent antioxidant properties found in tomato based products), selenium and soy may exert a protective effect. On the other hand, diets rich in fat and red meat, especially well-done meats, may exert a promotional effect [10-13]. Positive associations between high calcium intake and increased incidence and mortality from PCa have also been reported [14-16].

Androgens have an important role in development of PCa. The incidence is significantly decreased by inhibiting conversion of testosterone to dihydrotestosterone. Another important risk factor are higher concentrations of insulin-like growth factor-1 (IGF-1), which normally promote proliferation and apoptotic inhibition of normal prostate cells [17]. IGF-1 levels can be influenced both by diet or

genetics, hence the reason for higher or lower rates of PCa in certain populations and countries. BRCA1 and BRCA2 mutations increase the risk of developing PCa. BRCA1 (17q21) double the risk and BRCA2 (13q12) mutation carriers have a five to sevenfold increase in risk, an early onset of disease, a worse prognosis and a higher Gleason score [18-23].

It is very important that clinicians differentiate indolent tumours from those that are more aggressive. This way there is no overtreatment with the first and no undertreatment of the latter tumours. The current classification system was formed by the National Comprehensive Cancer Network (NCCN) which stratifies men into very-low, low-, intermediate-, high-, and very-high-risk groups, each having a different treatment approach. It is based on general tumour stage, Gleason score and PSA level. Gleason score is based upon PCa microscopic appearance and it ranges from 2 to 10, with 2 representing the most well-differentiated tumours and 10 the least-differentiated tumours. For example, men with low risk PCa comprise the majority of patients. PCa is localized to the prostate with a Gleason score ≤6, low-volume disease and serum PSA ≤10 ng/mL. Treatment options for low-risk PCa would be radical prostatectomy, external beam radiotherapy, brachytherapy and less commonly cryotherapy and high intensity focused ultrasound [24-29].

We have to determine on an individual basis whether treatment is at all appropriate. A 50-year-old man has a lifetime risk of 42% of developing PCa, but only a 9.5% risk of developing the disease clinically and being diagnosed and a 2.9% risk of dying from PCa [30]. If the treatment has not begun until the man develops clinical signs of progression, it is called watchful waiting and is sometimes used for older men with shorter life expectancies or with comorbidities, where curative treatment could pose a risk to person's health. It is appropriate for men who have less than 15 years of life expectancy, as PCa seems to rapidly progress after 15 years.

DIAGNOSTICS

Current course of diagnostics

Most prostate cancers are found during screening with a prostate-specific antigen (PSA) blood test, a digital rectal exam (DRE) or transrectal ultrasound (TRUS). DRE is a part of basic examination in men after 50 years of age. Doctor describes size and consistence and deviation from the normal is indication for biopsy [31,32].

PSA is secreted into the seminal fluid by luminal epithelial cells of the ducts and acini in the prostate. In small quantities it is present in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer or other prostate disorders such as prostatitis and BPH (benign prostatic hyperplasia). PSA allows finding prostate cancer when the disease is not yet tactile with DRE [33,34].

In USA FDA has approved the PSA test for annual screening of prostate cancer in men of age 50 and more. This kind of testing has resulted in earlier PCa detection, potentially in more curable stage. But there is also a negative side, because usage of PSA screening has led to overdetection, overtreatment and to an increase in the rate of negative biopsis. In Slovenia there is no national programme for screening, diagnostic approaches are used only when clinical signs are presented [38].

TRUS is a diagnostic procedure where doctor inserts a small probe in your rectum to the intestine and examines the prostate. The probe gives off sound waves that enter the prostate and create echoes. The probe picks up the echoes and a computer turns them into a black and white image of the prostate. Ultrasonicly suspicious changes are visible only in half of the patients with prostate cancer [31,32].

If cancer is suspected based on results of screening tests, biopsy is needed to confirm the diagnosis. Biopsy is the removal of small pieces of the prostate for microscopic examination. They are routinely done and usually do not require hospitalization. The most common is core needle biopsy which is usually done by urologist. Most of them take about 12 core samples from different parts of the prostate. Samples are send to the lab where are examined if they contain cancer cells or not [32].

TUMOUR MARKERS

It has been mentioned before that PSA – mostly used tumour marker enabled earlier PCa detection at potentionally more curable stage. Nevertheless PSA screening has led to overdetection and overtreatment, because PSA is not cancer-specific or a compensation for the behaviour of prostate cancer [38].

Companies all over the world have developed and are still developing different biomarkers and genetically-based tests for prostate cancer diagnosis, staging, prognostication and monitoring. A lot of molecular tests and algorithms have been evolved to improve pretreatment, to enhance diagnostics accuracy and to distinguish among aggressive and indolent disease to facilitate therapeutic decisions [38].

Nowadays blood and urine-based prostate cancer biomarkers help to decide if biopsy is necessary or not. This kind of tests are inexpensive and analytes can be measured by automated methods as is multi-analyte immunoassay. Such biomarkers are urine prostate cancer antigen 3 (PCA3) score, prostate health index (phi) and the four kalikrein panel (4K score) that can also be combined with other assays (Tabel 1) [38].

Numerus genomics and proteomic test are already commercially available but majority of them have not been approved by FDA. Based on the results of this tests doctors can more easily decide who to rebiopsy and to reduce the number of unnecesary biopsies. Some of this test are: confirm MDx, PCMT, Oncotype DX, Prolaris, ProMark, etc. Assays base on quantitative RT-PCR, multi-gene RT-PCR and other molecular techniques. Objects of interest are different genes, mtDNA or proteins (Table 1). Some of this tests are also useful to distinguish between aggressive and indolent tumours and to help doctors decide who to keep under surveillance and who to treat [38].

Table 1: Novel tests for prostate cancer detection

Assay	**Marker description**	**Assay type**	**FDA approved**
PCA3 score	PSA and PCA3 mRNA	In vitro RNA TMA assay	Only when repeat biopsyis considered
phi	PSA, FPSA, p2PSA	Multi-analyte immunoassay	No
4K score	Total PSA, fPSA, intact PSA, hK2	Multi-analyte immunoassay	No
Confirm MDx	3 genes	Quantitative methylation specific PCR	No
PCMT	mtDNA deletions	Quantitative PCR	No
Oncotype DX	17 genes	RT-PCR	No
Prolaris	46 genes	RNA expression	No
ProMark	8 proteins	Immunofluorescent imaging	No

Samples

For discovery of biomarkers in prostate cancer different kind of biological sources can be used.

1. Blood

Serum and plasma are the fractions that are most frequently used for routine blood testing in hospitals and clinics as they contain many proteins that are synthesized, secreted or lost from the cells and tissues throughout the body. In fact, the majority of serum or plasma proteins are made up of a few high abundant proteins such as albumin, immunoglobulins, alpha-1-antitrypsin and haptoglobins which can mask the presence of potentially significant low abundant proteins. This is the reason why several fractionations, depletions and enrichments are used to enhance detectability of low abundant proteins [33,35,36,37].

2. Tissue

In tissue and cell culture models it can be directly investigated the expression and the role of certain protein. Also it is possible to analyse single or mixed cell populations. The biggest problem with identifying protein biomarkers in tissue and cell cultures is that they cannot provide accurate insight into disease progression in vivo [33,39,40].

3. Urine

Urine is ultrafiltrate of blood and is commonly used for diagnostics and biomarker discovery. Urine has a lot of positive characteristics: it can be obtained non-invasively, it contains proteins and peptides of low molecular weight and it is also very stable body fluid. Like others also urine has some negative characteristics. It is difficult to control variability in sample collection if samples are not taken by a trained personnel, also there are other proteins besides biomarkers and urine composition is dependent on collection time, diet, exercise and stage of disease [33, 41].

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Molecular basis of prostate cancer

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Prostate cancer (PCa) is the most frequent cancer type in males in Europe and other continents. Although, its high occurrence in male population, its mortality rate is lower than in other cancers like lung cancer. In the late 1980s a screening method for the prostate specific antigen (PSA) was introduced. As a cause of the better diagnostic screening, the incidence rate of the disease has risen. However, the mortality rates remained the same or decreased [1].

In order to find new therapeutic targets for PCa it is necessary to understand the molecular drivers of cancer progression and tumorigenesis. PCa is caused by many alterations. Not only alterations in molecular signaling pathways but also DNA rearrangements and epigenetic modifications such as microRNA (miRNA) regulation are factors. Alterations in DNA repair mechanisms are found in genes involved in DNA double strand break (DSB) repair such as ATM, as well as in genes involved in homologous recombination such as BRCA1/2 and RAD51B/C. Moreover, Androgen Deprivation Therapy (ADT) is thought to interact with Ku70 which is known to bind DSB and initiate non homologous end joining (NHEJ) [2].

Whereas most activated pathways like the mitogen activated protein kinases (MAPK), Phosphoinositide 3-kinase (PI3K) and the Wnt signaling are commonly altered in many tumor entities, the Androgen Receptor (AR) signaling plays a very prominent role especially in PCa. Therefore, AR is involved in tumorigenesis, recurrence and resistance mechanisms of malignant PCa. Characteristically, alterations in AR signaling are found on gene-, transcription- and protein levels [3]. A multitude of copy number variations are found for the AR coding regions on Xq11-12. Amplifications of AR are described in 2% of primary PCa, but not in benign prostatic hyperplasia (BPH). Interestingly, up to 23.4% of CR-PC harbor extensive copy number amplifications of AR [2].
The amplified AR gene does not only result in elevated gene expression and protein levels, but also has an important impact on protein stability and half life time. Alterations in Posttranscriptional modifications as well as the interaction with chaperones are associated with stabilization of AR molecules [2].

With respect to the AR transcription factor, phosphorylation of the mutated N-terminal domain (NTD) of AR is described as an important stabilizing factor. Especially MAPK and PI3K are thought to play a prominent role in this stabilization. Experimental data suggest that Akt signaling is the primary activator of Ser213 phosphorylation and AR activation. Mutations in PTEN are frequently found in PCa which leads Akt signaling stimulation. Therefore, double mutants are connected to worse outcome of the disease [4][5]. Furthermore, mutations in the Ligand binding domain (LBD) are frequently found in PCa. Yet, it remains unclear how these mutations can contribute to the castration resistant prostate cancer (CR-PCa) formation. Over 14 different splice variants of AR are described. Some splice variants lack the LBD and are therefore hormone independent. These ligand independent AR are thought to drive CR-PCa. Moreover, AR interacts direct with several DNA repair mechanisms like base excision repair, mismatch repair, homologous recombination and NHEJ [2]. In addition, recent data suggest that AR signalling is not only involved in CR-PCa formation but also involved in ADT resistance [4][2].

Despite the signaling pathways, epigenetic factors are found to play a prominent role in PC tumor formation. miRNAs are short non-coding RNA molecules, that are involved in post-translation regulation in various types of diseases including cancer. They are key epigenetic mechanisms involved in tumorigenesis of prostate cancer [6]. Calin et al. [7] was the first one to describe the relationship between miRNAs and cancer. He discovered that genes for miR-15 and miR-16 (located chromosome 13q14) are downregulated or deleted in majority of chronic lymphocytic leukemia (CLL) patients (65%).

There are several major signaling pathways targeted by miRNAs in PCa such as apoptosis, cell invasion, migration, transforming growth factor-β, (TGF-β) pathway, extracellular signal-regulated kinases (ERKs), phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR, cell cycle, and epithelial-mesenchymal transition (EMT) [8] (Figure 1).

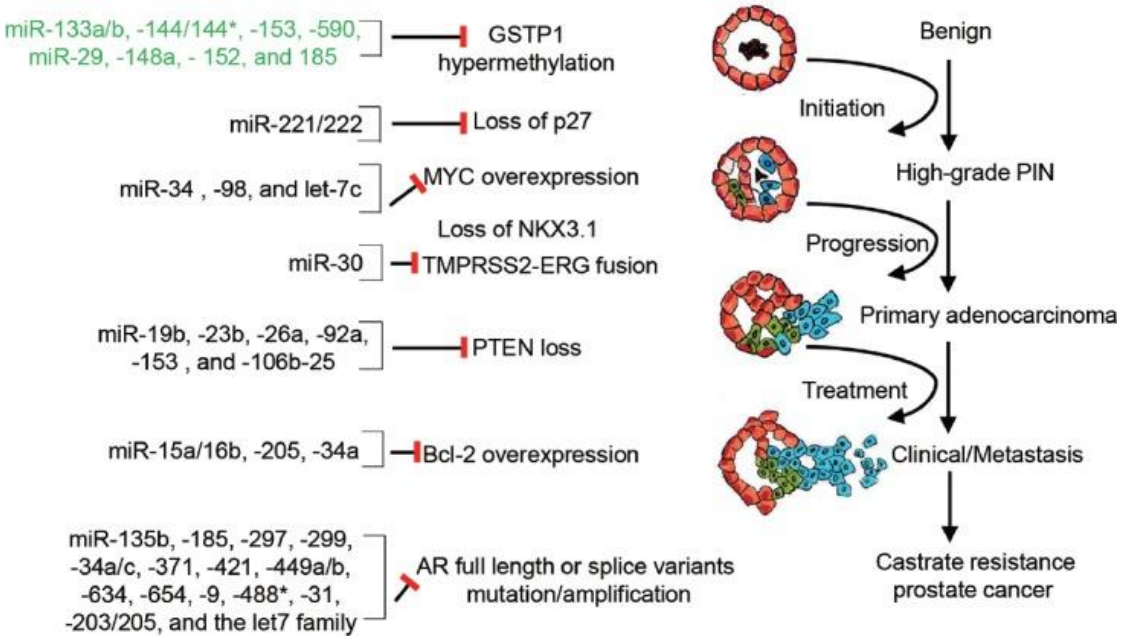

Figure 1: The scheme of key pathways involved in the molecular progression of prostate tumors and miRNAs specifically targeting molecules in those pathways [6].

Extensive research is done on miRNAs expression profile in prostate cancer in recent years. It is focused not only on deregulation of miRNAs in tumor tissue but also in bodily fluids, so the miRNAs could be used as predictive biomarkers and therapy agents (targeting molecules involved in PCa tumorigenesis pathways) (Figure 2) in the future [8].

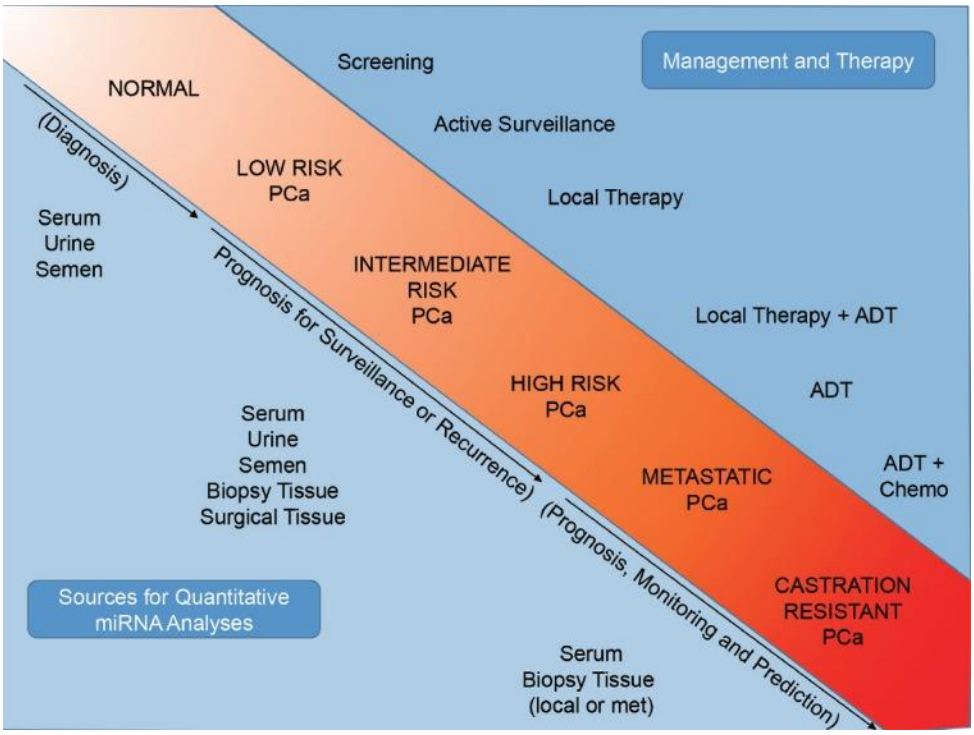

Figure 2: The scheme of key pathways involved in the molecular progression of prostate tumors and miRNAs specifically targeting molecules in those pathways [6].

Various miRNAs are upregulated in prostate cancer tissue such as miR-21, which is the most well studied oncoMir. It has been reported to be highly overexpressed in prostate cancer tissue [9], blood [10], or urine [11]. Its high overexpression was observed especially in drug resistant PCa [12]. Other miRNAs proved to be deregulated in tumor tissue an also in plasma: let-7c, let-7e, miR-103, miR-106a, miR-130b, miR-141, miR-16, miR-200b, miR-200c, miR-20a, miR-20b, miR-221, miR-223, miR-24, miR-26b, miR-30c, miR-346, miR-429, miR-92a and miR-93. Moreover, altered levels of several miRNAs were also detected urine samples of patients with PCa: miR-107 miR-205 miR-214 miR-375 miR-484 miR-574 [6].

A few miRNAs have been associated with AR. Some of them are affected by AR the others regulate AR mRNA or protein levels. 71 miRNAs are likely o by involved in AR regulation in prostate cancer [13]. For example, here is an androgen-response element incorporated into the promoter region of miR-21. As a result, miR-21 and AR regulates each other in a positive feedback loop. miR-21 can effect cell growth, cell migration, apoptosis and androgen insensitivity by influencing various pathways and it is upregulated by androgen [14]. Other miRNAs deregulated by androgen are miR-141, which is acting as oncomiR [15] or miR-34a/b/c cluster. AR is downregulated by miR-185 which is involved in cell cycle deregulation [16] and Let-7 family which targets c-Myc and thus suppresses AR expression and activity of PCa cells [17].

There many molecular alteration involved in PCa such as androgen receptor signaling pathway, DNA rearrangements and epigenetic modifications such as miRNA regulation. Understanding major molecular regulators involved in prostate cancer development and progression is important in order to find new biomarkers therapeutic approaches for the disease.

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Treatment of prostate cancer

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Introduction

Prostate cancer is a tumor whose growth is promoted by male hormons and androgens. Despite the fact, that characterizing the molecular landscape of prostate cancer is challenging it is important of understanding the process leading to development and progression of prostate tumors. It is even more important to study genomic basis of castration resistant prostate cancer, which is resistant to androgen deprivation therapy. This facts can help us to find new ways of diagnosis and therapy for prostate cancer.

Ways of treating prostate cancer:

- Castration - With castration we can block androgens. There are two types of castracion – prostatectomy and chemical castration.
- Antiandrogens - There are two types of antiandrogens. Antiandrogens which block androgen syntesis and antiandrogens which are connecting with androgen receptors. Some of antiandrogens: ciproterone acetate, nonsteriodic - flutamide, nilutamide, bicalutamide and enzalutamide.
- Hormon therapy – is the first step metastatic prostate cancer treatment. Usually therapy is antiandrogen with castration (completed androgenic block). It gives patients oportunity to live longer but still metastatic prostate cancer is not curable in this type of therapy.. Also if patient become rezistant tothis type of therapy it develops rezistant prostate cancer (CRPC). Some of medications in that moment are **abirateron and enzalutamid**.
- Chemotherapy **-** It is usually used with CPRC patients who don`t have good treatment results on hormon therapy or patinets who have metastasis. Chemotherapeutics in those cases are **docetaxel, cabazitaxel, abirateron acetate, enzalutamide and zoledronat acid**. When patient has bone metastazis then we use **^{223}Ra and ^{89}Sr**.
- Cell and imuno therapy **-** Immunotherapy becomes very important part for the metastatic prostate cancer treatment. We have evidence from phase III studies of **sipuleucel-T** (antigen-presenting cell vaccine. That study showed increased survival in hormone-refractory prostate cancer patients [1] Patiens with CRPC with some bone metastazis and no sympthoms were in that study. We can also use Sipuleucel-T for the metastatic castration-resistant prostate cancer (mCRPC). Treatment also extends survival.
  „In 2010, sipuleucel-T became the first US Food and Drug Administration (FDA)-approved immunotherapy following the demonstration of significant overall survival (OS) improvement in patients with primarily asymptomatic or minimally symptomatic mCRPC [2, 3, 4].“
  Mechanisam of Sipuleucel T – imunostimulans inhibitor of prostatic acid phosphatase (PAP). PAP is expressed by prostate cells so it is target therapy. Sipuleucel-T is composed by the patients own cells.

CAR (Chimeric Antigen-Receptor) - One of the new approaches in cell therapy is using CAR (Chimeric Antigen-Receptor). It can be used for treatment CRPC. It is the part of target therapy. We have first, secong and third generation of CARs. First-generation has only the CD3-ζ chain and second-generation has CD3-ζ and a domain from a costimulatory molecule. Third-generation has CD3-ζ and two costimulatory molecule domains.

Ipilimumab is human anti CTLA-4 monoclonic antibody. It has been produced by DNA recombinate tehnology. Ipilimumab inhibits CTLA-4 (cytotoxic T-lymphocyte-associated protein 4). After

inhibiation we have a grow number of reactive T cells. They can mobilize to the tumor and then we have direct imune attack of T cells to the tumor cells. T cells are infiltrating in prostate cancer tumor in most cases. Blocade of CTLA-4 can reduce function of regulatory T-cells and that can cause antitumor imune answer. When we reduce function of regulatory cells we are increasing ratio of intratumor T efector and regulatory T cells and that is the causing death of the tumor cell. There are aproches where we can combine ipilimumab with immune checkpoint blockade antibodies. Ipilimumab has two types of side effects. Life threatnig side effects (diarea or colitis 3. or 4. level, increased hepatic enzymes such as AST or ALT, skin rash 4. level and neuropatics 3. or 4. level) and moderate or minor side effects (diarea for few days, little increased ALT or AST, minor skin rash, minor neuropatic problems and endrochrinologic problems). In the case of major side effect patient should stop using this medication. Ipilimumab has serious major interactions with anticoagulans, antiagregatic, imunosupresive and medications for hypercholesteremia.

List of the medications which are in major interactions:

- ardeparine, argatrobane, bivalirudine, anisindione, apixabane, rivaroxabane, edoxabane, fondaprinux, enoxaparine, desirudine, dabigatrane, dalteparine, danaparoid, dicumarol, warfarine and lepirudine (gastrointestinal hemorhage).
- heparine and tinazaparine (gastrointestinal hemorhage).
- leflunomide and teriflunomide (hepatotoxic).
- lomitapide and mipomersen (hepatotoxic). {5} {6}

BRCA therapy

Mutations in the BRCA genes, mainly in BRCA2 increase the risk of developing PC. They also can give us information about prognosis. BRCA genes are in general autosomal dominant cancer-susceptibility genes and they are tumor suppressor genes. BRCA1 have a role in repairing DNA damage, regulation of transcription, and epigenetic effect on genomic stability. BRCA2 role is repairing the DNA damage by regulating the function of other protein such as RAD51.

Poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme which is important in base repairing of DNA breaks. Inhibition of PARP leads to double strand breaks. Cells are not able to repair those double strand breaks and it leads to a cell death. PARP inhibitor (olaparib) is used to treat *BRCA* mutation carriers. {9} {10} {11} {12}

Olaparib is one of the PARP inhibitors. It has major side effects (chest pain, chills, cough,ear congestion or pain, fever, head congestion, hoarseness or other voice changes, nasal congestion, pale skin,runny nose, sneezing, sore throat, troubled breathing with exertion, unusual bleeding or bruising and unusual tiredness or weakness) and minor side effects (acid in stomach, back pain, belching, blistering, crusting, irritation, itching, or reddening of the skin, blurred vision, burning, numbness, tingling, or painful sensations, cracked, dry, or scaly skin, decreased appetite, diarrhea, difficulty with moving, dry mouth, fear or nervousness, flushed, dry skin, headache, heartburn, increased hunger, increased thirst, increased urination, indigestion, lack or loss of strength, loss of bladder control, muscle pain or stiffness, nausea, pain in the joints, stomach discomfort, upset, or pain, sweating, swelling or inflammation of the mouth, trouble sleeping, unexplained weight loss, unsteadiness or awkwardness, weakness in the arms, hands, legs, or feet). {13}

List of the medications which are in major interactions:

- CYP 3A4 interactions: dexametasone, amprenavire, netupitant, phenobarbital, clarihromycin, amprenavir, aprepitant, itraconazole, lumacaftor, mibefradil, mitotone, modafinil, primidone, nafcilin, ketoconazole, atazanavir, boceprevir, tenofovir, crizotinib,delaviridine, flucoconazole, imatinib, etavirine, darunavir, indinavir, verapamil, diltiazem
- high risk of infections – adalimumab, leflunomide, teriflunomide, golimumab {14}

Conclusion

In the past few years there was a significant discover of new ways of treatment prostate cancer. Mostly monoclonal and polyclonal antibodies made most of the target therapy which is now the best way of treating prostate cancer.

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SUPPLEMENT – Lectures handouts

Molecular mechanisms in carcinogenesis

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Cancer

- a complex genetic disease
- it develops as a multi-step process during which accumulation of nonlethal mutations results in malignant transformation of the cells
- each cancer is characterized by distinctive set of mutations and consequently it is phenotypically specific and consequently, it should be considered as a unique disease
- genetic/phenotypic specificity of each cancer seeks for personalized approach in treatment and therapy

Cancer

- the consequence of breakage of the regulatory mechanisms that role cell behaviour
- cell proliferation, differentiation and survival are precisely regulated
- in cancer cells regulation is broken and they grow, divide and spread around uncontrolledly and disturb normal functions of healthy cells
- disturbance of the level/activity of proteins that play crucial roles in cell signalling, cell cycle regulation and control of programed cell death (apoptosis) leads to the uncontrolled proliferation, main characteristic of cancer cells

Cancer

- any cell type can be malignantly transformed
- tumour – every abnormal proliferation of the cells
- benign tumour *vs.* malignant tumours (cancers)
- benign tumour – remains limited on the location where it developed, no ability to metastasize
- malignant tumour – it may spread into surrounding tissues or distant parts of the body using lymphatic or blood circulation

Cancer classification

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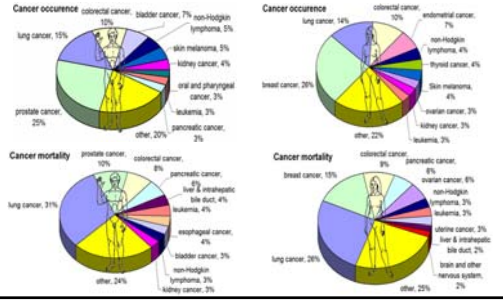
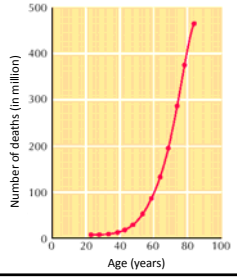
- benign tumour *vs.* malignant tumours (cancers)
- benign tumour – remains limited on the location where it developed, no ability to metastasize
- malignant tumour – it may spread into surrounding tissues or distant parts of the body using lymphatic or blood circulation
- malignant tumours: CARCINOMAS – malignant diseases of epithelial cells (90%)
  SARCOMAS – malignant diseases of connective tissues (3%)
  LEUKAEMIAS & LYPHOMAS – malignant diseases of haematopoietic or immune cells (7%)
- usually classified according the tissue (colon cancer) or the cell type (fibrosarcoma)

Cancer occurence and mortality



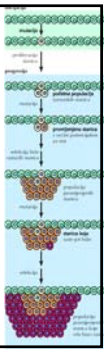
The incidence of cancer increases with age

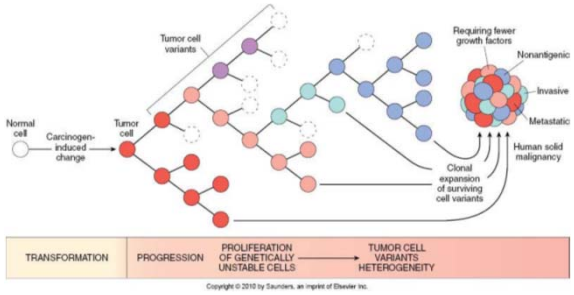
- carcinogenesis is complex, multi-step process that leads to transformation of normal cells which acquire physiological characteristics that together determine malignant phenotype
- accumulation of mutations may last for years, thus the tumour incidence increases with age and the prevalence of tumours is much higher in population over 60 years of age



Cancer – a multi-step process

- multi-step process that includes mutation and selection of the cells with pronounced ability to proliferate, survive, spread and metastasize
- TUMOUR INITIATION – result of the genetic change that leads to the proliferation of one cell
- proliferation leads to the excessive growth of monoclonal population of tumour cells
- TUMOUR PROGRESSION – accumulation of additional mutations in a population of tumour cells
- selective advantage of the cells with greater potential for proliferation - its descendants eventually become the dominant population in the tumour = clonal selection
- clonal selection is ever-present - all tumours grow faster and become more malignant





Carcinogenic agents

- mutations are the consequence of the action of environmental agents (*e.g.* chemicals, radiation, or biological agents) or may be inherited in the germ line
- in some cases, mutations may be spontaneous and stochastic
- three main classes of carcinogenic agents:
  - chemicals
  - radiation
  - microbial agents (mainly viruses)
- while chemicals and radiation are well documented causes of cancers in humans, oncogenic viruses are involved in development of at least some human tumours

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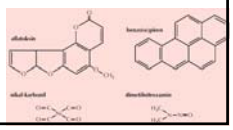
Chemical carcinogens

- most chemical carcinogens are mutagenic and considered to be initiators of carcinogenesis
- contain highly reactive groups that interact with DNA (as well as proteins and RNA)
- important targets of chemical carcinogens are oncogenes and tumour suppressors (such as Ras and p53) (*e.g.* aflatoxin B1 causes characteristic mutations in the p53 gene)
- promotors (*e.g.*, hormones, phenols, and drugs), which by themselves are non-tumorigenic (mutagenic), may induce cell proliferation and thus augment carcinogenicity of some chemical carcinogens
- it seems that an initiator may cause the mutational activation of an oncogene while subsequent application of promoters leads to clonal expansion of initiated (mutated) cells

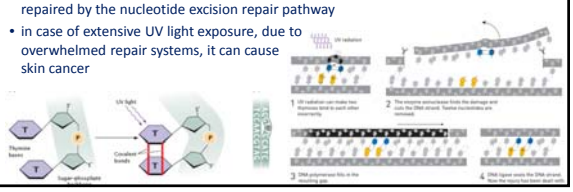
Chemical carcinogens (continued)

- there are two types of chemical carcinogens
- direct-acting carcinogens which require no metabolic conversion to become carcinogenic. They are in general weak carcinogens but are important because some of them are cancer chemotherapeutic drugs (*e.g.*, alkylating agents)
- indirect-acting agents - chemicals that require metabolic conversion to an ultimate carcinogen
  - **polycyclic hydrocarbons** *e.g.* benzopyrene, formed in the high-temperature combustion of tobacco in cigarette smoking or during the process of broiling meats
  - **aromatic amines** and **azo dyes** *e.g.* β-naphthylamine
  - **aflatoxin B1** produced by some strains of *Aspergillus*, a mold that grows on improperly stored grains and nuts
  - **vinyl chloride**, **arsenic**, **nickel**, **chromium**, and **insecticides** are potential carcinogens in the workplace and the house
  - **nitrites** - used as food preservatives.



Radiation

- radiation (UV rays of sunlight, x-rays, nuclear fission, radionuclides) causes chromosome breakage, translocations, and, less frequently, point mutations
- biologically, DNA breaks seem to be the most important form of DNA damage caused by radiation
- UV light has the ability to damage DNA by forming pyrimidine dimers, which can be repaired by the nucleotide excision repair pathway
- in case of extensive UV light exposure, due to overwhelmed repair systems, it can cause skin cancer



Microbial agents

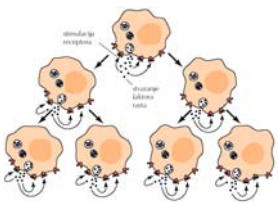
- several viruses have been linked with human cancer
- the viral agents causing cancer in eukaryotic cells by integrating in host genom
  - oncogenic RNA viruses *e.g.* human T-cell leukaemia virus-1 (HTLV-1), associated with a form of T-cell leukaemia/lymphoma
  - oncogenic DNA viruses (human papillomavirus (HPV), Epstein-Barr virus (EBV), Kaposi sarcoma herpesvirus (human herpesvirus 8, HHV8), hepatitis B virus (HBV)
- the precise molecular mechanisms underlying the carcinogenetic effects of different virus type are complex and still under nvestigation
  - *Helicobacter pylori* - the first bacterium classified as a carcinogen (increased epithelial cell proliferation in a background of chronic inflammation)

Characteritics of cancer cells

- Autocrine stimulation of growth and/or self-sufficiency in growth signals
- Insensitivity to growth-inhibitory signals
- Evasion of apoptosis
- Limitless replicative potential and disturbed cell differentiation
- Sustained angiogenesis
- Defects in DNA repair
- Ability to invade and metastasize

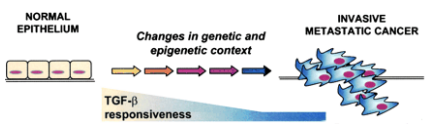
Autocrine stimulation of growth and/or self-sufficiency in growth signals

- Tumours have the capacity to proliferate without external stimuli, usually as a consequence of oncogene activation or they can produce all needed growth factors, by them self.



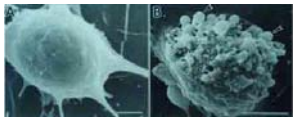
Insensitivity to growth-inhibitory signals

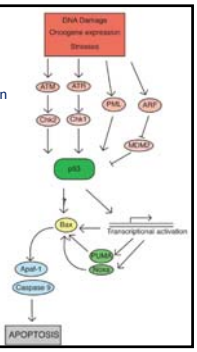
- Tumours may not respond to the molecules that inhibits normal cells growth such as transforming growth factor β (TGF-β) and direct inhibitors of cyclin-dependent kinases (CDKIs).



Evasion of apoptosis

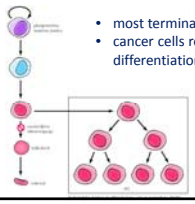
- Tumours may avoid programmed cell death, by inactivation of tumour suppressor p53 or activation of anti-apoptotic genes.





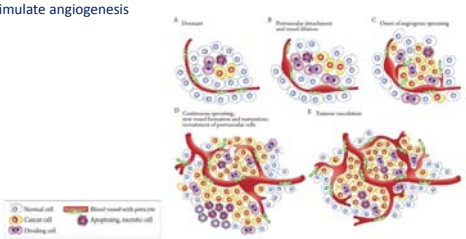
Limitless replicative potential and disturbed cell differentiation

- tumour cells have unrestricted proliferative capacity, avoiding cellular senescence and mitotic catastrophe
- in some cases, they lose ability to differentiate, so undifferentiated cells uncontrolledly proliferate
  - most terminally differentiated cell do not devide
  - cancer cells remain arrested in the early stage of differentiation (leukemias)



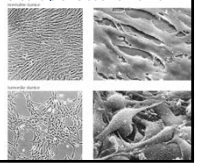
Sustained angiogenesis

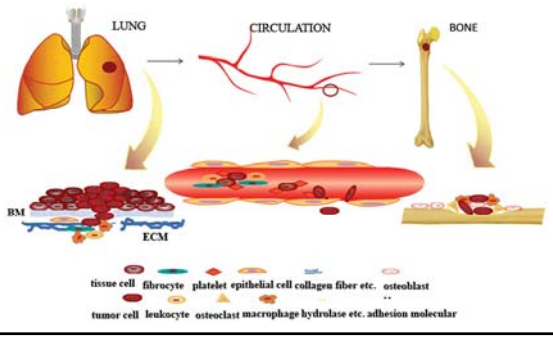
- Tumour cells, like normal cells, need nutrients and oxygen for their growth, thus they have to stimulate angiogenesis



Ability to invade and metastasize

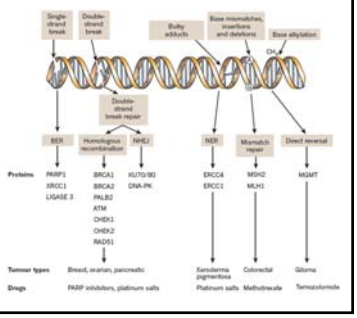
- Spreading into environment is essential for tumour growth, so tumour cells produce high quantity of proteases. Due to a loss of contact inhibition and reduced adhesiveness tumour cells can easily detach from tumour mass and enter into the lymph or blood circulation, thus spreading around the body.
- most cancer cells is not sensitive to inhibition dependent on the cell density (many cancer cells have a reduced need for growth factors)
- loss of contact inhibition - contact with neighbour cells inhibits movement and proliferation of normal cells, but not tumour cells
- cell interactions and interactions between cells and the extracellular matrix in cancer cells are reduced





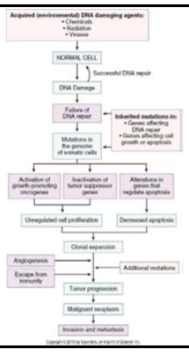
Defects in DNA repair

- Tumours may fail to repair DNA damage caused by carcinogens or incurred during unregulated cellular proliferation, leading to genomic instability and mutations in proto-oncogenes and tumour suppressor genes.



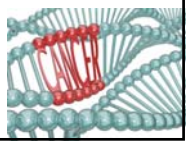
Cancer – a concequence of demage of good guys

- Four classes of normal regulatory genes are the principal targets of genetic damage
  - proto-oncogenes
  - tumour suppressor genes
  - genes that regulate programmed cell death (apoptosis)
  - DNA repair genes
- while mutant alleles of proto-oncogenes are considered dominant, both alleles of the tumour suppressor genes must be damaged before transformation can occur
- in some cases loss of a single allele of a tumour suppressor gene reduces level or activity of the protein enough that the brakes on cell proliferation and survival are released
- genes that regulate apoptosis may behave as proto-oncogenes or tumour suppressor genes
- mutations of DNA repair genes do not directly transform cells by affecting proliferation or apoptosis
- instead, DNA-repair genes affect cell proliferation or survival indirectly by influencing the ability of the organism to repair nonlethal damage in other genes, including proto-oncogenes, tumour suppressor genes, and genes that regulate apoptosis



Proto-oncogenes and oncogenes

- oncogenes are created by mutations in proto-oncogenes
- characterized by the ability to promote cell growth in the absence of normal growth-promoting signals
- their products, oncoproteins, resemble the normal products of proto-oncogenes except that oncoproteins are often devoid of important internal regulatory elements, and their production in the transformed cells does not depend on growth factors or other external signals
- in this way cell growth becomes autonomous, freed from checkpoints and dependence upon external signals

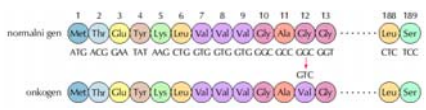


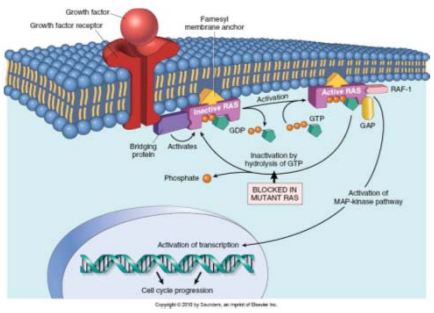
How good guys become bad guys?

- growth factors can be mutated in a way that they act as oncoproteins
  - PDGF-β chain
  - fibroblast growth factors,
  - TGF-α
- growth factor receptors, as well
  - EGF-receptor family members
  - PDGF receptor
  - receptor for neurotrophic factors

ras oncogenes

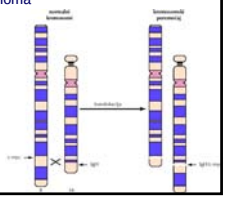
- *ras* protoonkogene is present in normal cells, whereas *ras* onkogene is not
- the reason of transformation from *ras* protoonkogene to *ras* onkogene are mutations occurring during tumour development
- the difference between *ras* oncogene and *ras* proto-oncogene is a mutation of individual amino acids at key locations
- mutation glycine → valine at the position 12, or some other
- mutated Ras is constitutively active (binds GTP) since GAP (protein that activates GTPase) has not effect on mutated Ras





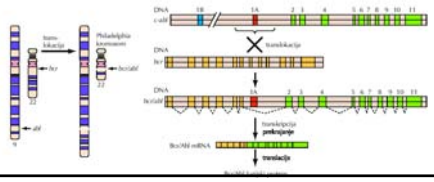
c-myc oncogenes

- Many cancer cells have disturbed structure of chromosomes (translocations, duplications, deletions)
- gene re-arrangements caused by chromosomal translocations often lead to the formation of oncogenes
- *c-myc* in human Burkitt lymphoma and murine myeloma
- chromosomal translocations that affect loci of immunogloubuline genes
- translocation of the part of chromosome 8 on one of the loci of immunoglobulines (chromosomes 2, 14 or 22)
- at the breakage point (chromosome 8) - protoonkogene *c-myc*



abl oncogenes

- translocation of protooncogene *abl* from chromosome 9 to 22 is associated with chronic myeloid leukaemia (CML)
- fusion of *abl* with *bcr* gene → the fusion protein Bcr/Abl in which the N-terminus of normal protoonkogenic protein Abl is replaced by N-sequence of the Bcr → dysregulation of the Abl tyrosine kinase activity and cell transformation



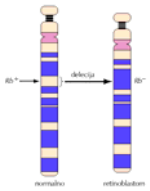
Tumour suppressor genes

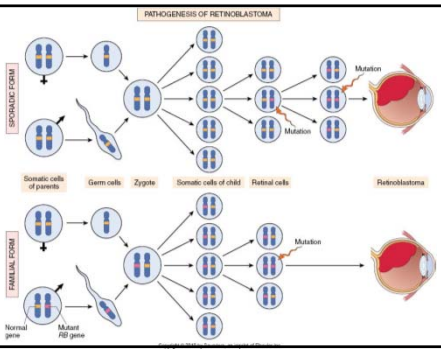
- inhibit cell proliferation and tumour formation
- in many tumours, tumour-suppressor genes are missing or have been inactive → negative regulation of cell proliferation does not function

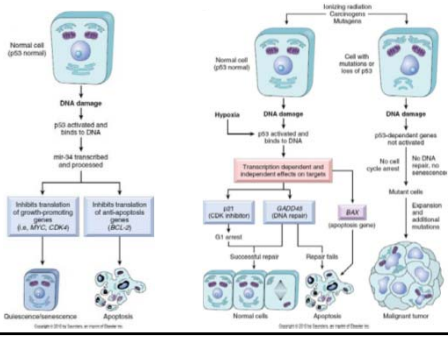
GENE	CANCER	CROMOSOM	ISOLATE
RB	Retinoblastoma	Crom.13q	1986
P53	Osteosaracoma	Crom.17p	1990
APC	Colon	Crom.5q	1991
VHL	Renal carcinoma	Crom.3p	1993
P16	Melanoma	Crom.9p	1994
BRCA1	Brest censer	Crom.17q	1994
BRCA2	Brest censer	Crom.13q	1995

Rb – tumour suppressor protein

- retinoblastoma - a rare eye cancer in children; treatment is available
- retinoblastoma occur in 50% of the offspring of diseased → for tendency for the formation of tumours is responsible one gene that is inherited in an autosomal dominant way
- *Rb* gene - a negative regulator of tumorigenesis
- deletions of chromosome 13q14
- lack of *Rb* gene is responsible for the occurrence of cancer of the bladder, breast, lung ...
- Rb protein is a key target of oncogenic proteins of some DNA viruses (SV40, adenovirus, human papilloma virus, herpes virus ...)
- Rb binds to the transcription factor E2F and prevents protein synthesis required for cell division







International CEEPUS Summer School 2016

Biochemical and inflammatory aspects of tumorigenesis

Jelena Kotur-Stevuljević

Faculty of Pharmacy – University of Belgrade, Serbia
Department for Medical Biochemistry

International CEEPUS Summer School 2016

Metabolism in cancer cells

- Cancer cells - abnormal metabolism
- Oncogenic mutations in metabolic enzymes
- Existing and emerging therapies aim to target this abnormal metabolism in various ways

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What is normal cell metabolism?

- Intracellular chemical reactions
- ATP – which two methabolic pathways?
- Oxygen level influences metabolic destiny of different substrates

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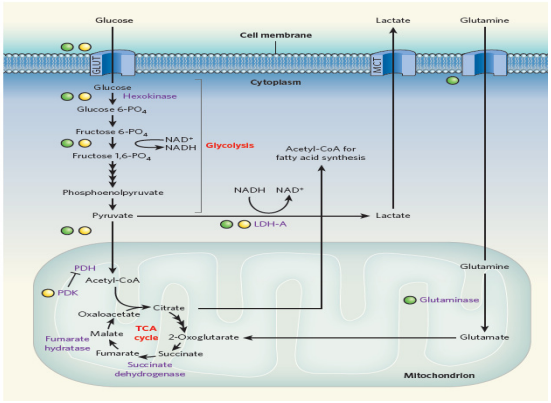

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How is cancer-cell metabolism different?

- Quantity of nutrients
- Glucose metabolism
- Glutamin use

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Do any normal cells share these properties?

- Proliferation phase
- Nutritients quantity dependent proliferation
- Ability to change proliferation status depends on tumor supressors proteins: p53 and LKB1
- Cancer cells don't have tumour-suppressor proteins – "addiction" to glucose and/or glutamine consumption.

How do these abnormalities occur?

- PI3K-AKT signalling pathway
- Tyrosin-kinases receptor mutation
- Excessive nutrients uptake
- Role of HIF and MYC
- Role of p53

How do cancer cells benefit from their abnormal metabolism?

- Suply of building blocks for biomolecules synthesis – NADPH and acetyl-CoA
- ***Stealing*** nutrients from ***neighboring*** cells
- ROS generation – promotion of cancer cells proliferation by inactivating growth-inhibitory phosphatase enzymes
- DNA damage

As anaerobic glycolysis is inefficient, how do cancer cells maintain ATP levels?

- Metabolising more glucose
- Need for NADPH, acetyl-CoA, ribose and nonessential amino acids.
- Uncoupling ATP production from mitochondrial electron transport
- Using NADH without ATP production i.e.

"diversion of glycolytic intermediates into non-ATP-generating bypass pathways"

Can cancer cells use fat as a fuel?

- Intensive lipid synthesis
- Phospholipids – why?
- Prostaglandins – why?
- FA synthesis

What is the effect of changing oxygen levels?

- Tumor blood supply – hypoxia
- HIF-200 genes
- PHD enzymes
- AKT activation
- ROS, TCA metabolities

Why is glutamine crucial for cancer cells?

- Glutamine is essential for cell growth
- Transport of reduced nitrogen
- Synthesis of nucleotides and AA
- Essential AA uptake
- TCA cycles roles
- MYC role

How does the Warburg effect come in?

- Cancer cells continue to perform glycolysis and lactate production even when oxygen levels are abundant
- Warburg effect is proofed by imaging studies

And how does it relate to the Pasteur effect?

- Reciprocal relationship between anaerobic glycolysis and oxidative phosphorylation
- The Warburg effect is a loss of the Pasteur effect
- HIF and oxygen
- Mutations at MYC and p53

Are metabolic enzymes frequently mutated in cancer?

- NO
- Metabolic pathway *vs.* single enzyme

How are cancer-associated metabolic changes detected?

- Mass spectrometry
- Magnetic resonance imaging
- Positron emission tomography (PET) -18F-fluorodeoxyglucose

Could targeting cancer-associated glycolysis be used therapeutically?

- *In vitro vs.* clinical studies
- Inhibition of the HK enzyme (2-deoxyglucose, Ionidamin) – toxic, ineffective
- PK inhibitors
- Problem: red blood cells, brain
- HIF inhibitors

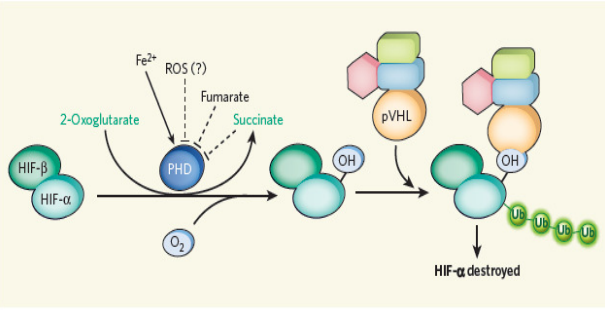
Do any anticancer agents exploit the metabolic addictions of cancer cells?

- YES
- Aminopterin – dihydrofolate reductase – nucleic acid synthesis
- Activation of this enzyme in anaerobic glycolisis conditions
- Asparaginase – L-asparagine

What about other potential therapies?

- LDH inhibition
- Glutaminase inhibitors
- FA synthase inhibitor

What are the latest advances?



Fumarat-hydratase
Succinate-dehydrogenase

Ubiquitin ligase complex - pVHL – tumour suppressing protein

OXIDATIVE STRESS, INFLAMMATION AND CANCER

Disorders Sharing Oxidative Stress and Cancer Proneness

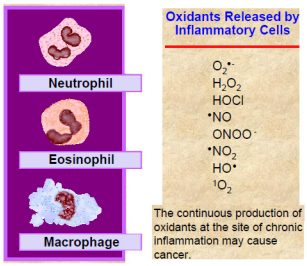
- Fanconi anaemia #
- Xeroderma pigmentosum
- Ataxia telangiectasia
- Bloom syndrome
- Down syndrome
- Cystic fibrosis

#Petrovic SZ, Leskovac AR, Kotur-Stevuljevic J, Joksic J et al. Gender-related differences in the oxidant state of cells in Fanconi anemia heterozygotes BIOL CHEM 2011; 392 (7): 625-632.

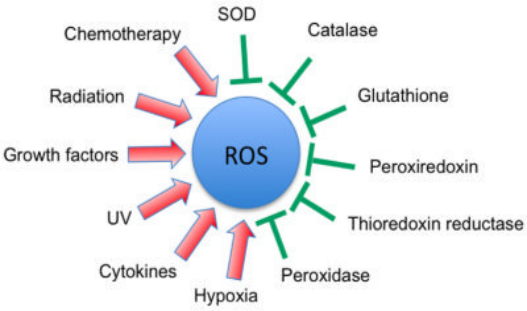
Chronic Inflammation is Associated with Malignancy

CANCER	INFLAMMATORY CONDITION
Lymphoma	HIV, Epstein-Barr and Herpes 8 virus, host vs. graft disease
Colon	Ulcerative colitis
Lung	Asthma, chronic bronchitis, emphysema
Ovarian	Ovarian epithelial inflammation
Bladder	Eosinophilic cystitis, schistosomiasis
Pancreatic	Pancreatitis
Esophago-gastric junction carcinoma	Barret's esophagus
Gastric	Heliobacter pylori infection
Liver	Sarcoidosis, hepatitis B virus
Cervical	Human papilloma virus
Mesothelioma	Asbestos fiber exposure

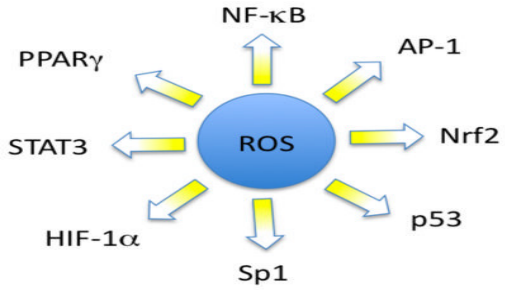
Oxidative stress and cancer



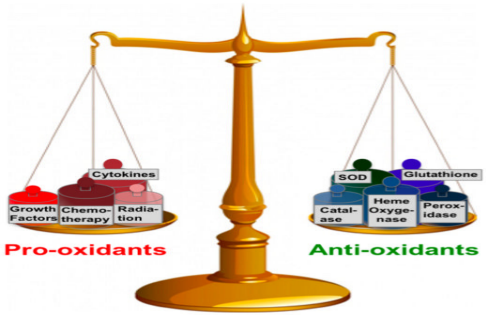
Activators and inhibitors of reactive oxygen species



Transcription factors that are modulated by reactive oxygen species



Model of a balance between pro-oxidants and anti-oxidants

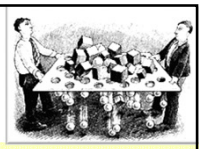


Oxidative stres and anti-cancer therapy

- Anticancer agents

Doxorubicin
Daunorubicin
Mitomycin C
Etoposid
Cisplatin
Arsenic trioxide
Ionizing radiation
Photodynamic therapy

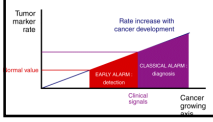
univerzitetni klinični center ljubljana



TUMOR MARKERS IN CLINICAL PRACTICE

Prof.Joško Osredkar, PhD

University Medical Centre Ljubljana
Clinical Institute of Clinical Chemistry and Biochemistry

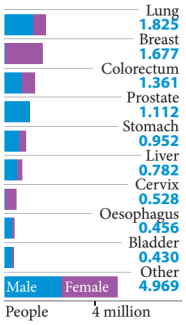


Incidence

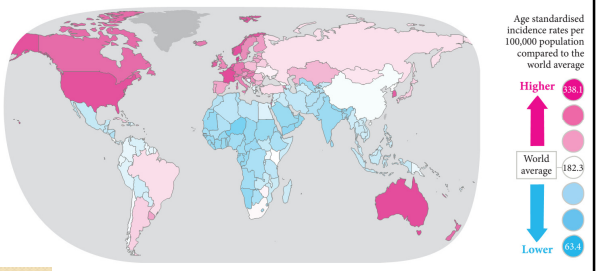
Cancer is a leading cause of disease world wide. An estimated 14.1 million new cancer cases occured in 2012.

An estimated 7.4 million males were diagnosed with cancer worldwide in 2012. Lung cancer was the most common, accounting for almost a fifth (17%) of all cases diagnosed. Prostate cancer was the second most common cancer diagnosed in males worldwide (15%).

Prostate cancer is the most common cancer in Europe for males, and the third most common cancer overall, with around 417,000 new cases diagnosed in 2012 (3% of male cases and 12% of the total). In Europe (2012), the highest World age-standardised incidence rates for prostate cancer are in Norway; the lowest are in Albania.



Incidence 2012 / Cancer incidence by country



World cancer trends

Approximately 44% of cancer cases and 53% of cancer deaths occur in countries at a low or medium level of the Human Development Index (HDI)

Projections to 2030

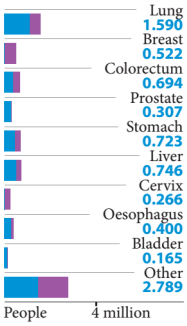
If recent trends in major cancers are seen globally in the future, the burden of cancer will increase to 23.6 million new cases each year by 2030. This represents an increase of 68% compared with 2012 [3]

Mortality

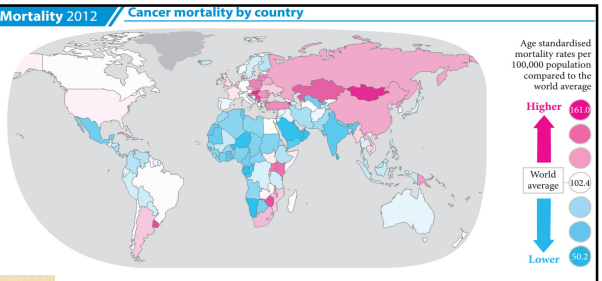
Cancer is a leading couse of deaths worldwide, with 8,2 million deaths in 2012. More than half of all cancer death each year are due to lung, stomach, liver, colorectal and female breast cancers.

In 2012, there were an estimated 8.2 million deaths from cancer in the world: 4.7 million (57%) in males and 3.5 million (43%) in females, giving a male:female ratio of 10:8.

The World age-standardised (AS) mortality rate shows that there are 126 cancer deaths for every 100,000 men in the world, and 83 for every 100,000 females.



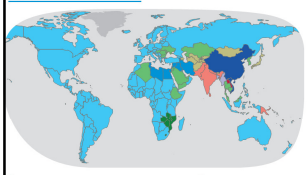
Mortality 2012 / Cancer mortality by country



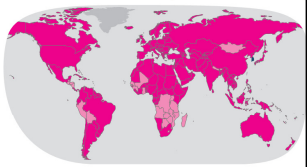
The World AS mortality rates in males vary more than two-fold across the different world regions, ranging from 173 per 100,000 in Central and Eastern Europe to 68 per 100,000 in Western Africa (2012). In females, rates vary more than three-fold, ranging from 119 per 100,000 in Melanesia to 65 per 100,000 in South-Central Asia (2012).

Prevalence 2012 / Most prevalent cancer by country

Males



Females

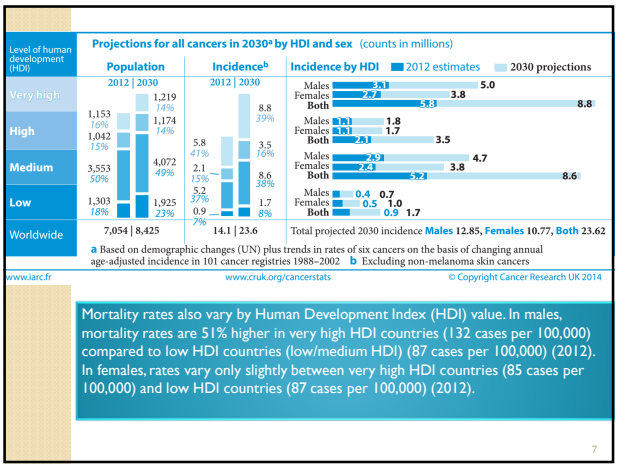


1. **Prostate** - 124 countries worlwide
2. **Bowel** - 23 countries in Africa, Asia and Eastern Europe
3. **Stomach** - 9 countries in Asia
4. **Lip, Oral Cavity** - 7 countries in South-Central Asia and Melanesia
5. **Bladder** - 7 countries in Northern Africa, Asia
6. **Kaposi Sarcoma** - Lesotho, Malawi, Mozambique, Swaziland, Zimbabwe, Zambia
7. **Liver** - Gambia, Laos
8. **Lung** - China, Vietnam
9. **Pharynx** - Bangladesh, Myanmar

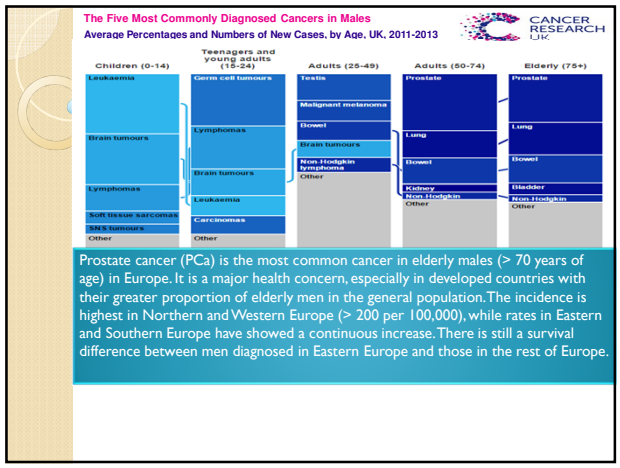
1. **Breast** - 151 countries worldwide
2. **Cervix** - 30 countries in Africa, the Americas and Asia
3. **Thyroid** - South Korea

Prevalence

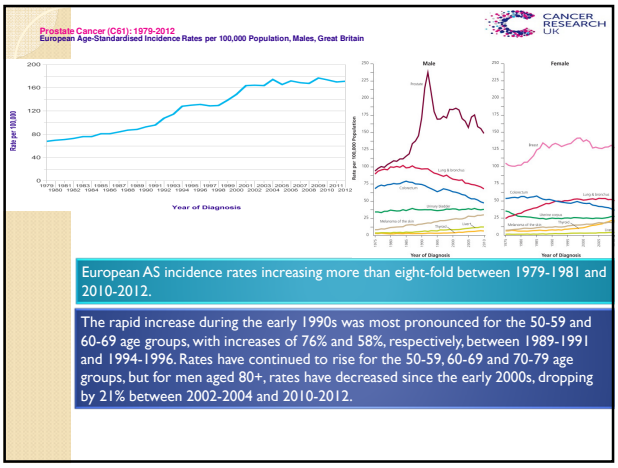
32.5 million people diagnosed with cancer within the five years previously were alive at the end of 2012. most were women after their breast cancer diagnosis (6.3 million), men after their prostate cancer diagnosis (3.9 million), and men and women after their colorectal cancer diagnosis (3.5 million).



Mortality rates also vary by Human Development Index (HDI) value. In males, mortality rates are 51% higher in very high HDI countries (132 cases per 100,000) compared to low HDI countries (low/medium HDI) (87 cases per 100,000) (2012). In females, rates vary only slightly between very high HDI countries (85 cases per 100,000) and low HDI countries (87 cases per 100,000) (2012).



Prostate cancer (PCa) is the most common cancer in elderly males (> 70 years of age) in Europe. It is a major health concern, especially in developed countries with their greater proportion of elderly men in the general population. The incidence is highest in Northern and Western Europe (> 200 per 100,000), while rates in Eastern and Southern Europe have showed a continuous increase. There is still a survival difference between men diagnosed in Eastern Europe and those in the rest of Europe.



European AS incidence rates increasing more than eight-fold between 1979-1981 and 2010-2012.

The rapid increase during the early 1990s was most pronounced for the 50-59 and 60-69 age groups, with increases of 76% and 58%, respectively, between 1989-1991 and 1994-1996. Rates have continued to rise for the 50-59, 60-69 and 70-79 age groups, but for men aged 80+, rates have decreased since the early 2000s, dropping by 21% between 2002-2004 and 2010-2012.

DIAGNOSTICS IN ONCOLOGY

RADIOLOGY

DIAGNOSTICS IN ONCOLOGY

RADIOLOGY

PATHOLOGY

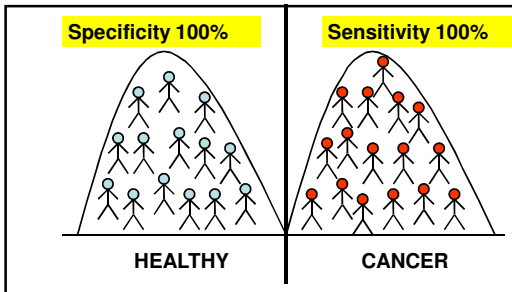
DIAGNOSTCS IN ONCOLOGY

RADIOLOGY

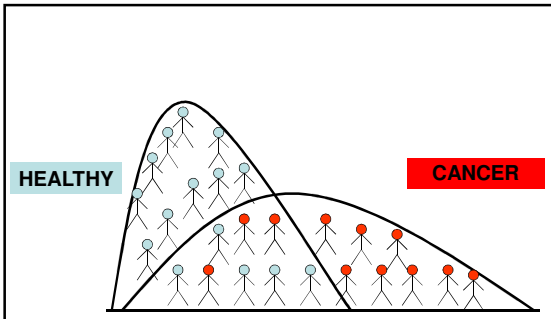
PATHOLOGY

LABORATORY MEDICINE

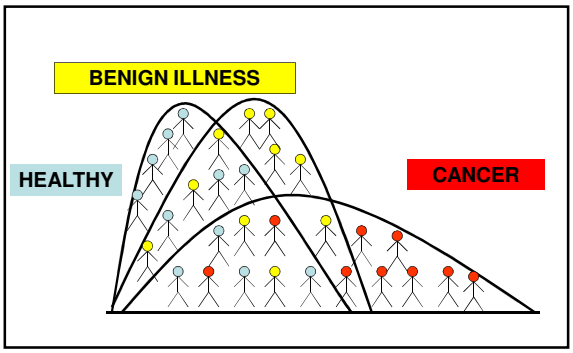
WE ARE STILL FAR FROM WHAT WE WOULD WISH...



REALITY OF BIOMARKERS IN ONCOLOGY



REALITY OF BIOMARKERS IN ONCOLOGY



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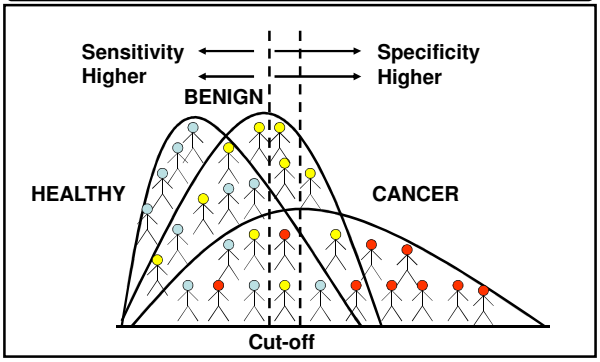
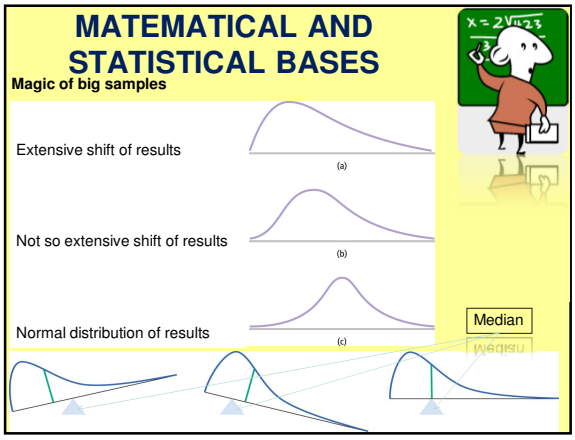
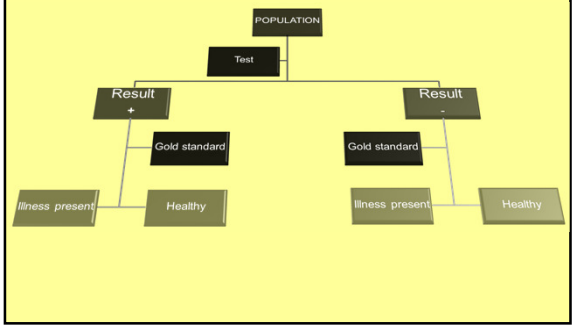


MATEMATICAL AND STATISTICAL BASES

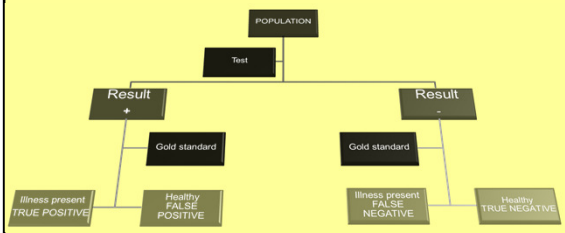
Magic of big samples



USEFULNESS OF THE TEST



WHAT IS THE RESULT



SENSITIVITY

Sensitivity = $\frac{\text{True positive}}{\text{All cases}} = \frac{a}{a+c}$

	Cancer	Healthy	
Positive	True positive (a)	FalsE positive (b)	a + b
Negative	False negative (c)	True negative (d)	c + d
	a + c	b + d	

Sensitivity is a proportion of THOSE, WHO REALY HAVE CANCER (TP + FN) and the results are in TP

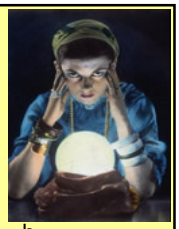
SPECIFICITY

Specificity = $\frac{\text{True negative}}{\text{All healthy}} = \frac{d}{b+d}$

	Cancer	Healthy	
Positive	True positive (a)	False positive (b)	a + b
Negative	False negative (c)	True negative (d)	c + d
	a + c	b + d	

Specificity is the proportion of THOSE, WHO ARE HEALTHY (TN + FP) and the results are in TN

POSITIVE PREDICTIVE VALUE

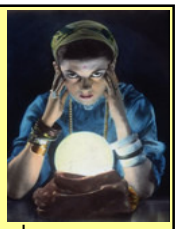


PPV = $\frac{\text{True positive}}{\text{All positive}} = \frac{a}{a+b}$

	Cancer	Healthy	
Positive	True positive (a)	False positive (b)	a + b
Negative	False negative (c)	True negative (d)	c + d
	a + c	b + d	

Positive predictive value is the proportion of THOSE, TESTED POSITIVE (TP + FP) and realy have cancer - TP

NEGATIVE PREDICTIVE VALUE



NPV = $\frac{\text{True negative}}{\text{All negative}} = \frac{d}{c+d}$

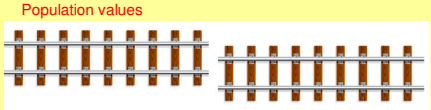
	Cancer	Healthy	
Positive	True positive (a)	False positive (b)	a + b
Negative	False negative (c)	True negative (d)	c + d
	a + c	b + d	

Negative predictive value is a proportion of THOSE, TESTED NEGATIVE (TN + FN) and are healthy - TN

PROBLEMS

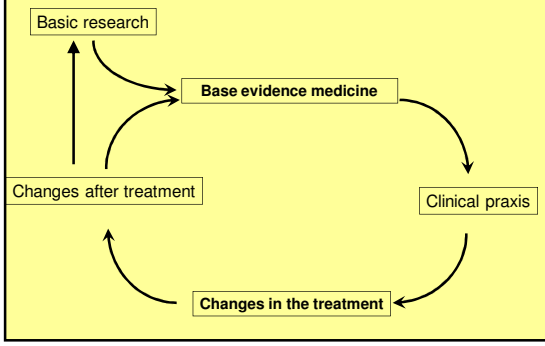
- Sensitivity and specificity are test dependent
- Predictive values are dependent of the prevalence of the disease

Population values



Our data

HOW TO CHOOSE A CERTAIN TUMOR MARKER?



TUMOR MARKERS - USE

- Diagnosis of illness - ☒
- Prognosis - ☒
- Staging - ☒
- Monitoring of therapy - ☑
- Early detection of recurrence - ☑
- Screening - ☒
- Predictive factor - ☒

Tumor markers are used to help detect, diagnose, and manage some types of cancer. Although an elevated level of a tumor marker may suggest the presence of cancer, this alone is not enough to diagnose cancer. Therefore, measurements of tumor markers are usually combined with other tests, such as biopsies, to diagnose cancer.

Tumor marker levels may be measured before treatment to help doctors plan the appropriate therapy. In some types of cancer, the level of a tumor marker reflects the stage (extent) of the disease and/or the patient's prognosis (likely outcome or course of disease).

TUMOR MARKERS - DETERMINATION

- Concentration is dependent of:
  - Number of cells where TM is synthesized, stadium;
  - Speed of synthesis;
  - Rate of release from the tumor cell;
  - Activity of TM;
  - Type of tumor;
  - Blood supply of the tumor;
  - Degree of necrosis of tumor tissue;
  - Half time of TM;
  - Role of antibodies.

TUMOR MARKER - IDEAL

- Present only in tumor cells;
- Specific for organ and tumor;
- Determined in biological samples all patients with the same type of tumor;
- Present in sufficient concentration;
- Detected in the early stage;
- Serum concentrations dependent of tumor growth or mass of the tumor;
- Serum concentrations as a prognostic factor of the disease.

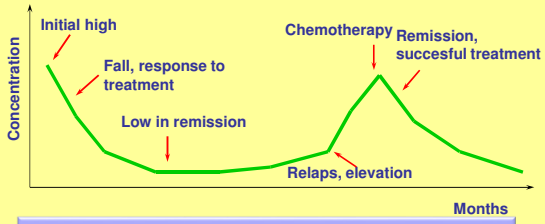
TUMOR MARKERS - WEAKNESSES

- Unsufficient specificity;
- Synthesis of high concentrations in non cancer illnesses (inflamation, benign tumors);
- Synthesis in different physiological states (pregnancy, menstriation, lactation);
- Synthesis in healthy tissues.

TUMOR MARKERS - WHEN?

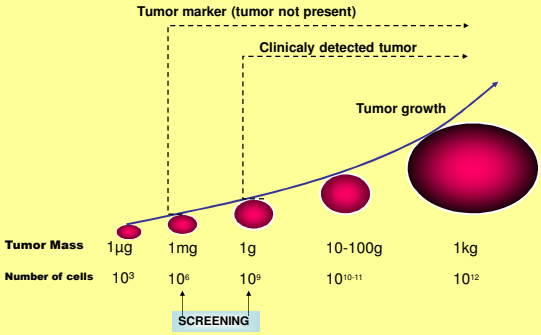
- Before surgery, before the begining of the treatment (radiation, chemotherapy, hormone or biological therapy);
- After surgery, during the treatment and after the treatment. Once in a 3 to 6 months in the first two years, and later once a year or at regular check ups;
- In case of suspected recurrence or progression of the disease;
- Before the change in the treatment;
- 2-3 weeks after elevated values of TM.

CHANGE IN THE CONCENTRATION OF TM THROUGH THE TREATMENT

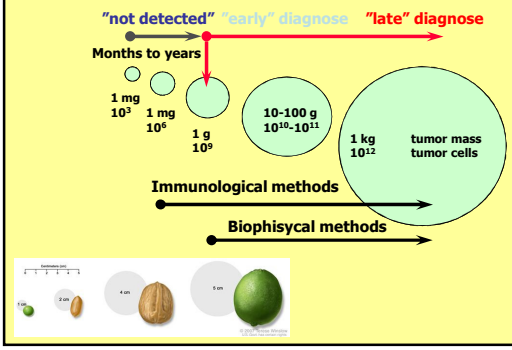


Tumor markers may also be measured periodically during cancer therapy. A decrease in the level of a tumor marker or a return to the marker's normal level may indicate that the cancer is responding to treatment, whereas no change or an increase may indicate that the cancer is not responding.

MEASURING OF TUMOR MASS



"DIAGNOSTIC WINDOW"



TUMOR MARKERS - DIVISION

- Upon chemical structure
- Place of the synthesis
- Type of tumor disease, etc.
- The most common is biochemically, place of origin and functionality.

TUMOR MARKERS

- **Epitelial markers**
  - **Cytokeratins**
  - **Epitelial membrane antigenes (EMA)**
  - **Desmoplacyn**
  - **Onkofekal proteins**
    - Carcinoembrional antigen – CEA
    - Alpha-fetoprotein - AFP
- **Mezenhimal markers**
  - **Muscle antigens**
    - **Desmin**
    - **Actin**
    - **Myoglobin**
    - **Myozin**
  - **Vascular antigens**
    - **CD 34**
    - **CD 31**
  - **Neuronal antigens**
    - **S 100**
    - **Neuron specific enolase (NSE)**
    - **Glialni fibrilar acid protein (GFAP)**
    - **Synaptophisyn**
    - **Receptor for neuron growth factor**

TUMOR MARKERS

- **Tumor antigens**
  - Carcinom antigen 15-3 – CA 15-3
  - Mucin carcinom antigen – MCA
  - Carcinom antigen 125 – CA 125
  - Carcinomi antigen 19-9 – CA 19-9
  - Prostate specific antigen - PSA
- **Special serum proteins**
  - Ferritin
  - Tyroglobulin
  - Beta-2-mikroglobulin
  - Glicoprotein
  - Immunoglobilins
- **Mixed markers**
  - Tissue polypeptid antigen – TPA
  - Spermin
  - Spermidin
  - Putrescin

TUMOR MARKERS

- **Prognostic markers**
  - **Adhesion molecules**
    - **Kadherins**
    - **Integrins**
    - **Selektins**
  - **Proliferative markers**
    - **PCNA**
    - **Ki67**
    - **AgNOR**
- **Hormones, hormone receptors and/or carcinoplacental antigens**
  - Chorionic gonadotropin –hCG
  - Estrogen receptor
  - Progesteron receptor
- **Biochemical markers**
  - **Enzymes and izoenzymes**
    - Prostatic acid phosphatase
    - Alkaline phosphatase
    - Lactat dehydrogenase
    - Gama GT
    - Tymidin kinase
    - Lyzozim

SCREENING

PRIMARY DIAGNOSIS : DIFFERENCIAL DIAGNOSIS

CONTROL OF THERAPY

FOLLOW UP – EARLY DETECTION OF METASTASES

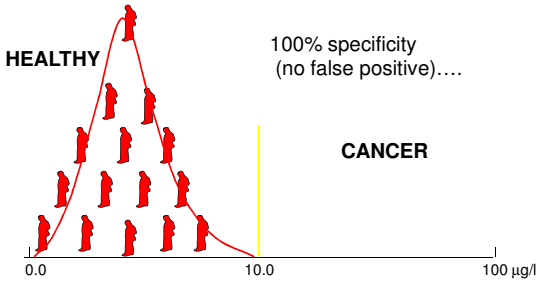
SCREENING

PRIMARY DIAGNOSIS : DIFFERENCIAL DIAGNOSIS

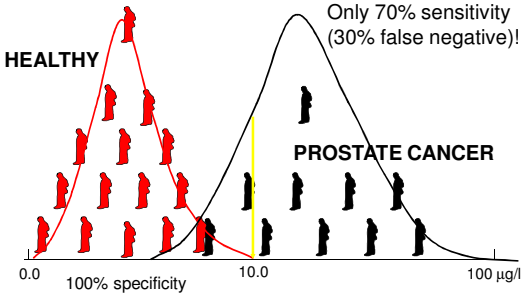
CONTROL OF THERAPY

FOLLOW UP – EARLY DETECTION OF METASTASES

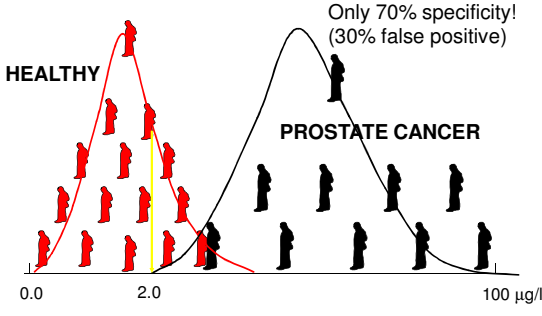
CUT OFF (PSA)



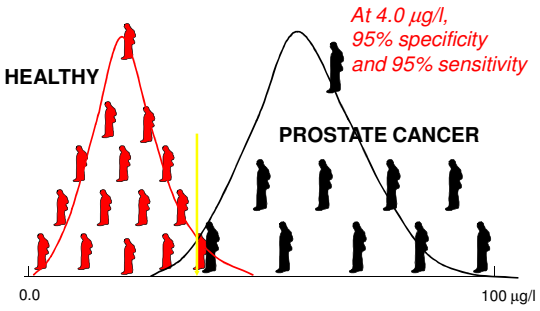
CUT OFF (PSA)



CUT OFF (PSA)



CUT OFF (PSA)



Signs, symptoms and diagnostic procedures

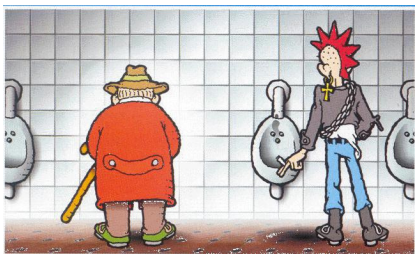
44

Signs, symptoms and diagnostic procedures

- ***Elevated prostate-specific antigen (PSA) level***
  - No PSA level guarantees the absence of prostate cancer.
  - The risk of disease increases as the PSA level increases, from about 8% with a PSA level of 1 ng/mL to about 25% with a PSA level of 4-10 ng/mL.
- ***Abnormal digital rectal examination (DRE) findings***
  - DRE is examiner-dependent, and serial examinations over time are best
  - Most patients diagnosed with prostate cancer have normal DRE results but abnormal PSA readings
- ***Biopsy***
  - Biopsy establishes the diagnosis
  - False-negative results often occur, so multiple biopsies may be needed before prostate cancer is detected

45

Prostate Cancer Screening



46

Prostate Cancer Screening

- DRE and PSA evaluation are the two components used in prostate cancer screening.
- Transrectal ultrasonography (TRUS) has been associated with a high false-positive rate, making it unsuitable as a screening tool, although it has an established role in directing prostatic biopsies.

- Screening for prostate cancer is a controversial topic. Screening offers the opportunity to find cancers at a more curable stage.

- Guidelines on prostate cancer screening have been issued by the following organizations:
  - American Cancer Society (ACS)
  - National Comprehensive Cancer Network (NCCN)
  - US Preventive Services Task Force (USPSTF)
  - American Urological Association (AUA)
  - American College of Physicians (ACP)

47

Prostate Cancer Screening

The Prostate Intervention Versus Observation Trial (**PIVOT**), which randomized men with localized prostate cancer to watchful waiting or radical prostatectomy, found no significant difference in either all-cause or prostate cancer mortality between the two groups through at least 12 years of follow-up

Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial was a trial comparing high-intensity PSA screening to less-intensive screening. This trial found no reduction in mortality with screening after 10 years

European Randomized Study of Screening for Prostate Cancer (ERSPC) trial found that, over a median follow-up of 9 years, PSA-screening at an average of every 4 years resulted in a 20% reduction in the rate of death from prostate cancer. However, the risk of overdiagnosis was high

48

Prostate Cancer Screening

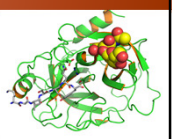
Conclusions: After about 10 years, PSA-based screening results in small or no reduction in prostate cancer-specific mortality and is associated with harms related to subsequent evaluation and treatments, some of which may be unnecessary.

Data Sources: MEDLINE (2002 to July 2011), the Cochrane Library Database (through the 2nd quarter of 2011) and reference lists.
Study Selection: Randomized trials of PSA-based screening; randomized trials and cohort studies of prostatectomy or radiation therapy versus watchful waiting for localized prostate cancer; and large (n>1000), uncontrolled observational studies of perioperative harms.

Biochemistry: from PSA to PHI

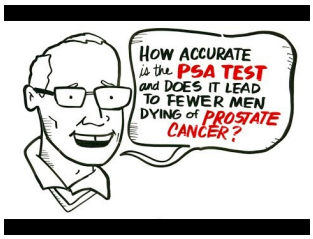
Biochemistry: from PSA to PHI

- PSA exists in serum in multiple forms: complexed to alpha-1-anti-chymotrypsin (PSA-ACT complex), unbound (free PSA), and enveloped by alpha-2-macroglobulin (not detected by immunoassays).



Prostate-specific antigen
- The use of PSA as a serum marker has revolutionised PCa diagnosis.
- PSA is organ - but not cancer specific, therefore, it may be elevated in benign prostatic hypertrophy (BPH), prostatitis and other nonmalignant conditions. As an independent variable, PSA is a better predictor of cancer than DRE or transrectal ultrasound (TRUS).

Biochemistry: from PSA to PHI



Biochemistry: from PSA to PHI

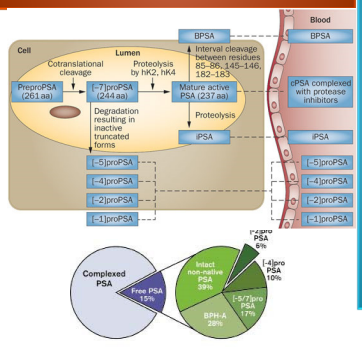
PSA level (ng/ml)	Risk of Pca (%)	Risk of Gleason > 7 Pca (%)
0.0 – 0.5	6.6	0.8
0.6 – 1.0	10.1	1.0
1.1 – 2.0	17.0	2.0
2.1 – 3.0	23.9	4.6
3.1 – 4.0	26.9	6.7

Table demonstrates the occurrence of Gleason >7 PCa at low PSA levels, precluding an optimal PSA threshold for detecting non-palpable but clinically significant PCa.

Free:total PSA	50-59 years	60-69 years	> or =70 years
< or =0.10	49.2%	57.5%	64.5%
0.11-0.18	26.9%	33.9%	40.8%
0.19-0.25	18.3%	23.9%	29.7%
>0.25	9.1%	12.2%	15.8%

Based on free:total PSA ratio: the percent probability of finding prostate cancer on a needle biopsy by age in years

Biochemistry: from PSA to PHI



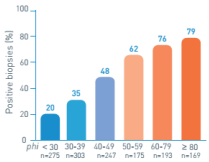
[-2]proPSA levels were significantly higher in the cancer group (n=208) than in the benign group (n=173). Already 4 years before diagnosis [-2]proPSA differed significantly between PCa and benign prostate in all measured time points, however, highest prediction value was 2 and 1 years before diagnosis (P<0.001). When stratified [-2]proPSA levels according to GS of RP specimens, [-2]proPSA was highest in patients with GS8 and lowest in those with GS6.

Biochemistry: from PSA to PHI

p2PSA appears to have the highest predictive ability when associated with other variables. Beckman Coulter Inc. Developed the PHI, a mathematical algorithm that is defined as

$$PHI = \frac{[-2]proPSA}{fPSA} \times \sqrt{tPSA}$$

The higher the PHI index, the greater the risk of having prostate cancer:



Stephan et al, Clinical Chem Acta, 2013

Biochemistry: from PSA to PHI

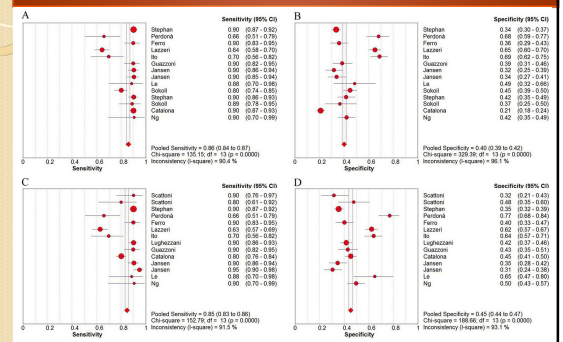
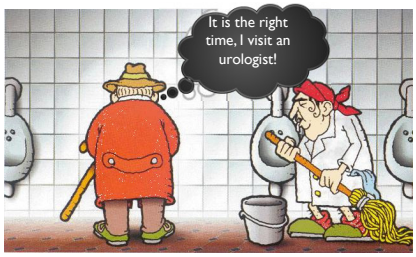
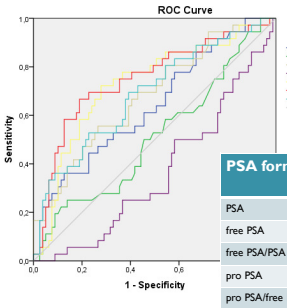


Figure 2 | Forest plot of sensitivities and specificities of %p2PSA and Phi for the diagnosis of PCa. (A) sensitivity for %p2PSA; (B) specificity for %p2PSA; (C) sensitivity for Phi; (D) specificity for Phi.

Results of our study and comparison with others



Results of our study and comparison with others



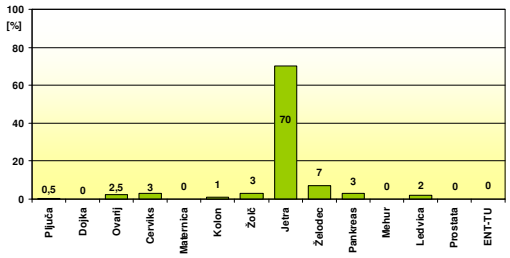
Number of cases: 115
- **Pca: 36**
- **BHP: 79**

PSA form	Area under the curve - ROC
PSA	0,64
free PSA	0,51
free PSA/PSA	0,36
pro PSA	0,68
pro PSA/free PSA	0,73
PHI	**0,75**
PSA velocity	0,69

Alpha-fetoprotein (AFP)

- Alpha-fetoprotein (AFP) is a protein produced primarily by the liver in a developing baby (fetus). AFP levels are typically elevated when a baby is born and then decline rapidly. Liver damage and certain cancers can increase AFP concentrations significantly.
- AFP is produced whenever liver cells are regenerating. With chronic liver diseases, such as hepatitis and cirrhosis, AFP may be chronically elevated. Very high concentrations of AFP may be produced by certain tumors. This characteristic makes the AFP test useful as a tumor marker. Increased amounts of AFP are found in many people with a type of liver cancer called hepatocellular carcinoma and in a liver cancer occurring in infants called hepatoblastoma. They are also found in some people with cancers of the testicles or ovaries.

AFP in different cancers

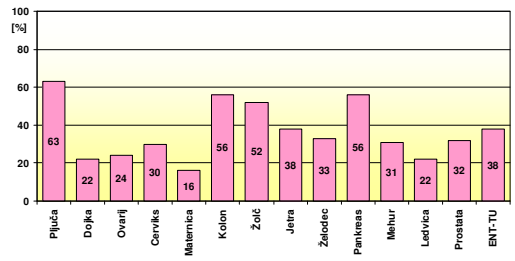


Cut off AFP: 15 µg/l

Carcinoembryonic Antigen (CEA)

- The carcinoembryonic antigen (CEA) test measures the amount of this protein that may appear in the blood of some people who have certain kinds of cancers, especially cancer of the large intestine (colon and rectal cancer). It may also be present in people with cancer of the pancreas, breast, ovary, or lung.
- CEA is normally produced during the development of a fetus. The production of CEA stops before birth, and it usually is not present in the blood of healthy adults.
- The carcinoembryonic antigen (CEA) test is used to:
- Find how widespread cancer is for some types of the disease, especially colon cancer.

CEA in different cancers

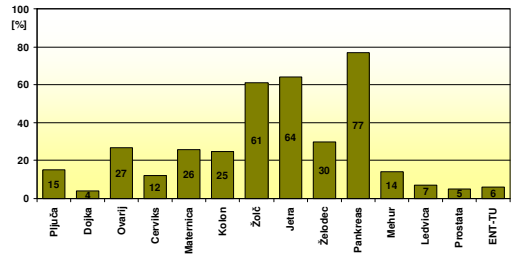


Cut off CEA: 3 µg/l

CA 19-9

- Cancer antigen 19-9 (CA 19-9) is a protein that exists on the surface of certain cancer cells. CA 19-9 does not cause cancer; rather, it is shed by the tumor cells, making it useful as a tumor marker to follow the course of the cancer.
- CA 19-9 is elevated in 70% to 95% of people with advanced pancreatic cancer, but it may also be elevated in other cancers, conditions, and diseases such as colorectal cancer, lung cancer, gallbladder cancer, bile duct obstruction (e.g., gallstones), pancreatitis, cystic fibrosis, and liver disease. Small amounts of CA 19-9 are present in the blood of healthy people.

CA 19-9 in different cancers

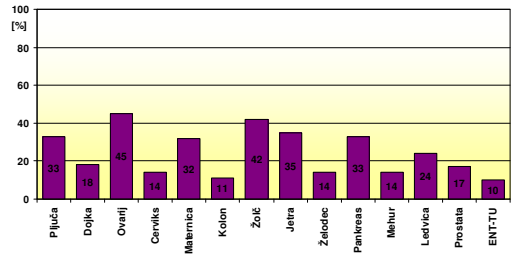


Cut off CA 19-9: 37 kU/l

CA 15-3

- Cancer antigen 15-3 (CA 15-3) is a protein that is produced by normal breast cells. In many people with cancerous breast tumors, there is an increased production of CA 15-3. CA 15-3 does not cause cancer; rather, it is shed by the tumor cells and enters the bloodstream, making it useful as a tumor marker to follow the course of the cancer.
- CA 15-3 is elevated in only about 10% of women with early localized breast cancer but is elevated in about 80% of those with metastatic breast cancer.
- CA 15-3 may also be elevated in healthy people and in individuals with other cancers (e.g., colon, lung, pancreas, ovary, or prostate malignancies) or certain conditions (e.g., cirrhosis, hepatitis, and benign breast disease).

CA 15-3 in different cancers

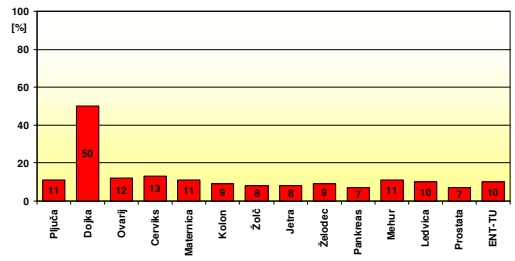


Cut off CA 15-3: 28 kU/l

HER2/neu

- HER2-positive breast cancer is a breast cancer that tests positive for a protein called human epidermal growth factor receptor 2 (HER2), which promotes the growth of cancer cells.
- In about 1 of every 5 breast cancers, the cancer cells have a gene mutation that makes an excess of the HER2 protein. HER2-positive breast cancers tend to be more aggressive than other types of breast cancer. They're less likely to be sensitive to hormone therapy, though many people with HER2-positive breast cancer can still benefit from hormone therapy.

HER2-neu in different cancers

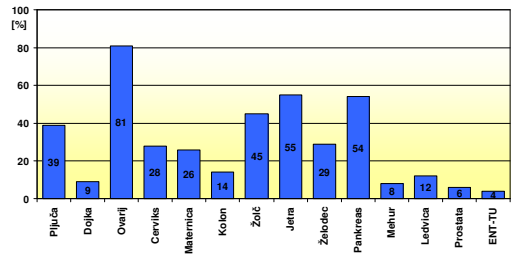


Cut off Her2-neu: 10 µg/l

CA 125

- Cancer Antigen 125 (CA-125) is a protein that is present on the surface of most, but not all, ovarian cancer cells. This makes the test useful as a tumor marker in specific circumstances.
- Significantly elevated concentrations of CA-125 may be present in the blood of a woman who has ovarian cancer.
- Currently, less than 20% of ovarian cancers are found in the early stages before they have spread outside the ovary. The primary reason they go undetected is that the symptoms of ovarian cancer are fairly non-specific.
- CA-125 is not recommended as a screening test for asymptomatic women because it is non-specific. Small quantities of CA-125 are produced by normal tissues throughout the body and by some other cancers. Levels in the blood may be moderately elevated with a variety of non-cancerous conditions, including menstruation, pregnancy, and pelvic inflammatory disease.

Pogostnost izločanja CA 125 pri različnih rakih

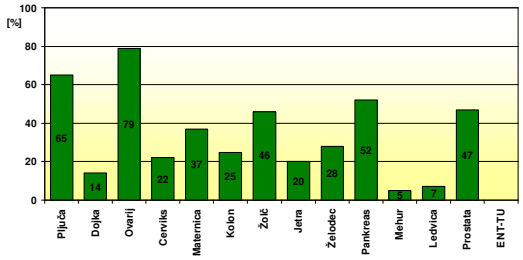


Cut off CA 125: 35 kU/l

HE 4

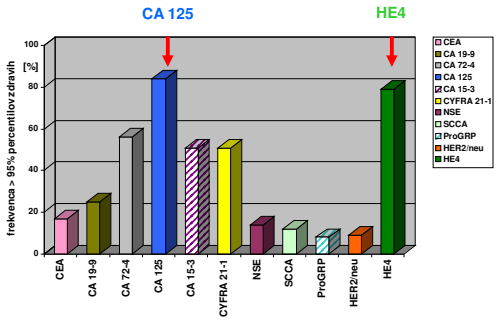
- Human epididymis protein 4 (HE4) is a protein that is produced by most, but not all, epithelial ovarian cancer cells. This makes the test useful as a tumor marker in specific circumstances.
- Significantly elevated concentrations of HE4 are frequently present in the blood of a woman who has epithelial ovarian cancer.
- There are several different subtypes of epithelial ovarian cancer, including: serous, endometrioid, mucinous, and clear cell, with serous being the most common. Some studies have shown that HE4 is elevated in more than 90% of serous and endometrioid epithelial ovarian cancers and about 50% of clear cell tumors, but it is not usually elevated in mucinous cancers.
- HE4 is not recommended as a screening test for asymptomatic women because it is non-specific. Small quantities of HE4 are produced by normal tissues throughout the body and mild elevations may be seen with a variety of non-cancerous conditions.

HE4 in different cancers



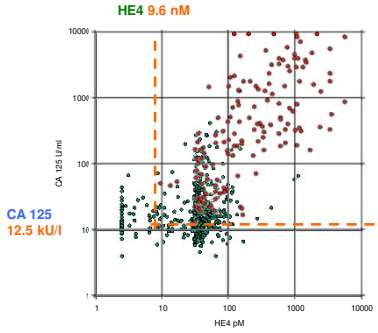
Cut off HE4: 70 pM

Biomarker release in primary ovary cancer



marker	CEA	AFP	CA 19-9	CA 72-4	CA 125	CA 15-3	CYFRA 21-1	NSE	SCC	ProGRP	HER2/neu	HE4
Cut off	3 µg/l	15 µg/l	35 kU/l	3 kU/l	35 kU/l	30 kU/l	3 µg/l	20 µg/l	2 µg/l	30 ng/l	14 µg/l	70 pM

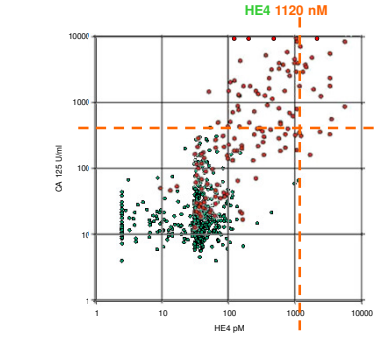
Correlation CA 125 and HE4



NPV CA 125 in HE4

- CA 125 <12.5 kU/l
  23% (n=147/651) TN
- HE4 <9.6 nM
  9% (n=59/651) TN
- CA 125 <12.5 kU/l and/or HE4 <9.6 nM
  29% (186/651) TN =>
  benign gynecologic illness

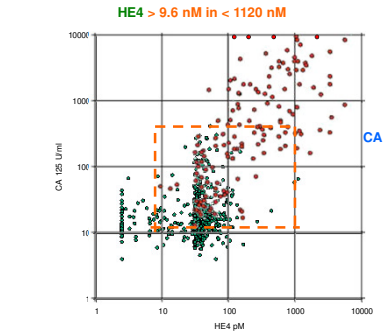
Correlation CA 125 and HE4



PPV CA 125 in HE4

- CA 125 >416 kU/l
  10% (n=64/651) TP
- HE4 >1120 nM
  3% (n=21/651TP
- CA 125 >416 kU/l and/or HE4 >1120 nM
  11% (n=69/651) TP

Correlacija CA 125 with HE4



Regression algoritem

Risk of Ovarian Malignancy Algorithm (ROMA)

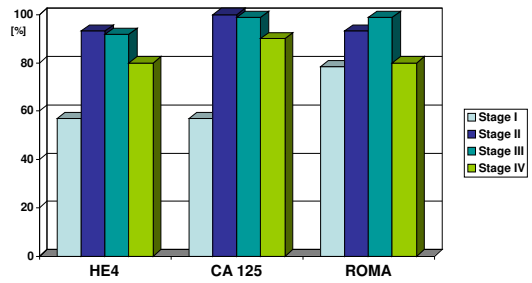
Premenopauzal women
PI = -12.0 + 2.38*LN[HE4] + 0.0626*LN[CA 125]

Postmenopauzal women
PI = -8.09 + 1.04*LN[HE4] + 0.732*LN [CA 125]

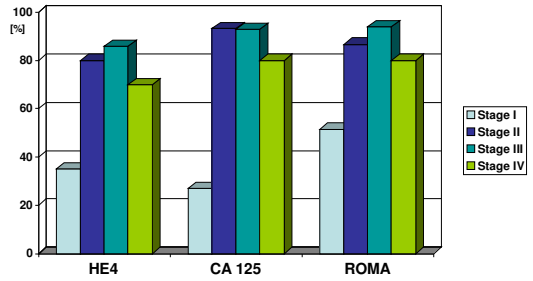
ROMA = exp(PI) / [1 + exp(PI)]x 100

PI: Predictive Index

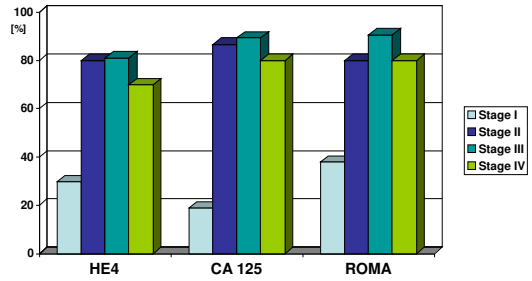
Sensitivity HE4, CA 125 and ROMA index
(Benigna ginekološka obolenja proti raku ovarija glede na stadij)
Specifificity 75%



Sensitivity HE4, CA 125 and ROMA index
(Benigna ginekološka obolenja proti raku ovarija glede na stadij)
Specificity 90%



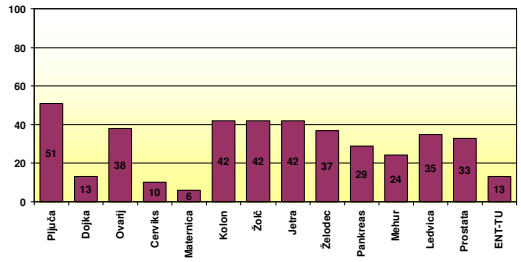
Sensitivity HE4, CA 125 and ROMA index
(Benigna ginekološka obolenja proti raku ovarija glede na stadij)
Specificity 95%



NSE in different cancers

- **NSE:** Neuron-specific enolase (NSE) is a substance that has been detected in patients with certain tumors, namely: neuroblastoma, small cell lung cancer, medullary thyroid cancer, carcinoid tumors, endocrine tumors of the pancreas, and melanoma.
- Studies of NSE as a tumor marker have concentrated primarily on patients with neuroblastoma and small cell lung cancer. Measurement of NSE levels in patients with these two diseases can provide information about the extent of the disease and the patient's prognosis (outlook), as well as about the patient's response to treatment.

NSE in different cancers

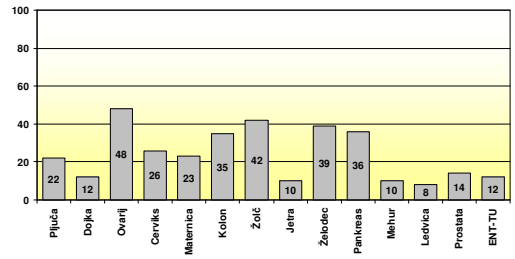


Cut off NSE 12,5 µg/l

CA 72-4

- Diagnosing gastric carcinoma is often complicated and can be extremely difficult due to presentation of non-specific symptoms that are sometimes associated with non-malignant disease.
  Although endoscopy, coupled with histologic evaluation of biopsy specimens, is most often used to make definitive diagnosis, the search for additional non-invasive diagnostic procedures has longed continued: strong new clinical evidence has recently emphasized the clinical value of the CA 72-4 serum tumor marker assay in diagnosis and monitoring of gastric cancer..
  We use it, to be largely complete, with CEA and CA 19-9.

CA 72-4 in different cancers

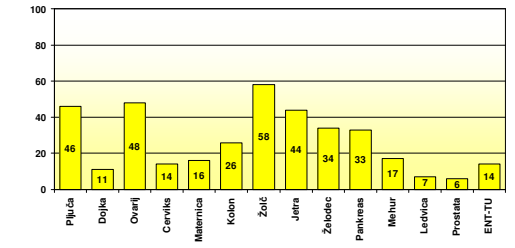


Cut off CA 72-4: 3,0 kU/l

Cyfra 21-1 in different cancers

- The “cytokeratin fragment” is the most stunning marker ever discovered. Very few labs monitor it at present. It is specially interesting for lung cancer, diagnostic and follow-up of epidermoidis forms.
  Among all forms of lung cancer Cyfra 21-1 delivers an appreciated sensitivity of 65%, and an exceptional specificity of 95%.
  Regular evaluation of the Cyfra allows easier follow-up treatment, and even better when associated with other TMs.
  CA 15-3, Cyfra are now used in breast cancer: its elevation in follow-up demonstrates, very early, the apparition of metastases.
  Cyfra can also be used in uterine cancer, oesophagus cancer, bladder cancer.

Cyfra 21-1 in different cancers



Cut off CYFRA 21-1: 3,0 µg/l

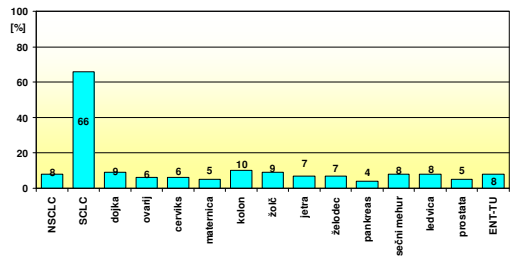
Lung cancer

- Lung cancer is one of the most common cancers in the world with 1.35 million new cases diagnosed every year
- The two main histological types of the disease are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). It is important to distinguish between these two subtypes as they have different treatments and prognoses
- NSCLC (approx. 80% of cases) is curable with surgery in the early stages. SCLC, however, is an aggressively spreading neoplasm of rapid growth that is usually only treatable with chemo- and radiotherapy.

ProGRP

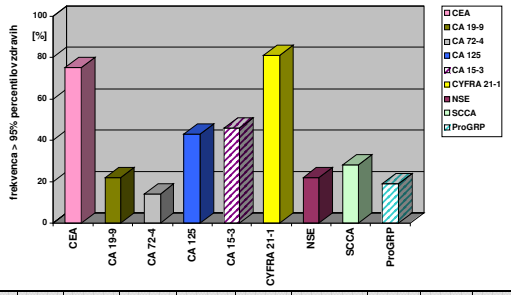
- Pro-gastrin releasing peptide (ProGRP) is a novel tumor marker with benefits for lung cancer patient management. It is the tumor marker of choice for SCLC as it supports quick and accurate discrimination between SCLC and NSCLC for a faster decision on patient treatment
- ProGRP can also be used to assess response to therapy and monitor recurrence of the disease
- The 85.7 pg/mL cut-off value is based on a 95 % specificity from the NSCLC collective.

ProGRP in different cancers



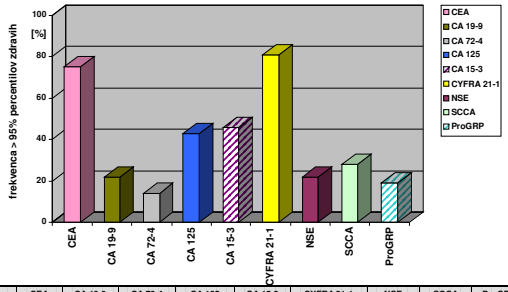
Cut off ProGRP: 30 ng/l

Biomarkers in lung cancer



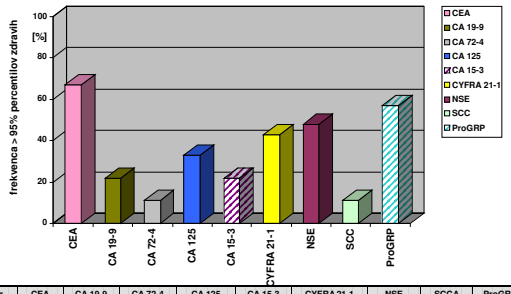
marker	CEA	CA 19-9	CA 72-4	CA 125	CA 15-3	CYFRA 21-1	NSE	SCCA	ProGRP
Raz.vr.	2.3 µg/ml	28.4 kU/l	5.9 kU/l	31.5 kU/l	23.1 kU/l	1.3 µg/l	20.0 µg/l	1.5 µg/l	30.0 ng/l

Biomarkers in NSCLC phase I-IIIA



marker	CEA	CA 19-9	CA 72-4	CA 125	CA 15-3	CYFRA 21-1	NSE	SCCA	ProGRP
Raz.vr.	2.3 µg/ml	28.4 kU/l	5.9 kU/l	31.5 kU/l	23.1 kU/l	1.3 µg/l	20.0 µg/l	1.5 µg/l	30.0 ng/l

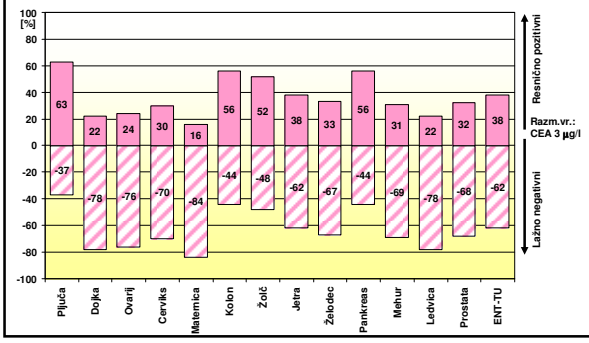
Biomarkers in SCLC Phase LD (limited growth)



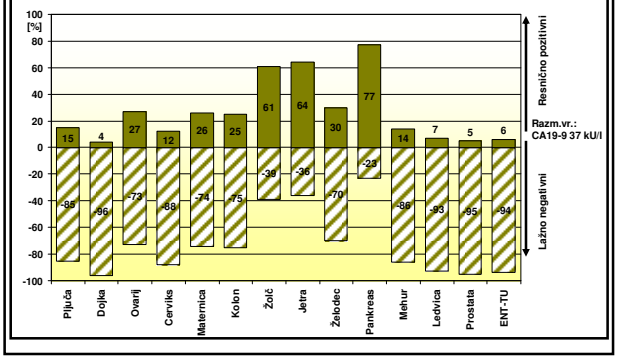
marker	CEA	CA 19-9	CA 72-4	CA 125	CA 15-3	CYFRA 21-1	NSE	SCCA	ProGRP
Raz.vr.	2.3 µg/ml	28.4 kU/l	5.9 kU/l	31.5 kU/l	23.1 kU/l	1.3 µg/l	20.0 µg/l	1.5 µg/l	30.0 ng/l

The use of only one TM for screening is not recomended because of high FP and FN results

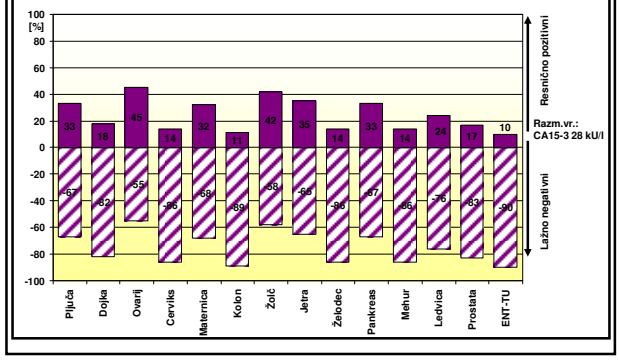
CEA < cut of for different cancers



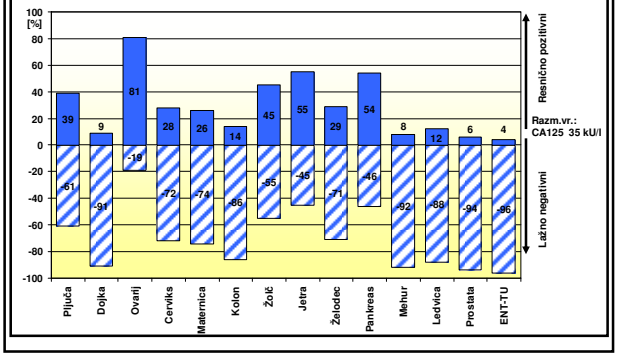
CA 19-9 < cut off for different cancers



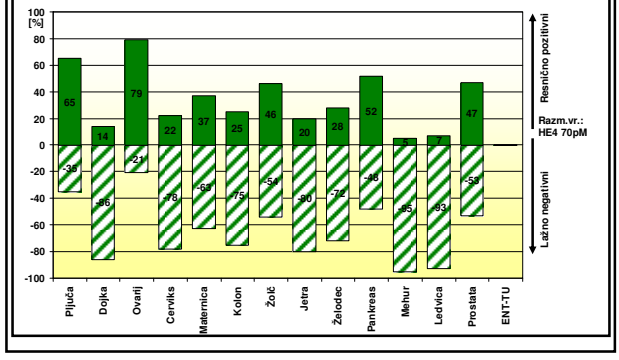
CA 15-3 < cut off for different cancers



CA 125 < cut off for different cancers



HE4 < cut off for different cancers



SCREENING

PRIMARY DIAGNOSIS : DIFFERENCIAL DIAGNOSIS

CONTROL OF THERAPY

FOLLOW UP – EARLY DETECTION OF METASTASES

Can we diagnose cancer on the base of tomor non specific marker?

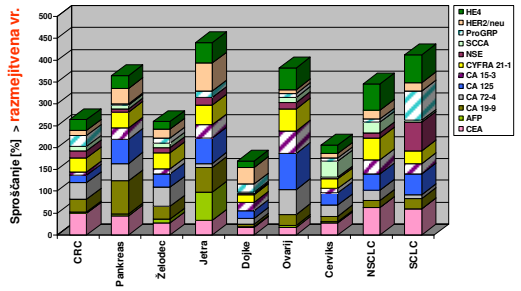
Higher concentrations

are leading to increased specificity for the presence of tumor

Tumor specificity > 99%

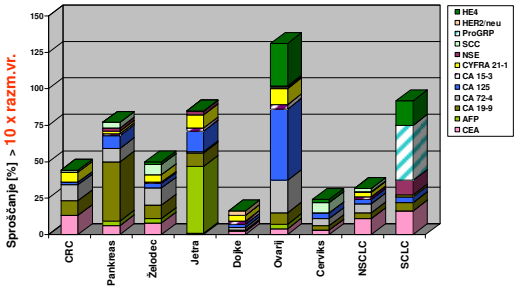
CEA:	> 20 µg/l
CA 19-9:	> 1000 kU/l
AFP:	> 1000 µg/l
CA 125:	> 1000 U/l
CA 15-3:	> 100 kU/l
CA 72-4:	> 100 kU/l
CYFRA21-1:	> 20 µg/l
SCC:	> 10 µg/l
NSE:	> 50 µg/l
ProGRP:	> 200 ng/l
S100:	> 1 µg/l
PSA:	> 50 µg/l

Biomarkers in different cancers - MODERATE



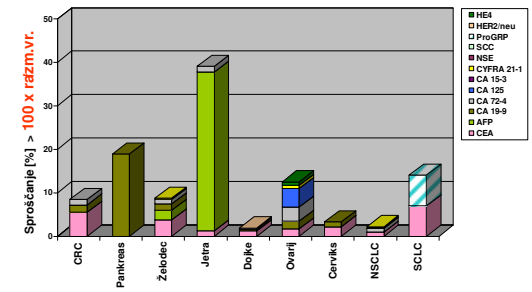
marker	CEA	AFP	CA 19-9	CA 72-4	CA 125	CA 15-3	CYFRA 21-1	NSE	SCC	ProGRP	HER2/neu	HE4
Raz.vr.	3 µg/l	15 µg/l	35 kU/l	3 kU/l	35 kU/l	30 kU/l	3 µg/l	20 µg/l	2 µg/l	30 ng/l	14 µg/l	70 pM

Biomarkers in different case - HIGH



marker	CEA	AFP	CA 19-9	CA 72-4	CA 125	CA 15-3	CYFRA 21-1	NSE	SCC	ProGRP	HER2/neu	HE4
Raz.vr.	3 µg/l	15 µg/l	35 kU/l	3 kU/l	35 kU/l	30 kU/l	3 µg/l	20 µg/l	2 µg/l	30 ng/l	14 µg/l	70 pM

Biomarkers in different cancers – VERY HIGH

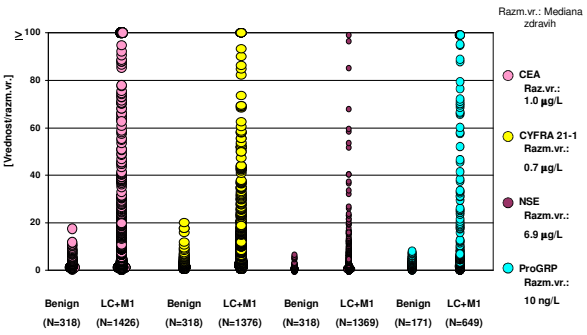


marker	CEA	AFP	CA 19-9	CA 72-4	CA 125	CA 15-3	CYFRA 21-1	NSE	SCC	ProGRP	HER2/neu	HE4
Raz.vr.	3 µg/l	15 µg/l	35 kU/l	3 kU/l	35 kU/l	30 kU/l	3 µg/l	20 µg/l	2 µg/l	30 ng/l	14 µg/l	70 pM

Diagnosis of tumor with biomarkers

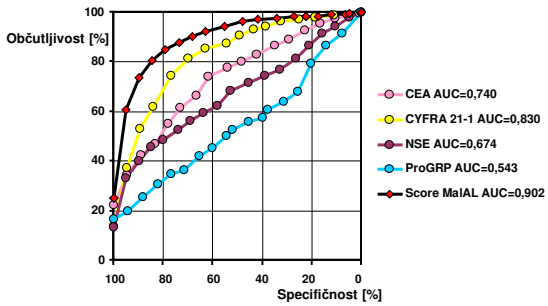
Is a <u>combination</u> of biomarkers able to incerase diagnostic capacity?

CEA, CYFRA 21-1, NSE, ProGRP
Benign vs malign lung disease

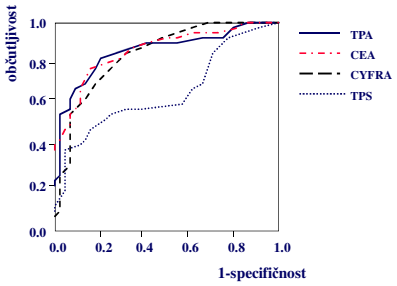


From certain point all markers become tumor specific

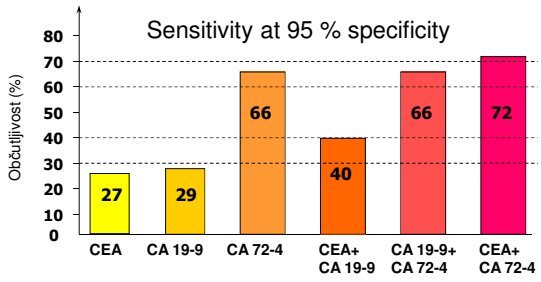
ROC curves
Benign vs malign lung diseases



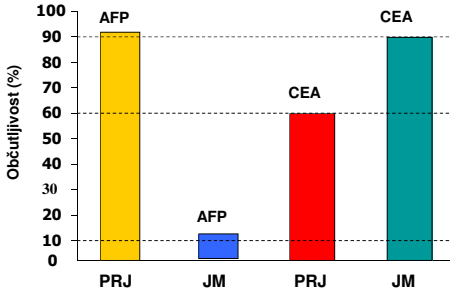
ROC - TPA, CEA, CYFRA in TPS pri kolorektalnem raku



TM in stomach cancer



AFP and CEA in liver cancer

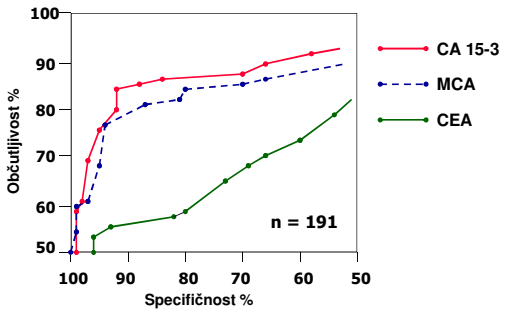


PRJ – primary liver cancer JM – liver metastases

Sensitivity TPA, CYFRA, CA 15-3 and TPS in breast cancer

	Specifičnost (%)	Občutljivost (%)
TPA	95	17
	90	22
CYFRA	95	11
	90	21
CA 15.3	95	11
	90	15
TPS	90	10
	95	19

ROC curve in breast cancer



EARLY DETECTION

Early detection of metastases in breast cancer and relaps

TO	Sensitivity %	Specificity %
TPA	63	98
CA 15-3	46	98
CEA	7	99
CA15-3+TPA	83	96
CEA+TPA	70	98

SCREENING

PRIMARY DIAGNOSIS : DIFFERENCIAL DIAGNOSIS

CONTROL OF THERAPY

FOLLOW UP – EARLY DETECTION OF METASTASES

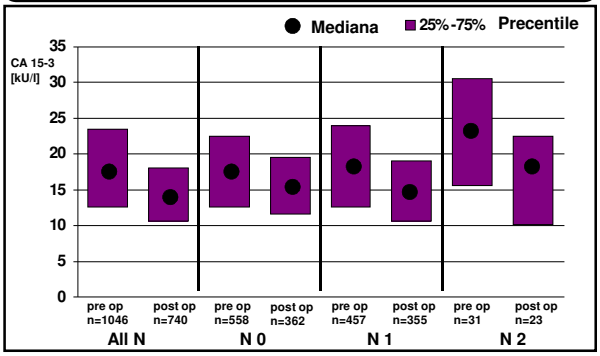
Meaning of individual basical values of concrete patient

BREAST CANCER – BEFORE SURGERY

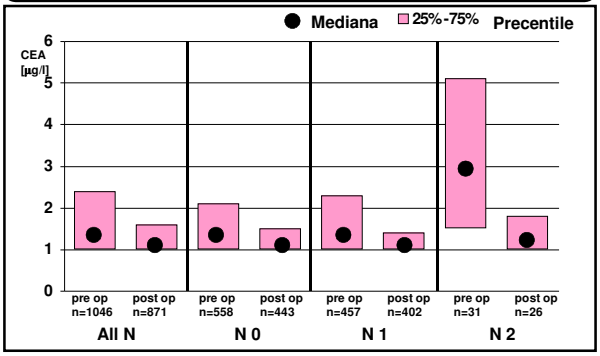
CEA	2,4 µg/l
CA 15-3	23,1 kU/l

TM are normal

After surgery fall of CA 15-3 in breast cancer



After surgery fall of CEA in breast cancer



Concentration of Biomarkers falls

(depending on half time)

after succesful surgery or therapy

to individual basic values

of the patient

What is

„normal“

for a certain patient

can be established solely by following individual basic values

This

individual basic values

of a certain patient are real results for the interpretation during the treatment

Thus the control measurement of TM after first stage of treatment is ABSOLUTELY NECESSARY

-

irrespective of whether the primary value is within or outside the reference range

SCREENING

PRIMARY DIAGNOSIS : DIFFERENCIAL DIAGNOSIS

CONTROL OF THERAPY

FOLLOW UP – EARLY DETECTION OF METASTASES

Common thinking about tumor markers:

Tumor markers have been “normal” at the time of diagnosis, and can’t be used to assess the effectiveness of therapy

…..

BUT:

Diagnostic use of biomarkers

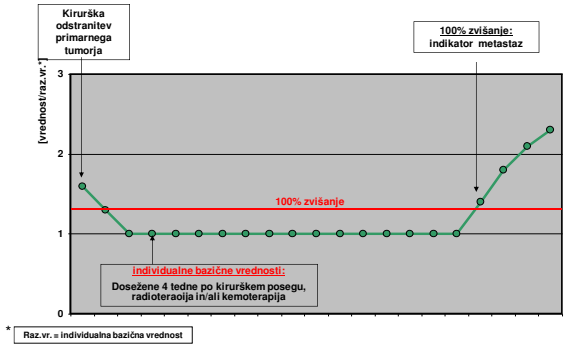
for the early recurrence detection or

metastases is not dependent of the release of tumor biomarkers from the primary tumor!

100% elevation from the individual base value

means almost 100% tumor specificity

100% elevation of CEA, CA 15-3, CA 125



Breast cancer, 869 patients

in 91 patients
metases present

When metastses were detected:

In 50% within first 4,3 years

in 75% within first 7 years

in 100% in the period of 23 years

91 patients with metastases

66 patients with elevated TM

25 patients without elevation of TM

Metastases and elevation of TM (n=66) Which marker was elevated?

CA 15-3	30
CEA	16
CA 125	10
HER2	1
CA 15-3 + CEA	1
CA 15-3 + CA 125	4
CEA + CA 125	2
All	2

Elevation of TM without metastases (4% patients)

CA 15-3	0
CEA	13
CA 125	14
CEA + CA 125	2

Secundary cancers during the study (n=24)

Ovariy CA	6 (3+)
Colorectal CA	6 (1+)
Stomach CA	3 (1+)
Limfom	3
Kidney CA	1
Anus CA	1
Lung CA	1
GIT	1 (1+)
Endometrium CA	1 (1+)
Clitoris CA	1

What elevation do we need to have tumor specificity > 99% :

CEA	100%
CA 15-3	75%
CA 125	150%
S-HER-2/neu	50%

RULE:

- Serial determinations of TM are comarable
- If the identical method and analyzer are used
- Beside the result laboratory should report the method (analyzer)
- Method should be if possible the same in the laboratory
- In the case of the change the new method has to be validated

THERAPEUTIC APPROACHES TO DIFFERENT CANCER DISEASES

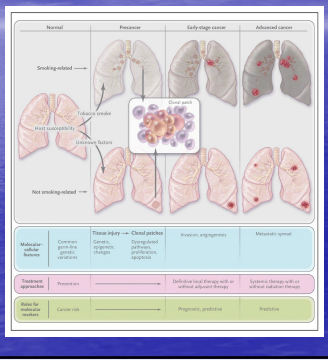
Prof Dr Besim Prnjavorac
Faculty of Pharmacy, Sarajevo BiH
General Hospital Tešanj

POSSIBILITIES FOR CANCER TREATMENT (choice of optimal approach)

- Surgery – any time as the best choice
- Radiotherapy – Not many invasive
  - Brachitherapy
- Chemotherapy – Different invasive levels
- Immunotherapy – wide spectrum fo therapeutic possiblities
  - Decrease of immune response (IMMUNOSUPRESSION)
  - Increase of immune response (IMMUNOSTIMULATION)
  - IMMUNOMODULATION (targeted to different cytokines)
- Combination of different approach

CANCER PROMOTION AND GROWTH –MATHER OF TIME

(One milion mistakes of DNA replication daily – tumor promotion is the result of cummulativ mistake – yes or no ...)
(immunologic surweilance – recognition of cancer cell...)



CLASIFICATIONS OF CHEMOTHERAPEUTICS (Classic historical approach)

- The majority of chemotherapeutic drugs can be divided in to:
  - Alkylating agents,
  - Antimetabolites,
  - Mitotic inhibitors,
  - Antracyclines (adriamycin)
  - Antibiotics
  - Vinca – Plant alkaloids (Vincristine)
  - Taxanes
  - Hormons, hormonal antagonist and enzymes
  - Other antitumour agents.
- All of these drugs affect cell division or DNA synthesis and function in some way.

Carcinogenesis, 16 (3): 437-441

There are three basic treatment possibilities for cancer: surgery, radiotherapy, and chemotherapy.

Some cancers where chemotherapy works very well:

- Childhood leukemia
- Retinoblastoma
- Osteosarcoma
- Testicular cancer(hormone sensitive cancers)
- Hodgkin's Disease
- Some lymphomas
- Some early breast cancers (hormone sensitive cancers)

Cancers that are very difficult to treat with chemotherapeutics (need surgery or radiotherapy first): (combination of approach)

- Colon
- Lung
- Pancreatic cancer
- (late stage of breast cancer –the same tumor cell, but stroma...) different approach of the same cancer.

(WHY???).

PROBLEMS ASSOCIATED WITH CHEMOTHERAPY

(Resistance to chemotherapy) (imperative to change approach)

Resistance to chemotherapy may develop by several mechanisms:

- Decrease in the amount of drug uptake by cancer cells

E.G. Methotrexate (some kind of adaptation)

- Increase in the amount of drug removed by cancer cells.

E.G. Vinblastine ,doxorubicin, bleomycin ,etapsoid....

- Decrease or alteration in target molecule sensitivity – this is caused by mutation in the molecule targeted by the drug – drug receptors

E.G. Methotrexate,Mercaptopurine,doxorubicin

- Increase in DNA repair ability of the cell via an increased expression of DNA repairing enzymes.

E.G. Alkylating agent

Cell cycle – approach fot target phase of cell cycle

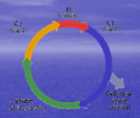
Cell cycle can be divided into:.

Gap 0 (G0): There are times when a cell will leave the cycle and quit dividing. This may be a temporary resting period or more permanent. An example of the latter is a cell that has reached an end stage of development and will no longer divide (e.g. neuron).

Gap 1 (G1): Cells increase in size in Gap 1, produce enzymes needed for DNA synthesis

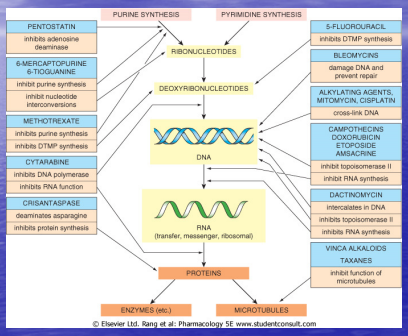
S Phase: To produce two similar daughter cells, the complete DNA instructions in the cell must be duplicated. DNA replication occurs during this S (synthesis) phase.

Gap 2 (G2): It is the gap between DNA synthesis and mitosis, the cell will continue to grow and produce new proteins & RNA.



Mitosis or M Phase (formation of mitotic spindle): Cell growth and protein production stop at this stage in the cell cycle. All of the cell's energy is focused on the complex and orderly division into two similar daughter cells.

POINT OF ACTION OF ANTINEOPLASTIC DRUGS

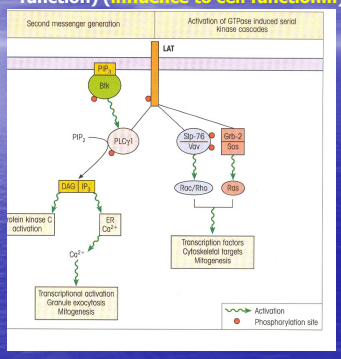


Cytostatic works as antibiotics (Anti Bios) (Better term for use as usual-anti microbics)

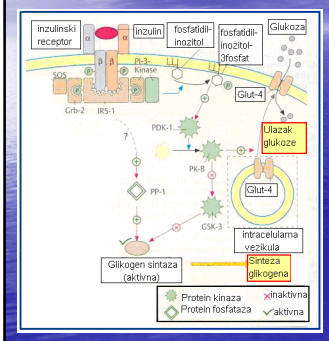
- Chemotherapeutics impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules.
- Mechanismof action depend of phase of cell cycle

SECOND MESSENGER FORMATION

(Forforilation is protein kinase (PK) job... necessair for cell function) (influence to cell function...)



ENTRY OF GLUCOSE INTO THE CELL (Cancer promotion and growth is mather of cell metabolism)



1. Linkage of inzulin with receptor
2. Autofosforilation of receptor.
3. Activation of molecuIs – second messengers
4. Induction of protein synthesis (GLUT-4)
5. Activation of fosfatidilinozitol-3-kinaze (PI3K) lead to movemen of glucose transporter (GLUT-4) to cell membrane.
6. Entry glucose into the cell .
7. Activation of PI3K in cell matri and stimulation of glicogene

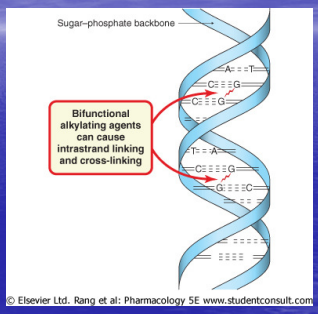
CLASSIC APPROACH FOR TREATMENT OF CANCER

- Chemotherapy – up to date in common use
- Oriented to cellmetabolism and growth
- No targeted

ALKYLATING AGENTS:

Alkylating agents are so named because of their ability to alkylate many nucleophilic functional groups under conditions present in cells.

Alkylating agents, or their reactive intermediates, form covalent bonds with deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein to form an adduct in which a methyl or ethyl group is added.



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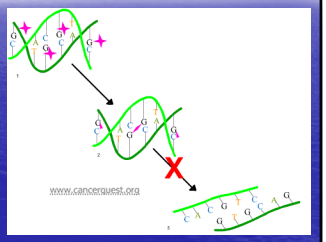
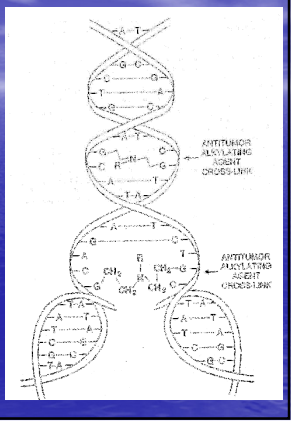
ALKYLATING AGENTS

- DNA adducts (Complex of two compounds) are believed to play a major role in mutagenesis and blastogenesis, as well as in carcinogenesis
- Common locations include the N-7 and O-6 of guanine which are shown to be associated with mutagenesis and carcinogenesis.
- Brake of DNA synthesis develop



DNA Cross Linkage

- Arrests DNA replication
- Can result in DNA damage and chromosome breaks
- Also mutagenic!



ALKYLATING AGENTS:

- As a result of this "alkylation", there are a few consequences:
  1) Miscoding (In transcription)
  2) Cross linking- this only occurs if the drug is bifunctional

The net result is cancer cell undergo apoptosis (Cell death)

- Some of the drugs:
  (*Busulfan (Myleran), Carboplatin (Paraplatin), Chlorambucil, Cisplatin, Cyclophosphamide (Cytoxan), Dacarbazine (DTIC-Dome), Estramustine Phosphate, Ifosphamide, Mechlorethamine (Nitrogen Mustard), Melphalan (Phenylalanine Mustard), Procarbazine, Thiotepa, Uracil Mustard*

ANTIMETABOLITES

- An antimetabolite is a chemical with a similar structure to a metabolite required for normal biochemical reactions, yet different enough to interfere with the normal functions of cells, including cell division.
- All antimetabolites are used in cancer treatment, as they interfere with DNA production and therefore cell division and the growth of tumors (mainly in S-phase specific).

- They are classified into:
  1- Folic acid analogues
  2- Purine analogues
  3- Pyrimidine analogues

- Purin and pyrimidine antagonists are phosphorelated inside the body into nucleotid form in order to be cytotoxic

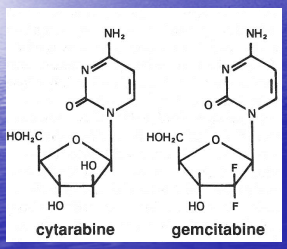
ANTIMETABOLITES:

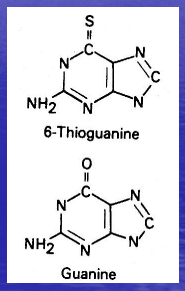
- Some of antimetabolites: Cladribine, Cytarabine (Cytosine Arabinoside), Floxuridine (FUDR, 5-Fluorodeoxyuridine), Fludarabine, 5-Fluorouracil (5FU), Gemcitabine, Hydroxyurea, 6-Mercaptopurine (6MP), Methotrexate (Amethopterin), 6-Thioguanine, Pentostatin, Pibobroman, Tegafur, Trimetrexate, Glucuronate

Clinical uses: leukemia, non-Hodgkin's lymphoma

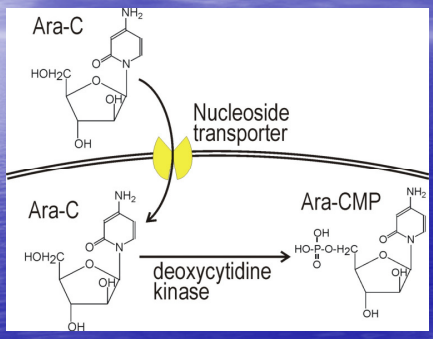
- inflammatory bowel disease such as Crohn's Disease and ulcerative colitis.
- It is widely used as immunosuppressant in transplantations to control rejection reactions, autoimmune disese,like Rh.art.
- Overlapp of tumor and autoimmune disease developent

NUCLEOSIDE ANALOGUES
- BIOCHEMICAL PHARMACOLOGY MIRRORS THE UPTAKE AND METABOLISM OF NORMAL NUCLEOSIDES

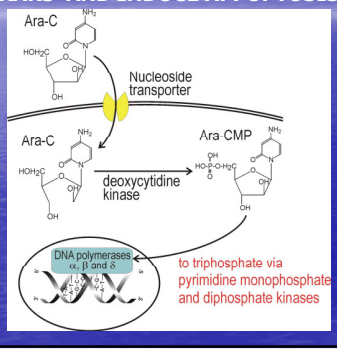




CYTOSINE ARABINOSIDE (CYTARABINE; ARA-C)
- MAJOR CHEMOTHERAPY DRUG TO TREAT ACUTE LEUKEMIAS



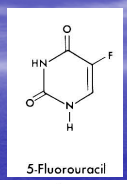
ARA-CTP COMPETE WITH NORMAL DNA METABOLISM AND INHIBIT DNA POLYMERASE. INCORPORATION INTO DNA PRODUCES STRAND BRAKS AND INDUCE APPOPTOSIS.

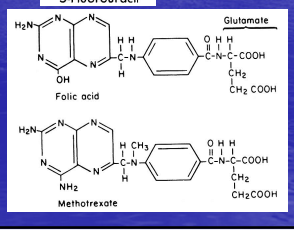


21

INHIBITION OF THYMIDYLATE SYNTHESIS

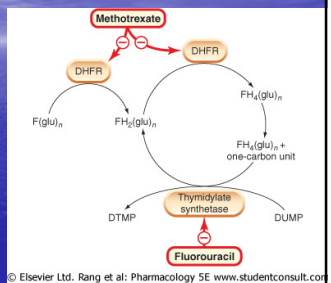
- Pyrimidine base 5-Fluorouracil (5FU) inhibits thymidylate synthase
- Methotrexate inhibits dihydrofolate reductase, reducing flow of methyl group carried by reduced folate





FOLIC ACID ANALOGUES (Antimetabolit)

1-Methotrexate compete with folic acid for DHFR (dihydrofolate reductase) and inhibits it . Therefore, it inhibits the synthesis of DNA, RNA and proteins.
2-Also,DHFR catalyses the conversion of dihydrofolate to the active tetrahydrofolat which is needed for the *de novo* synthesis of the deoxynucleoside thymidine phosphate DTMP (required for DNA synthesis)



ANTIBIOTICS:
(Classic approach – antibiotics-chemotherapeutics)

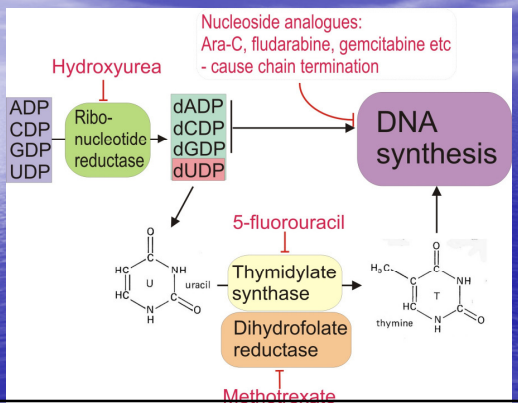
- Antitumor antibiotics interfere between DNA base pairs and disturb the synthesis or function of nucleic acids.
- Bleomycin is some different bindings to DNA results in single-strand breaks and double-strand scissions, thereby disrupting DNA synthesis. K1
- Doxorubicin intercalates between base pairs, and also alkylates macromolecules.
- Daunorubicin, doxorubicin, and their derivatives, belong to a subclass of antitumor antibiotics called anthracyclines.
- *Some drugs:*
  Aclarubicin, Bleomycin, Dactinomycin (Actinomycin D), Daunorubicin, Doxorubicin (Adriamycin), Epirubicin, Idarubicin, Mitomycin C, Mitoxantrone, Plicamycin (Mithramycin)

24

MITOTIC INHIBITORS
(Inhibition of formation of mitotic spindle)
(Protein oriented approach)

- Mitotic inhibitors include among others the vinca alkaloids (vincristine and vinblastine) which are mitotic spindle inhibitors and the epipodophyllotoxins (teniposide and etoposide) which are DNA topoisomerase II inhibitors.
- *Mitotic spindle inhibitors* bind to microtubular proteins and block their ability to polymerize or depolymerize, a process which brake nuclear division.
  DNA topoisomerase II inhibitors block religation of double strand DNA breaks (i.e., sister chromatid separation or cleaved DNA)
- Examples:
  Etoposide (VP-16, VePesid), Teniposide (VM-26, Vumon), Vinblastine, Vincristine, Vindesine

ANTIMETABOLITE BASICS
(Ribonucleotid approach)

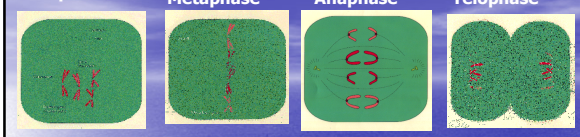


Impair of cytoskeleton formation (microtubules in cell division- mitotic spindle)

- Plant alkaloids and terpenoids (L01C)

  These alkaloids are derived from plants and block cell division by preventing microtubule function. Microtubules are vital for cell division, and, without them, cell division cannot occur. The main examples are vinca alkaloids and taxanes.

MITOSIS (M phase)



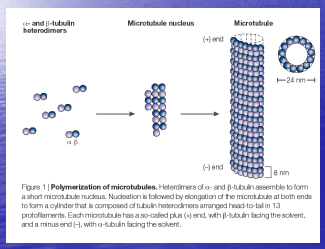


Figure 1 | **Polymerization of microtubules.** Heterodimers of α- and β-tubulin assemble to form a short microtubule nucleus. Nucleation is followed by elongation of the microtubule at both ends to form a cylinder that is composed of tubulin heterodimers arranged head-to-tail in 13 protofilaments. Each microtubule has a so-called plus (+) end, with β-tubulin facing the solvent, and a minus end (–), with α-tubulin facing the solvent.

Antimitotic agents bind to microt

Supression of microtubuli dynami

Metaphase arrest

TAXANES

- The prototype taxane is the natural product paclitaxel, originally known as Taxol and first derived from the bark of the Pacific Yew tree. Docetaxel is a semi-synthetic analogue of paclitaxel. Taxanes enhance stability of microtubules, preventing the separation of chromosomes during anaphase. (brake cell division)
- Used drug - paclitaxel

HORMONES, HORMONAL ANTAGONISTS AND ENZYMES (exept imunosupression of steroids)

- Hormones or hormone-blocking agents either exert a corticosteroid effect, such as prednisone, or manipulate the hormone environment in hormone-responsive tumors.
- The antiandrogenic agent, flutamide, which is used to treat prostate cancer, is believed to block androgen receptor sites.
- The antiestrogenic agent, tamoxifen, binds to intracellular estrogen receptors, then enters the nucleus where the tamoxifen-estrogen-receptor complex inhibits DNA and protein synthesis.
- Some of the drugs:
- *Equine Estrogen (Premarin), Cortisone, Chlorotriansene , Dexamethasone , Diethylstilbestrol Ethinyl Estradiol , Fluoxymesterone , Hydroxyprogesterone Caproate , Medroxyprogesterone Acetate (Provera), Megestrol Acetate (Megace), Prednisone, Tamoxifen (Nolvadex), Testosterone*

TYROSINE KINASES AS TARGETS FOR CANCER THERAPY
(Approach to influence on second messenger)
(Tumor is the mather of metabolism)

- Protein tyrosine kinases (TKs) are enzymes that catalyze the transfer of phosphate from ATP to tyrosine residues in polypeptides. (including energy transfer)
- The human genome contains about 90 TK and 43 TK-like genes, the products of which regulate cellular proliferation, survival, differentiation, function, and motility.
- In human genome only 3% of gene are "structural genes", and 97% are "regulatory" (their function is only in chromosome).
- "Very high level" of metabolic and functional role is just a challenge for therapeutic targets (Statins in the begenning...)

31

TYROSINE KINASES AS TARGETS FOR CANCER THERAPY

- Discavery of monoclonal antibody give the new (great) influence in chemotherapeutic strategy.
- The landscape was changed radically by the success of imatinib mesylate (gleevec), an inhibitor of the BCR-ABL TK in chronic myeloid leukemia (CML) — a result heralded as a proof-of-principle and a triumph of targeted cancer therapy.
- TKs are now regarded as excellent targets for cancer chemotherapy, but reality lies somewhere between the extremes of triumph and tribulation.
- (N Engl J Med 2005;353:172-87)

32

TYROSINE KINASES AS TARGETS FOR CANCER THERAPY

- Some newer agents do not directly interfere with DNA.
- These include monoclonal antibodies and the new tyrosine kinase inhibitors e.g. imatinib mesylate (Gleevec), which directly targets a molecular abnormality in certain types of cancer (chronic myelogenous leukemia, gastrointestinal stromal tumors). These are examples of targeted therapies.

  In addition, some drugs that modulate tumor cell behaviour without directly attacking those cells may be used. Hormone treatments fall into this category. (Not so agressive)

33

IMPAIR OF CYTOSKELETON FORMATION (MICROTUBULES IN CELL DIVISION)

- Vinca alkaloids (L01CA)

  Vinca alkaloids bind to specific sites on tubulin, inhibiting the assembly of tubulin into microtubules (M phase of the cell cycle). They are derived from the Madagascar periwinkle, Catharanthus roseus (formerly known as Vinca rosea).
- The vinca alkaloids include:
  - Vincristine
  - Vinblastine
  - Vinorelbine
  - 34Vindesine

34

THERAPY AGAINST TUMOR STROMA AND VASCULARISATION

- EGFR (epidermal growth factor) is activated by binding specific ligands, including epidermal growth factor and transforming growth factor-α. Activation of EGFR promotes cell proliferation and survival, as well as angiogenesis, leading to tumor growth and metastasis.
- Anti-hEGFR-hIgG4 (S228P) features the constant region of the human IgG4 (S228P) isotype and the variable region of cetuximab. Cetuximab is a chimeric human/mouse IgG1 monoclonal antibody that targets epidermal growth factor receptor (EGFR), a cell surface receptor overexpressed in many types of cancer.

Clin Cancer Res.
2007: 3(5):1552-61

35

Anti-hEGFR-hIgG4 (S228P)

- Binding of cetuximab to EGFR blocks ligand/receptor binding and induces receptor internalization and subsequent degradation. Consequently, cetuximab blocks downstream pathways which regulate cell growth and angiogenesis.
- Cetuximab induces cell death through antibody-dependent cell-mediated cytotoxicity (ADCC) (with activation of complement-very impotynt for all immunity) .
- Cetuximab has been approved by the FDA for the treatment of metastatic colorectal cancer and metastatic squamous cell carcinoma of the head and neck.
  Anti-hEGFR-hIgG4 (S228P) was generated by recombinant DNA technology. It has been produced in CHO cells (Hamster Chinese ovary-cell line) and purified by affinity chromatography with protein G.

36

Anti-hEGFR-hIgG4 (S228P) THERAPY

- Gefitinib(Antibody for EGFR), a quinazolin derivative that specifically inhibits the activation of EGFR tyrosine kinase through competitive binding to the ATP-binding domain of the receptor, has received approval of FAD for patients with advanced NSCLC refractory to chemotherapy

Vincenzi B. et al., 2010. Cetuximab: from bench to bedside. Curr Cancer Drug Targets. 10(1):80-95.

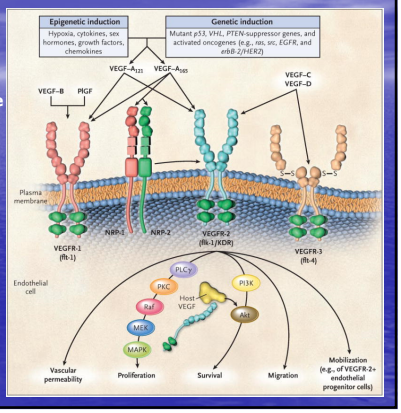
Vascular Endothelial Growth Factor (VEGF) and It's Role in Non-Endothelial Cells: Autocrine Signaling by VEGF (Potential target for tumor cells)

- Vascular endothelial growth factor (VEGF) is a potent angiogenic factor and was first described as an essential growth factor for vascular endothelial cells.
- VEGF is up-regulated in many tumors and its contribution to tumor angiogenesis is well defined.
- VEGF and VEGFR receptors are expressed on numerous non-endothelial cells including tumor cells.
- So, it is to considered it as well target for cancer therapy.
- Anti-VEGF strategies to treat cancers were designed to target the pro-angiogenic function of VEGF and thereby inhibit neovascularization.

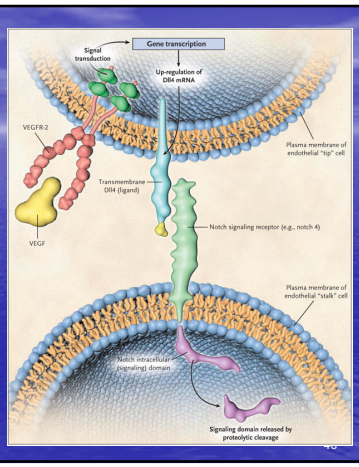
(Cancer Res. 1995;55: 3964–3968)

Left side:
-Cancer promotion and growth is mather of time
-- Changes of metabolism

-Target for cancer cell (only)
- Consideration of TM marker and oncogens



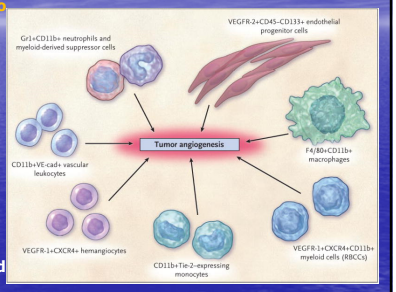
-Gene transcription – after signal transduction – second messenger activation (PIP_3, DAG, PKC)
- Cytoskeleton and membrane receptors pass throgh complete cell membrane, intracelluar space and throgh next neibghur cell.
-("silent speah of cells") (intercellular comunications- integrin,cytoskeleton, receptors)
(No strenght connestion between tm cells)



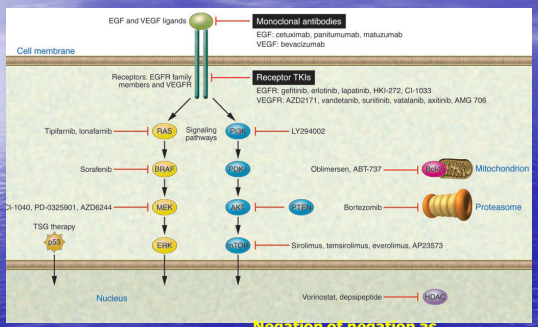
- VEGF AS TARGET…

For tumors of 2-3 mm - to growth - angiogenesis is essential for adequate oxygenation and nutrition.
VEGF is the most important growth factor for angiogenesis in normal and tumor cells.
- The VEGF family consists of six growth factors (VEGF-A, -B, -C, -D, and -E and placental growth factor [PlGF]) and three receptors

J. Natl. Cancer Inst. 82:4–6.



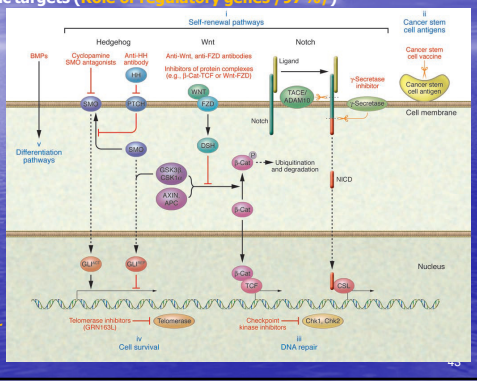
Inhibition of normal repair of the cell – to destroy cancer cells.
Tumor suppressor gene (TSG) therapies, inhibitors of antiapoptotic proteins (Bcl-2), HDAC (histone-deacetylase), proteasome , pyruvate dehydrogenase kinase isozyme 1; PI3K (second messenger)



J. Clin. Invest. 117:2740–2750 (2007

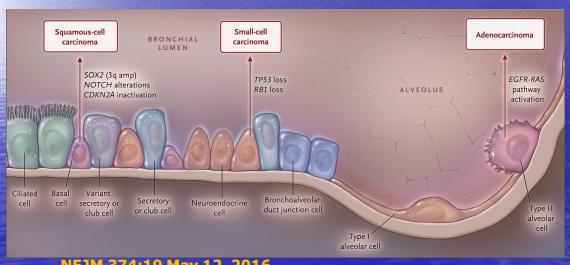
Negation of negation as approach – apoptosis of tm cell is well job

Cancer stem cell–specific therapeutic approaches. Hedgehog (HH), Notch, and Wnt signaling - key stem cell **self-renewal pathways** that are deregulated in lung cancer and thus represent potential therapeutic targets (**Role of regulatory genes /97%/**)



Breast Cancer Res. 7:86–95.

The likely cells of origin for the three common histologic subtypes of lung cancer — adenocarcinoma, squamous-cell carcinoma, and small-cell carcinoma — are depicted. **(Note - nearly anytime in point of junction of two cell types) (Neuroendocrin cells near to β2 receptor)**

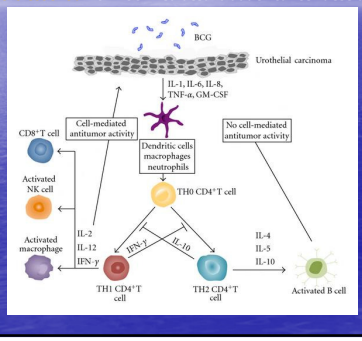


NEJM 374;19 May 12, 2016

INDUCING OF LOCAL INFLMAMATION (as possible antitumor therapy) (Approach to proinflammatory and antiinflammatory cytokines –modulation of action)

- Idea – activate inflamatiomn – cytokine sturm
- Bacill Calmetet Guerin – in use since 1976.

Intravesical BCG instillation – immune response. Triggers release of cytokines and chemokines from these cells. Differentiation of naïve **CD4+** T cells into TH1 and/or TH2 cells that direct immune responses toward cellular or humoral immunity, respectively. The ffect of BCG depends on TH1 immune responses. IL-10 inhibits TH1 immune responses, whereas IFN- inhibits TH2 immune responses. Blocking IL-10 or inducing IFN- lead to a **TH1-dominated immunity that is escential** for BCG-mediated bladder cancer destruction.



BCG for bladder cancer

- This complex and robust immune reaction evoked by BCG is evidenced by a massive transient secretion of cytokines in voided urine, including interleukin (IL)-1, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, IL-18, interferon-inducible protein (IP)-10, tumor necrosis factor (TNF)-α, granulocyte-monocyte colony stimulating factor (GMCSF), and interferon (IFN)-γ.
- Given that the effect of BCG is immune mediated, decades of research have focused on adjunctive immunotherapies including IFN-, IL-2, IL-10, and IL-12
- Combination of BCG anf IFN-α has the better results than any of them alone.

EVALUATION OF BCG AND IFN-α THWRAPY

- In vivo monotherapy with IFN-α2b for bladder cancer in humans has been explored by multiple groups.
- In 1990, Glashan published data from a randomized controlled trial evaluating high dose (100 million unit) and low dose (10 million unit) IFN-α2b regimens in patients with CIS [29]. Patients were treated weekly for 12 weeks and monthly thereafter for 1 year.
- The high and low dose groups had **complete response rates of 43% and 5%, respectively.** Of the high dose patients achieving a complete response, 90% remained disease-free at a notably short 6 months of follow-up

APPROACH WITH INTERFERONS

- Interferons (IFNs) are glycoproteins initially isolated in the 1950s and valued for their antiviral properties.
- Three types have been isolated, IFN- (which is actually a family of interferons), IFN-β, and IFN-γ. IFN- and IFN-β are grouped as "Type I" interferons, whereas IFN-γ is a "Type II" interferon.
- The Type I interferon receptor has 2 components, IFNAR-1 and IFNAR-2, which subsequently bind and phosphorylate Jak molecules initiating a cascade resulting in gene transcription .
- The IFN-α family is well known to stimulate natural killer (NK) cells, induce MHC class I response, and increase antibody recognition .
- They have antineoplastic properties by direct antiproliferative effects and complex immunomodulatory effects

APPROACH AD IL-2 AS POTENTIAL TARGET

- The cytotoxic antitumor capabilities induced in lymphocytes by IL-2 make it a potential cancer immunotherapeutic agent. To date, multiple studies have demonstrated regression of metastatic disease following systemic IL-2 treatment in some cancers

A. Rosenberg, M. T. Lotze, and L. M. Muul, "A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone," New England Journal of Medicine, vol. 316, no. 15, pp. 889–897, 1987

MONOCLONAL ANTIBODIES

Monoclonal antibodies are proteins produced in the laboratory from a single clone of a B–cell. When used as a treatment for cancer, there are three general strategies with monoclonal antibodies:

1. Uses the ability of the antibodies to bind to the cancer cells having the tumor antigens on their surface. The immune system will see the cancer cells marked with bound antibodies as foreign and destroy them.
2. A second strategy is to use the antibodies to block the binding of cytokines or other proteins that are needed by the cancerous cells to maintain their uncontrolled growth. Monoclonal antibodies designed to work like this bind to the cytokine receptors that are on the tumor cell surface.
3. A final strategy involves special antibodies that are linked (conjugated) to a substance that is deadly to the cancer cells. E.G. radioactive isotopes, cytostatics, have been successfully conjugated to antibodies. (monoclonal antibody as the carriers)

PRODUCTION OF MONOCLONAL ANTIBODY

- Human-mouse kimera

  (Lethal irradiation of mouse and than reconstruction immune system by human bone marrow). After revitalisation - injection of Ag to wich production of Ab is looking for.)
- Recombinnat DNA technology

WHICH APPROACH IS THE BEST ONE

- Syrgery of solid tumors
- Target therapy for leukemia-like tumors (no solid)
- Which one is the most promising?
- Targeted therapy if possible.
- Biotechnology for production of cancer cell line, with manipulation of them after successful culture could be promising.
- Have you better idea?

THANK A LOT FOR YOUR ATTENTION

CLINICAL PHARMACY SERVICES IN ONCOLOGY

Invited lecture at the
International CEEPUS Summer School 2016

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Goals of clinical pharmacy

The aim of clinical pharmacists is to ensure the correct and safe use of medicines and other medical products through the provision of various clinical pharmacy services.

Their objective is to:

• maximise treatment efficacy by choosing the most appropriate medication for each condition and for each individual patient

• minimise the risk for adverse drug events by providing drug monitoring and assessing patient's medication adherance

• reduce drug expenditure by choosing the most cost-effective among all suitable strategies

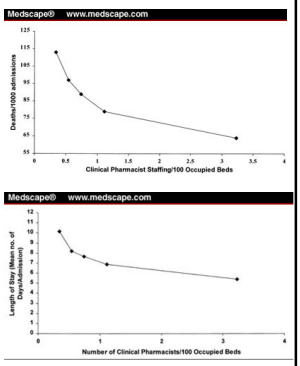
Clinical pharmacist

- Clinical pharmacist's role is to optimise drug treatment across the institutions of health care system, i.e. in:
  - hospitals, clinics (secondary and tertiary level) and
  - pharmacies, community health centres, nursing homes (primary level)
- Clinical pharmacist is entitled a pharmacist with Master of Pharmacy degree (MPharm) and a three-year specialisation in clinical pharmacy

Clinical outcomes of clinical pharmacists interventions

Number of clinical pharmacists is in correlation with clinical outcomes

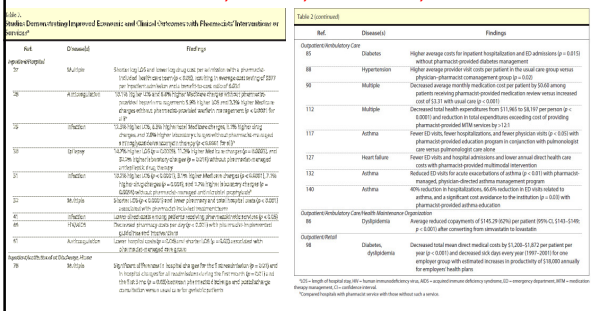
- Increase of clinical pharmacists from 0,34 to 3,23/100 beds **decreases number of deaths** in the hospital for 43% and **decreases length of stay** in the hospital for 47%
- **1,00 dollar spent** on clinical pharmacist **brings 4,86 dollars saving** to the system



Bond et al. Pharmacotherapy 2001; 21 (2): 129-141

Clinical and economic outcomes of clinical pharmacists interventions in USA: a systematic review

Positive in 15,9% studies, Mixed in 42,1% studies,
No effect in 4,8% studies, Uncertain in 37,3% studies



Burns et al. Am J Health-Syst Pharm 2010; 67: 1624-1634

Outcomes of clinical pharmacists interventions in Slovenia: a clinical study

T Roblek, A Detiček, B Leskovar, S Šuškovic, M Horvat, A Belič, A Mrhar, M Lainščak

Clinical-pharmacist intervention reduces clinically relevant drug–drug interactions in patients with heart failure: A randomized, double-blind, controlled trial, Int J Cardiol 2016; 203: 647–652

Pharmacist intervention significantly reduces the number of patients with clinically relevant drug-drug interactions, but not clinical endpoints 6 months from discharge.

Study performed at University Clinic Golnik, Golnik, Slovenia

Outcomes of clinical pharmacists interventions in Slovenia: a clinical study

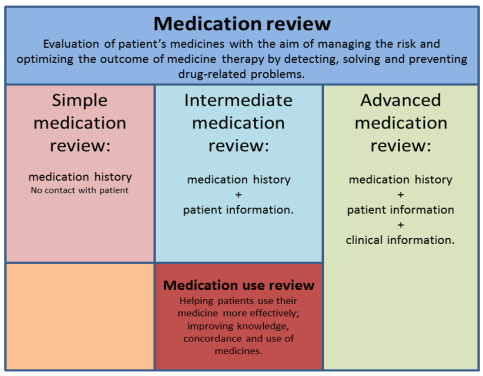
R Režonja Kukec, I Grabnar, T Vovk, A Mrhar, V Kovač, T Čufer

Febrile neutropenia in chemotherapy treated small-cell lung cancer patients,
Radiol Oncol 2015; 49: 173-180

Neutropenic events are assumed to be related to increased etoposide plasma concentrations after a standard etoposide and cisplatin dose. While the mean etoposide peak plasma concentration in the first cycle of chemotherapy was 17.6 mg/l, the highest levels of 27.07 and 27.49 mg/l were determined in two patients with febrile neutropenia.

Study performed at University Clinic Golnik, Golnik, Slovenia

Methodological issues



Cases of clinical pharmacy services in oncology in Slovenian settings

Tertiary hospital: *University Clinic Golnik*

A. Mrhar, L. Knez

CLINICAL PHARMACY SERVICES IN GENERAL:

EXPERIENCES & RESULTS

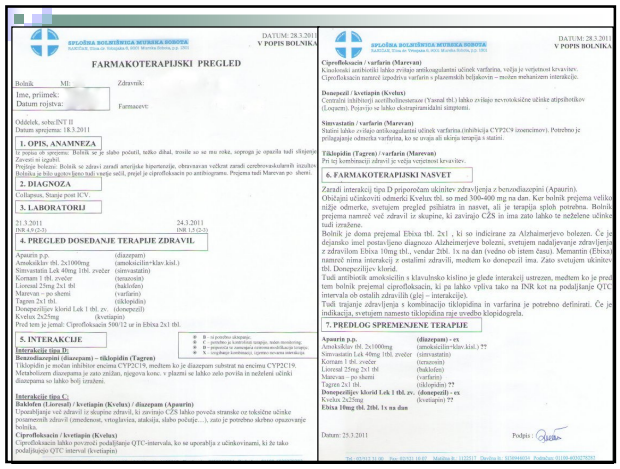
The beginning (10 years ago)!
What has been done so far in the hospital?

Clinical pharmacists are integrated in the treatment of:

- Patients at higher risks for adverse drug reactions
- Oncology patients
- Patients with tuberculosis
- Patients with hereditary angioedema

Advanced medication review
(Pharmacotherapeutic review) is obligatory in patients at higher risks for adversed drug reactions:

- Decreased renal function (<30 mL/min)
- Prescribed with strong inhibitors or inducers
- Prescribed with strong opioids
- Prescribed with drugs where TDM is performed
- Prescribed drugs with a feeding tube
- Prescribed medicines with restrictions in their use


SPLOŠNA BOLNIŠNICA MURSKA SOBOTA

FARMAKOTERAPIJSKI PREGLED

1. OPIS. ANAMNEZA
2. DIAGNOZA
3. LABORATORIJ
4. PREGLED DOSEDANJE TERAPIJE ZDRAVIL
5. INTERAKCIJE
6. FARMAKOTERAPIJSKI NASVET
7. PREDLOG SPREMENJENE TERAPIJE

CLINICAL PHARMACY SERVICES IN ONCOLOGY:

EXPERIENCES & RESULTS

OVERVIEW

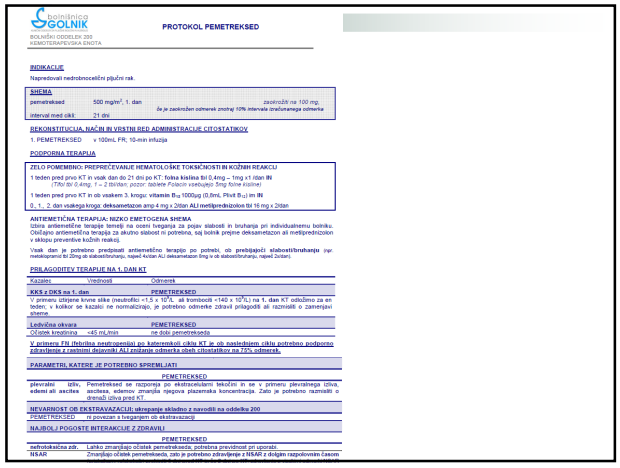
I. **Implementation of clinical pharmacy services**

II. **Evaluation of implemented services**

III. **Current activities & future plans**

I. IMPLEMENTATION

- **Self-initiated clinical screening of chemotherapy (CT) prescriptions**
  - Review of dose calculation
  - Review of laboratory investigations
  - Review of support therapy
- **ADE expended the service & made it obligatory**
  - Prevention of application of CT to neutropenic patient
  - Prevention of ADE (paclitaxel hypersensitivity) occured due to lack of supportive therapy (dexamethasone)
- **Joint commitment towards quality improvement**
  - Standardisation of CT protocols (basic)
  - Improvement of CT order form (upgrade)


PROTOKOL PEMETREKSED

INDIKACIJE

SHEMA

REKONSTITUCIJA, NAČIN IN VRSTNI RED ADMINISTRACIJE CITOSTATIKOV

PODPORNA TERAPIJA

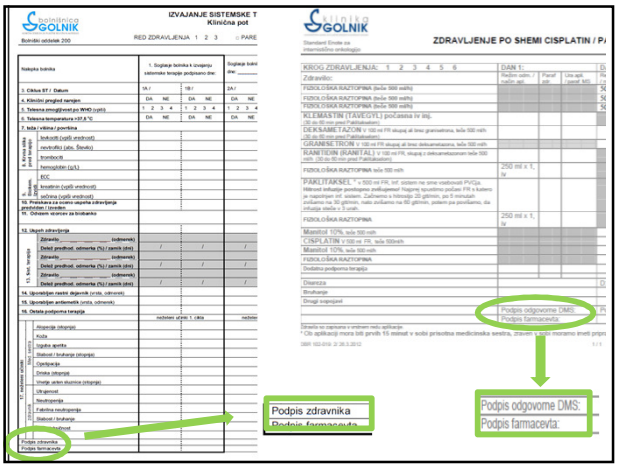
ANTIEMETIČNA TERAPIJA: NIZKO EMETOGENA SHEMA

PRILAGODITEV TERAPIJE NA 1. DAN KT

PARAMETRI, KATERE JE POTREBNO SPREMLJATI

NEVARNOST OB EKSTRAVAZACIJI:

NAJBOLJ POGOSTE INTERAKCIJE Z ZDRAVILI



I. IMPLEMENTATION

CLINICAL SCREENING OF CT PRESCRIPTIONS BY PHARMACISTS IS **OBLIGATORY** & IMPLEMENTED INTO **ROUTINE CLINICAL PRACTICE**.

Pharmacists have to check:

- Physician's signature & completness of all administrative data
- Calculation of CT doses & need for dose adjustment
- Prescription of support therapy
- Drug formulation and administration

II. EVALUATION

- **TO EVALUATE CLINICAL INTERVENTIONS MADE BY PHARMACISTS DURING PRESCRIPTION SCREENING**
- PERFORMED A CROSS-SECTIONAL OBSERVATION STUDY DURING A 5-MONTH PERIOD

Knez L, Jošt M, Toni J, Triller N, Čufer T. Implementing new clinical pharmacy services parallel to the introduction of centralized preparation of anti-cancer drugs. Slovenian Journal of Public Health 2011; 50: 12-23

II. EVALUATION:
ALL INTERVENTIONS

- **211 INTERVENTIONS PER 506 CT ORDERS (41,7%)**
- **DRUGS INVOLVED:**
  - Cancer drugs 65/211 (30,8%)
  - Antiemetic drugs 87/211 (41,2%)
  - Other support therapy drugs 26/211 (12,3%)
- **PROBLEMS INVOLVED:**
  - Administrative 32/211 (15,2%)
  - Drug (omission) 45/211 (21,3%)
  - Dose (frequency, regimen) 133/211 (63,0%)
- **IMPLEMENTATION:**
  - Implemented 160/211 (75,8%)
  - Implemented with changes 10/211 (4,7%)
  - Not implemented 35/211 (16.6%)

II. EVALUATION
INTERVENTIONS ON CANCER DRUGS

Problem category

- **DRUG RELATED:** 7/211 (3,3%)
- **DOSE, FREQEUNCY & DURATION:** 56/211 (26,5%)
  - Doses differ >10% 20/211 (9,5%)
  - Need for dose adjustment 14/211 (6,6%)
  - Chosen drug dose not written 10/211 (4,7%)
  - Other 12/211 (5,7%)
- **ADMINISTRATIVE & ADMINISTRATION:** 1/211 (0,5%)

Implemented 45/65 (69,2%)

II. EVALUATION

- The results of this study confirm the importance of integration of pharmacists' clinical roles for the provision of high quality oncology services
- ***Actions to tackle identified problems should be taken***
- ***Clinical (and economic) significance of the recorded interventions should be defined***

III. PRESENT & FUTURE

- Pharmacists participation in obtaining **COMPREHENSIVE MEDICATION HISTORY** & check for **DRUG INTERACTIONS** with cancer drugs **prior to the start of treatment**
- Continue the collaboration in IMPROVING THE QUALITY OF CARE and in ONCOLOGY RESEARCH

MANAGEMENT OF COMPREHENSIVE MEDICATION HISTORY (CHM)

Methods:

- Patients admitted to Universitiy Clinic Golnik were randomly selected and included in the study.
- For each patient a CMH was obtained by a research pharmacist using various sources of information.
- Next, the medication history in the hospital medical record was reviewed.
- The prescribed drugs were assessed for completeness of information, and possible discrepancies between both medication histories were recorded and classified.

Results:

- Overall, 108 patients with a median age of 73 years were included in the study.
- The research pharmacist recorded the use of 651 medicaments, with all relevant details being available for 94.9% of these drugs.
- **Of the 464 medicines listed in the hospital medical record, only 42.0% were considered complete.**
- A comparison of the medication history and the medical record with the CMH revealed at least one discrepancy in 72.4% of the drugs listed.
- **The majority of the identified discrepancies were often present both in the medication order on the drug chart (76.2%) and in the discharge letter (69.9%).**
- Most medication discrepancies were due to drug omissions (20.9%) and commissions (6.5%).

Conclusion:

- **The high rate of discrepancies between the recorded drug history and CMH reported in our study stresses the need for the implementation of medication reconciliation.**
- The participation of pharmacists in the reconciliation process, described in this study, resulted in more complete and accurate drug histories acquired.

Režonja R, Knez L, Šuškovič S, Košnik M, Mrhar A. Comprehensive medication history: the need for implementation of medication reconciliation processes. Slovenian Journal of Public Health 2010; 49: 202-210

MANAGEMENT OF DRUG INTERACTION

DRUG INTERACTIONS ARE REVIEWED FOR EACH PATIENT STARTING A NEW LINE OF SYSTEMIC CANCER THERAPY

- COMPREHENSIVE MEDICATION HISTORY IS OBTAINED
- DRUG INTERACTIONS WITH PLANNED CANCER THERAPY (ANTICANCER DRUGS AND SUPPORT CARE DRUGS) ARE CHECKED
- CLINICALLY IMPORTANT DRUG INTERACTIONS ARE IDENTIFIED
- CHANGES TO DRUG THERAPY ARE PROPOSED
- THE OBTAINED INFORMATION IS RECORDED IN THE MEDICAL RECORD AND PRESENTED TO THE PATIENT'S MEDICAL ONCOLOGIST

EVALUATION OF
OUR MANAGEMENT OF DRUG INTERACTIONS

THE MANAGEMENT OF DRUG INTERACTIONS WAS REVIEWED IN ALL LUNG CANCER PATIENTS IN WHOM ANTICANCER THERAPY WAS INITIATED IN 2012 (n = 223)

- DRUG INTERACTIONS IDENTIFIED BY THE PHARMACISTS WERE REVIEWED, DESCRIBED AND ANALYSED
- A SEPARATE SEARCH FOR DRUG INTERACTIONS WAS PERFORMED FOR THE SAME PATIENTS, USING 3 DIFFERENT COMPENDIA (Stockley's Drug Interaction, Lexi-Comp, Drugs.com) AND REVIEWING THE RELEVANT SmPCs

CHALLENGES IN
THE MANAGEMENT OF DRUG INTERACTIONS

1st CHALLENGE: OBTAIN PATIENT'S MEDICATION HISTORY

2nd CHALLENGE: SEARCH FOR DRUG INTERACTIONS

3rd CHALLENGE: IDENTIFY RELEVANT DRUG INTERACTIONS

1st CHALLENGE:
OBTAIN PATIENT'S MEDICATION HISTORY

- OBTAINING A COMPREHENSIVE MEDICATION HISTORY IS CHALLENGING…
  - Drug information on hospital admission is very often
    - **INCOMPLETE** in 72 %
    - **INCORRECT** in 71 %
- …BUT OF PARAMOUNT IMPORTANCE FOR FUTURE DRUG TREATMENT

WHAT DID OUR AUDIT REVEAL?

1st CHALLENGE: COMPLEX PATIENTS AND TREATMENTS

PATIENTS OF HIGHER AGE
- median 63 years, IQR: 56 - 69

DIAGNOSED WITH LUNG CANCER
- 78 % NSCLC
- 22 % SCLC

TAKE MANY Rx DRUGS
- median 4, IQR: 2-6, range: 0-13

USE OTC, FOOD SUPPLEMENTS, HERBAL REMEDIES

ARE TREATED WITH COMPLEX ANTICANCER REGIMENS WITH
- 8 drugs in 29 %
- 6 drugs in 64 %
- 1 drug in 7 %

AND THESE REGIMENS INCLUDE HIGH RISK DRUGS

OUR PATIENTS ARE AT HIGH RISK FOR ADVERSE DRUG EVENTS!

2nd CHALLENGE: SEARCH FOR DRUG INTERACTIONS

- THE NUMBER OF POSSIBLE DRUG INTERACTIONS IS OVERWHELMING
- DRUG INTERACTION COMPENDIA ARE „SAME SAME BUT DIFFERENT“
- INFORMATION IN SmPC IS VAGUE

WHAT DID OUR AUDIT REVEAL?

2nd CHALLENGE: SEARCH FOR DRUG INTERACTIONS

THE NUMBER OF POSSIBLE DRUG INTERACTIONS IS OVERWHELMING

1416 DRUG INTERACTIONS IN 223 PATIENTS
- *excluding drug interactions between chronic therapy drugs!*
- median: 5, IQR: 2 – 10, range: 0 – 23 int/pt

82 % INTERACTIONS WITH SUPPORT CARE DRUGS
- glucocorticoids: 42 %, aprepitant: 27 %, setrons: 13 %

81 % INTERACTIONS WOULD AFFECT OUTCOMES OF CHRONIC THERAPY DRUGS
- most often from **ATC C** (30 %: statins, antihypertensive, diuretics), **ATC N** (30 %: analgesics, anxiolytics and sedatives), **ATC A** (14 %: PPI, antidiabetics), **ATC M** (11 %: NSAR)

75 % OF AT LEAST MODERATE CLINICAL IMPORTANCE
- in 1 % combined use is contraindicated

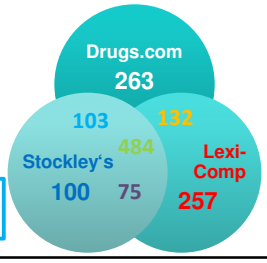
LOOK FOR THE RELEVANT DRUG INTERACTIONS!

2nd CHALLENGE: SEARCH FOR DRUG INTERACTIONS

DRUG INTERACTION COMPENDIA ARE „SAME SAME BUT DIFFERENT“

NOT ALL DRUG INTERACTIONS ARE INCLUDED IN ALL COMPENDIA
- only 34 % DI included in all 3 compendia
- whereas 44 % DI included in only 1 compendia

MORE IMPORTANT INTERACTIONS RECORDED IN MORE COMPENDIA
- Chi^2, $p < 0,01$
- still, 367 DI of moderate importance were recorded in only 1 compendia



ALWAYS CHECK 2 COMPENDIA & DO NOT RELY ON CLASSIFICATIONS

2nd CHALLENGE: SEARCH FOR DRUG INTERACTIONS

INFORMATION IN SmPC IS VAGUE

MOST INTERACTIONS (77 %) ARE DESCRIBED IN SmPC…
- still 325 drug interactions are not included

…BUT IN 90 % ARE DESCRIBED AS GENERAL DRUG INTERACTIONS
- e.g. strong CYP 3A4 inhibitors may increase plasma concentration of drug A

SmPC CAN SERVE ONLY AS A STARTING POINT…
…THAT TAKES YOU TO A VERY LONG JOURNEY

3rd CHALLENGE: IDENTIFY RELEVANT DRUG INTERACTIONS

- HOW TO IDENTIFY RELEVANT DRUG INTERACTIONS FOR THAT VERY PATIENT?
- HOW TO MANAGE THAT VERY DRUG INTERACTION BEST?
- HOW TO ENSURE THAT VERY DRUG INTERACTION IS BEING MANAGED AS AGREED?

WHAT DID OUR AUDIT REVEALED?

3rd CHALLENGE: IDENTIFY RELEVANT DRUG INTERACTIONS

HOW TO IDENTIFY RELEVANT DI FOR THAT VERY PATIENT?

PHARMACISTS IDENTIFIED ONLY 4 % (52/1416) OF POSSIBLE DRUG INTERACTIONS AS CLINICALLY RELEVANT

- median: 0 DI; 1 DI in 16 % pts, 2 DI in 3 % pts, 3 DI in 1 pt

PHARMACISTS IDENTIFIED AS RELEVANT MORE OFTEN DRUG INTERACTIONS…

- …involving anticancer drugs (85 % of identified DI; Chi^2, $p < 0,01$)
- …that affected outcomes of anticancer therapy (79 % of identified DI; Chi^2, $p < 0,01$)
- …recorded in all 3 reviewed compendia (50 % of identified DI; Chi^2, $p < 0,01$)

…BUT FAILED IN IDENTIFYING 16 MAJOR DRUG INTERACTIONS

- concurrent treatment with granisetron and escitalopram increase risk for arrythmia (10 DI)
- aprepitant increases fentanyl plasma concentrations (6 DI)

3rd CHALLENGE: IDENTIFY RELEVANT DRUG INTERACTIONS

HOW TO MANAGE THAT VERY DRUG INTERACTION BEST?

- **IN 75 % (39/52) OF CASES A CHANGE IN CHRONIC THERAPY WAS SUGGESTED**
- **in 2 cases it was suggested to reconsider the choice of anticancer agent:**
  - Because carbamazepine increases the clearance of vinorelbine, gemcitabine was chosen over vinorelbine.
  - Because nephrotoxicity of cisplatin is worsen during furosemide therapy, carboplatin was chosen over cisplatin.
- **pharmacists offered no suggestion in 7 cases**

3rd CHALLENGE: IDENTIFY RELEVANT DRUG INTERACTIONS

HOW TO ENSURE THAT THE VERY DRUG INTERACTION IS BEING MANAGED AS AGREED?

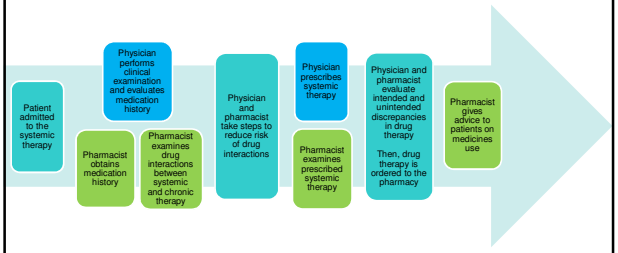
- **59% (23/41) IMPLEMENTED**
- **IN 41 % (17/41) OF CASES THE PROPOSED CHANGE IN THERAPY WAS NOT IMPLEMENTED**
  - the reasons for this were not recorded
  - remember: drugs are not being prescribed only by medical oncologists, but can be prescribed also by GPs and bought as OTC by patients

CONCLUSIONS

PHARMACISTS DO HAVE TO TAKE AN ACTIVE ROLE IN THE PREVENTION OF DRUG INTERACTIONS IN CANCER PATIENTS!

- **OUR PATIENTS ARE AT INCREASED RISK FOR DRUG INTERACTIONS**
- **IDENTIFYING CLINICALLY RELEVANT DRUG INTERACTIONS REQUIRES EXPERTISE (≠ drug interaction compendia!)**
- **ADEQUATE MANAGEMENT OF DRUG INTERACTIONS DEMANDS GOOD COLLABORATION BETWEEN PHARMACISTS, MEDICAL ONCOLOGIST, GENERAL PRACTITIONERS AND PATIENTS**

Implementation of medication reconciliation model for all oncology patients



Tertiary hospital: *Institute of Oncology Ljubljana*

A. Mrhar, S. Rožman

Treatment of *Candida* species urinary tract infection in an oncological patient

(Case report)

Patient details

- GM - 71 year old female patient, 163 cm, 94 kg
- Allergy to penicillin

- History of present illness
  - 10/2012: surgery (abdominoperineal excision) due to colorectal carcinoma after adjuvant chemo-radiotherapy
  - 09/2015: relapse of disease in the abdomen, according to PET/CT pathological nodes also around the aorta
  - 10/2015: explorative surgery – radical excision was not performed due to disease progression
  - 10/2015: after surgery → transfer to the Intensive Care Unit (ICU)
  - Supportive therapy in the ICU:
    - Parenteral and enteral feeding
    - Intravenous fluids
    - Antithrombotic prophylaxis with low-molecular weight heparin
    - Epidural and intravenous analgesia
    - Antiulcer prophylaxis
    - Antibiotic prophylaxis

Patient details

- History of present illness
  - 6th October 2015 – transfer from the ICU to surgical ward
    - Hemodynamically stable
    - Without vasoactive and oxygen support
    - Spontaneous diuresis
    - Inflammation parameters in decline
    - Normal stoma output
    - Feeding per mouth with the addition of intravenous amino acids
    - After mild acute on chronic kidney failure, serum creatinine (SCr) and blood urea nitrogen (BUN) return to preoperative values
      - SCr = 160 µmol/L [Normal value: 38-80 µmol/L])
      - BUN = 15 mmol/L [Normal value: 2.8–7.5 mmol/L])
      - EGFR ~ 35 mL/min [Estimated glomerular filtration rate, normal value: >90 mL/min]
  - 13th of October 2015 – ***yeasts*** found in urine (1.000.000 CFU/mL), patient developed symptomatic urinary tract infection, which prompted treatment

Yeast infection

- Yeast infection – typically caused by *Candida species* (*Candida albicans* being most common)

Summary of suggested antifungal drugs against treatable pathogenic fungi (The Sanford Guide to Antimicrobial Therapy, 44th Ed., 2014)

	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Echinocandin	Amphotericin B
Candida albicans	+++	+++	+++	+++	+++	+++
Candida glabrata	±	±	+	+	+++	++
Candida tropicalis	+++	+++	+++	+++	+++	+++
Candida krusei	-	+	++	++	+++	++

Definition: ± possible activity, + active (3rd line therapy), ++ active (2nd line therapy), +++ active (1st line therapy)

Echinocandins: anidulafungin, micafungin, caspofungin

Is an echinocandin a reasonable option?

Yeast infection

- Due to yeast isolate, the therapy chosen was anidulafungin (a member of echinocandins), as it covers all *Candida sp.*

- Afterwards, the pathogen isolated was *Candida glabrata* – anidulafungin was continued, as it is the most active drug against resistant *Candida species*

	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Echinocandin	Amphotericin B
Candida albicans	+++	+++	+++	+++	+++	+++
Candida glabrata	±	±	+	+	+++	++
Candida tropicalis	+++	+++	+++	+++	+++	+++
Candida krusei	-	+	++	++	+++	++

- History of present illness
  - 6th October 2015 – transfer from the ICU to surgical ward
  - 13th of October 2015 – ***yeasts*** found in urine (1.000.000 CFU/mL)
  - **15th of October 2015 – patient was seen by clinical pharmacist**

1st Intervention

- Treatment of Candida glabrata urinary tract infection with anidulafungin **is not appropriate**, if we understand pharmacokinetic properties of the drug:
  - Excretion of anidulafungin: less than 1% in urine
  - All echinocandins are excreted primarily in the feces. They do not excrete into urine and do not achieve measurable concentrations in the urine. Therefore, we cannot treat urinary tract infection with anidulafungin (or other echinocandins).
  - Symptoms of urinary tract infection persisted, treatment was not efficient
- 1st intervention – cessation of anidulafungin (the cost of drug 410 € per day!)

Susceptibility results

- 15th of October – antifungal susceptibility testing results available

Candida glabrata, 1.000.000 CFU/mL

Antifungal	MIC	Sensitivity
Fluconazole	16 µg/mL	Sensitive dose-dependent
Amphotericin B	0.38 µg/mL	Sensitive

- According to Clinical and Laboratory Standards Institute, the breakpoints for fluconazole in Candida sp. are:
  - Susceptible: MIC ≤ 8 µg/mL
  - Susceptible-dose dependent: MIC: 16 – 32 µg/mL
  - Resistant: MIC ≥ 64 µg/mL

Treatment of Candida UTI

- Pharmacist recommends **fluconazole 400 mg/day** for Candida glabrata UTI. Initially, the recommendation is not accepted, as it is believed that we are not able to achieve appropriate levels in urine.
- Candida glabrata has MIC of 16 µg/mL, while fluconazole plasma levels typically achieved are around 8 µg/mL. Usually, we do not treat Candida sp. with fluconazole, if the MIC is > 8-16 µg/mL.
- Physician recommends treatment with amphotericin B, even though the patients has chronic kidney injury and **amphotericin B is known to be nephrotoxic** (as there is no other option).

2nd Intervention

- Pharmacist recommends against the use of amphotericin B due to:
  - Nephrotoxicity
  - High cost (1300 €/day)
  - Better treatment alternatives
- Understanding pharmacokinetics of fluconazole, one can see:
  - Most of the drug excreted in urine
  - Fluconazole is concentrated in the urine – yielding urine levels > 100 µg/mL, which is 10-fold the simultaneous plasma levels.
  - Therefore, the expected concentrations in urine exceed the MIC not only for susceptible yeasts (MIC≤ 8 µg/mL), but also for organisms that are susceptible but dose-dependent (MIC 16-32 µg/mL) and sometimes even those that are resistant (MIC ≥ 64 µg/mL)

Reference: Fisher JF et al. *Candida* urinary tract infections – Treatment. Clinical Infectious Diseases 2011:52 (Supp 6)

Rationale for the dose selection

- In normal renal function, we would recommend 800 mg of fluconazole per day. Due to EGFR ~ 35 mL/min, a 50% reduction in the dose is recommended → 400 mg/day
- Anticipated success is 86%

Fluconazole dose, Candida MIC and anticipated success

Dose (mg/day)	MIC (µg/mL)	Dose/MIC	Anticipated success (%)
800	8	100	95
	16	50	86
	32	25	72
400	8	50	86
	16	25	72
	32	12.5	68

Reference: Pfaller MA et al. Interpretive breakpoints for fluconazole and Candida revisited: a blueprint for the future of antifungal susceptibility testing. Clinical Microbiology Reviews 2006: 435-447.

Conclusion

- After initiation of fluconalzole 400 mg/day, symptoms and signs of urinary tract infection improved
- Treatment was continued for 16 days
- Lessons learned
  - Pharmacokinetics – pharmacist`s strength. Use it!
  - Echinocandins not excreted in urine
  - Fluconazole concentrated in urine
  - Fluconazole the drug of choice for Candida urinary tract infection (also for organisms that are susceptible but dose-dependent, and sometimes even those that are resistant)

Take-home message

- Clinical pharmacy services in oncology is of utmost importance
- Clinical pharmacy developed a number of services specific for oncology patients
- Clinical pharmacists contribute significantly in improving clinical, humanistic and economic outcomes of pharmacotherapy in cancer patients

Univerza v Ljubljani
Fakulteta za farmacijo

IMMUNOTHERAPY OF CANCER

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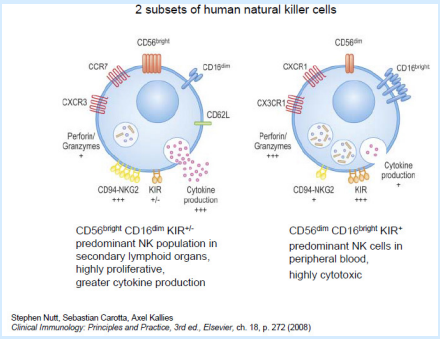
IMMUNE RECEPTORS OF INNATE AND ADAPTIVE IMMUNITY

Receptor characteristic	Innate immunity	Adaptive immunity
Specificity inherited in the genome	Yes	No
Expressed by all cells of a particular type (e.g. macrophages)	Yes	No
Triggers immediate response	Yes	No
Recognizes broad classes of pathogens	Yes	No
Interacts with a range of molecular structures of a given type	Yes	No
Encoded in multiple gene segments	No	Yes
Requires gene rearrangement	No	Yes
Clonal distribution	No	Yes
Able to discriminate between even closely related molecular structures	No	Yes

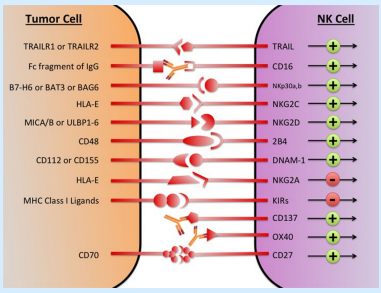
- Receptors expressed on or within the cells of innate immunity are evolutionary old, non-polymorphic and encoded en-block. They are called Pattern Recognition Receptors (PRRs), because they recognize conserved Pathogen-Associated Molecular Patterns (PAMPs) and cell Death Associated Molecular Patterns (DAMPs).
- Receptors expressed od natural killer (NK) cells are not reactive to normal cells, but attack virus infected, tumor and stress-altered cells.

NK CELLS



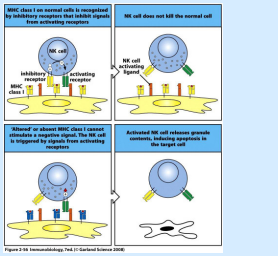
Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18, p. 272 (2008)

MAJOR NK RECEPTORS AND THEIR LIGANDS

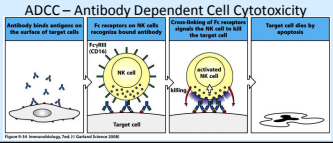


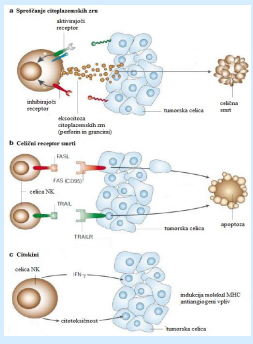
Chester C, et al. *Frontiers in immunology* 2015; 6 (Article 601)

MECHANISMS OF NK CELL CYTOTOXICITY



ADCC – Antibody Dependent Cell Cytotoxicity





ANTIGEN PRESENTING CELLS (APCs)

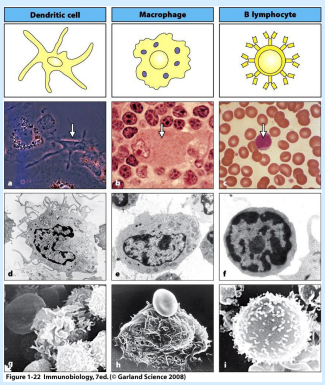
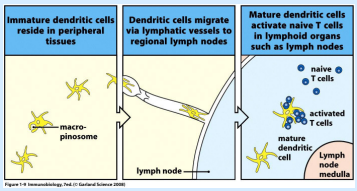
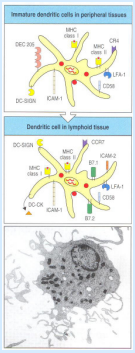


Figure 1-22 Immunobiology, 7ed. (© Garland Science 2008)

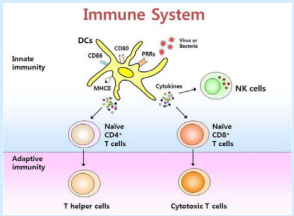
DCs ARE PROFESSIONAL APCs

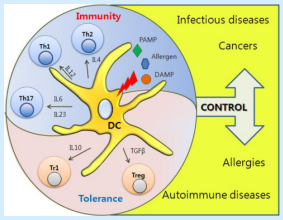




- Immature DCs reside in peripheral tissues and are not able to activate T cells (antigen uptake, processing and binding of peptides to MHC molecules, cytokine production).
- Only mature DCs can fully activate T cells (antigen presentation, expression of costimulatory molecules, cytokine production).

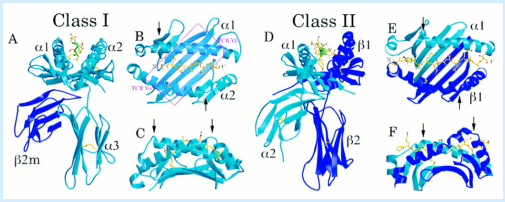
DCs ARE LINKING INNATE AND ADAPTIVE IMMUNITY AND CAN ACT IN A BIPOLAR WAY





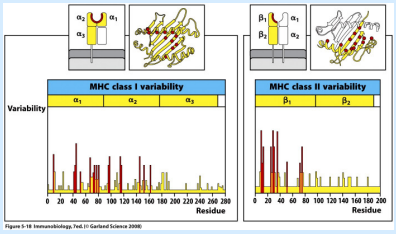
Gi M, Im W, Hong S. *Sensors* 2009; 9(9), 6730-6751

MHC (HLA) MOLECULES



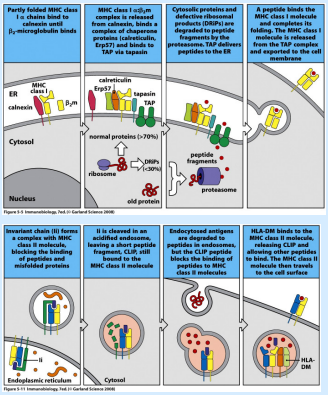
- Class I HLA molecules: A, B and C (classical) are expressed on all nucleated somatic cells. They bind and present intracellular antigenic peptides (8 – 10 aa) to TCRs present on $CD8^+$ cytotoxic T-cells (CTLs).
- Class II HLA molecules: DR, DQ and DP are constitutively expressed on APCs. They bind and present extracellular antigenic peptides (at least 13 aa) to TCRs present on $CD4^+$ T-cells (T helper cells, Th).

HLA POLYMORPHISM



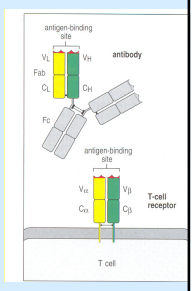
16. 8. 2016	Number of alleles
HLA class I alleles	11.100
HLA class II alleles	3.920
Total	15.020

ANTIGEN PROCESSING AND PRESENTATION

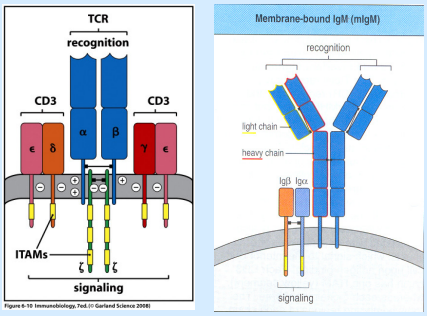


EXTREME POLYMORPISM OF ANTIGEN-SPECIFIC T-CELL RECEPTORS (TCRs), ANTIBODIES AND BCRs

Element	Immunoglobulin		α:β T-cell receptors	
	H	κ+λ	β	α
Variable segments (V)	40	70	52	~70
Diversity segments (D)	25	0	2	0
D segments read in three frames	rarely	–	often	–
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N- and P-nucleotides	2	50% of joints	2	1
Number of V gene pairs	1.9×10^6		5.8×10^6	
Junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Total diversity	$\sim 5 \times 10^{13}$		$\sim 10^{18}$	

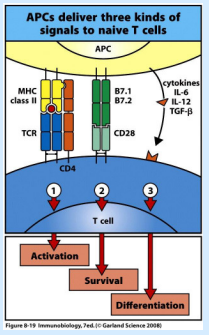


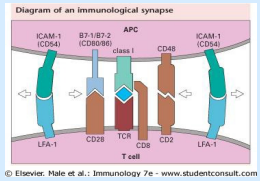
FUNCTIONAL TCR AND BCR COMPLEXES



ITAM = Immunoreceptor Tyrosine-based Activation Motifs

ACTIVATION OF T LYMPHOCYTES BY MATURE APCs & IMMUNOLOGICAL SYNAPSE





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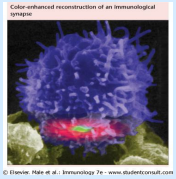

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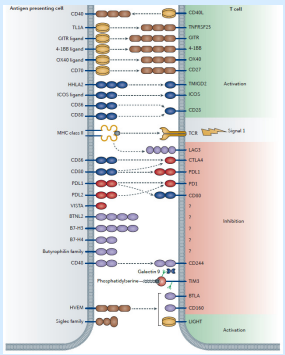
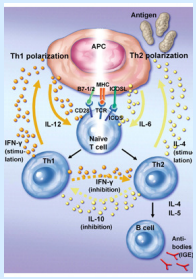
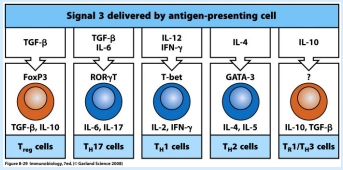
T-CELL ACTIVATION IS A COMPLEX MULTIPLE-SIGNAL PROCESS



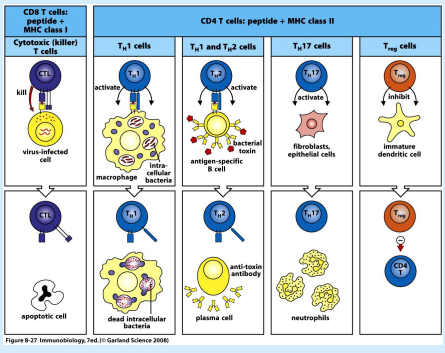
Mahoney KM, et al. *Nat Rev* 2015; 14:561-584

CYTOKINES PRODUCED BY APCs INDUCE FUNCTIONAL DIFFERENTIATION OF CD4+ Th CELLS

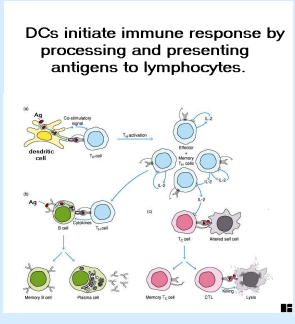


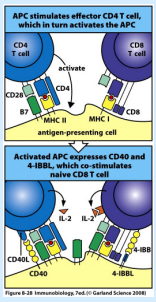


DIFFERENT EFFECTOR ROLES OF T LYMPHOCYTE SUBTYPES

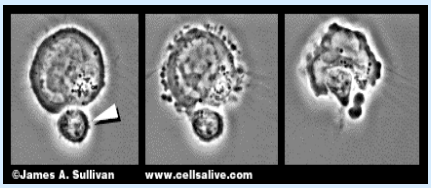


CD4+ Th CELLS ARE NEEDED FOR FUNCTIONAL MATURATION OF CD8+ CYTOTOXIC T CELLS (CTLs)





CD8+ CYTOTOXIC T CELLS (CTLs)

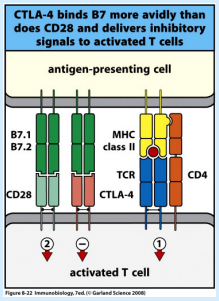


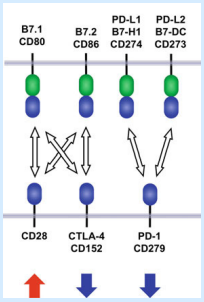
- Mature effector CTLs kill their target cells only after they recognize specific antigenic peptides bound to class I HLA molecules on their surfaces, via TCRs.
- CTLs can use several different killing mechanisms:

  - Ca^{++} dependent release of modified lysosomes, i.e. lytic granules, that contain perforin, granzymes (a family of serine proteases – proapoptotic actin) and granulysin (antimicrobial and proapoptotic).

  - binding of Fas ligands (FasL or CD178), members of the TNF (Tumor Necrosis Factor) molecular family, to Fas (CD95) molecules, expressed on target cells → activation of caspases (cisteine proteases), leading to apoptosis.

POSITIVE AND NEGATIVE REGULATION OF T CELL IMMUNE RESPONSES

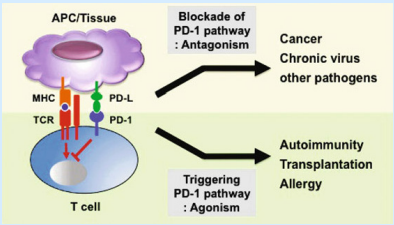




CTLA-4: Cytotoxic T-Lymphocyte Antigen-4

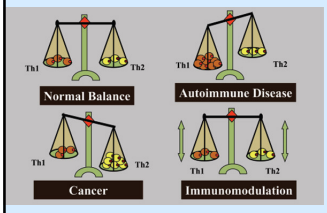
PD-1: Programmed Death-1

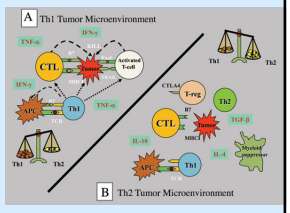
IMUNOSSUPRESSIVE FUNCTION OF PD-1



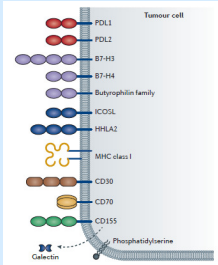
- While CTLA-4 negatively regulates T-cell immune responses following their initial activation, thereby PD-1 inhibits effector functions of already activated T cells (for example tumor specific T cells).
- Unlike CD28 molecules, the CTLA-4 ligands, PD-1 ligands, i.e. PDL-1 and PDL-2 are abundantly expressed in tumors, where they inhibit effector T cells that are in a long-lasting contact with tumor antigens.

CONSEQUENCES OF INAPPROPRIATE HOMEOSTASIS OF THE IMMUNE SYSTEM





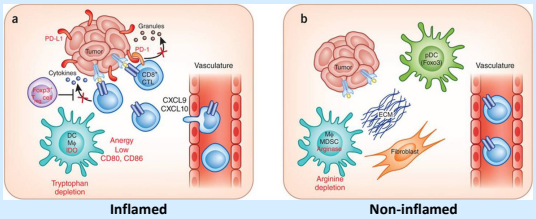
TUMORS CAN EXPRESS BOTH, CO-INHIBITORY AND CO-STIMULATIORY LIGANDS



Ligands	Tumor Expression	Refs
PDL1	Melanoma, renal cell, head and neck, cervical, glioblastoma, bladder, oesophageal, breast, hepatocellular, Hodgkin lymphoma, mediastinal large B-cell lymphoma, among others	109, 196, 223, 230–232
PDL2	Oesophageal, ovarian, pancreatic, hepatocellular, breast, Hodgkin, mediastinal large B-cell lymphoma, among others	233–238
B7-H3	Prostate, renal cell, non-small cell lung, pancreatic, gastric, ovarian, colorectal, urothelial cell, among others	239–246
B7-H4	Breast, renal cell, ovarian, oesophageal, gastric, pancreatic, melanoma, among others	247–257
HHLA2	Breast, lung, thyroid, melanoma, pancreas, ovary, liver, bladder, colon, prostate, kidney, oesophagus	129
Galectins	Non-small cell lung, colorectal, gastric, among others	250–261
CD30	Hodgkin lymphoma, embryonal, anaplastic large cell lymphoma	262
CD70	Non-Hodgkin lymphoma, renal cell	263, 264
ICOSL	Glioblastoma, melanoma	265, 266
CD155	Kidney, prostate, pancreatic, glioblastoma	267

Mahoney KM, et al. *Nat Rev* 2015; 14:561-584

IMMUNE EVASION MECHANISMS IN INFLAMED VS. NON-INFLAMED CANCERS

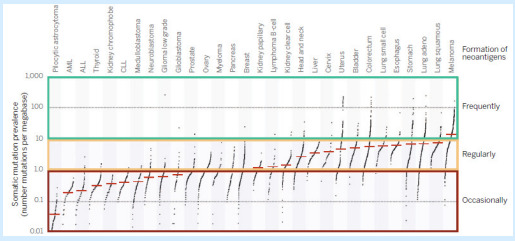


Inflamed

Non-inflamed

Gajewski, Schreiber, Fu. Nat Immunol 2013

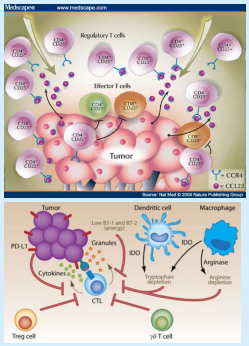
SOMATIC MUTATION PREVALENCES IN DIFFERENT TYPES OF CANCER (IMMUNOGENICITY)



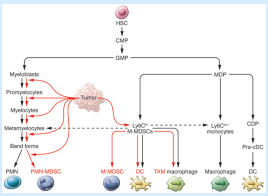
Schumacher TN, Schreiber RD. *Science* 2015; 348:69-74

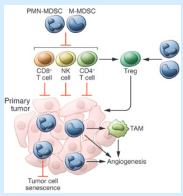
IMMUNOLOGICAL PROPERTIES OF TUMOR TISSUES

- Tumors are more or less immunogenic. Some tumor specific (TA) and tumor associated antigens (TAA) are well defined, and many are not. Melanoma is the most immunogenic cancer.
- Often, the immunogenicity of TA and especially TAA is weak,. Additionally their expression is largely fluctuating and their coding genes are prone to freequent mutations.
- Tumor cells seldom lose the capability to express certain HLA molecules or even a whole HLA haplotype, they lack costimulatory molecules and produce suppressive (anti-inflammatory) cytokines (VEGF, TGF-β, IL-10, IL-4), chemokine CCL22, which attracks and cumulates regulatory T cells (Tregs) expressing its receptor (CCR4), and indolamine-2,3,dioxyigenase (IDO).
- Tumors are additionally protected against the immune system by various types of immunosuppresive cells: Treg, Tr1, Th3, Ts (CD8$^+$), tumor-associated macrophages (TAM or M2) and tolerogenic DCs (DC2).
- Important immunosuppressors are also myeloid-derived suppressor cells (MDSCs) and even NK cells (IL-10, TGF-β, arginase, iNOS).



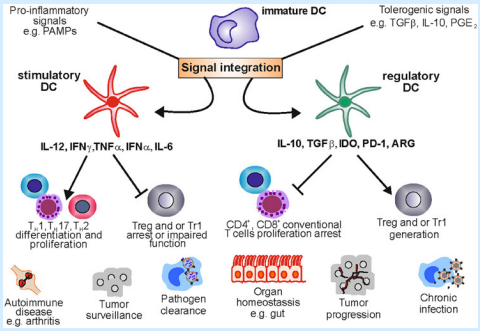
MYELOID-DERIVED SUPPRESSOR CELLS (MDSCs)



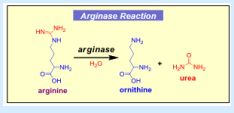


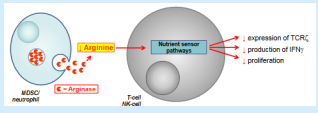
- MDSCs are not completely differentiated/matured myeloid cells which are morphologically and phenotypically similar to monocytes and neutrophils.
- They are being attracted into tumors, where they actively protect tumor cells against immune responses, remodulate microenvironment, establish pre-metastatic niches and via direct interactions with tumor cells induce their stemness.
- MDSCs produce numerous immunosuppressive factors (arginase, iNOS, TGF-β, IL-10, COX2) and induce immunosuppressive natural regulatory T cells (Treg).
- They are susceptible to gemcitabine, 5-fluorouracil and irradiation.

DC POLARIZATION DEPENDS ON THE NATURE OF THEIR MICROENVIRONMENT

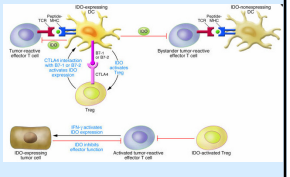


IMUNOSUPPRESSIVE ACTION OF ARGINASE AND IDO

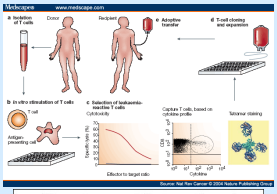




- Indolamine-2,3-dioxygenase (IDO) is an enzyme that degrades typtophan, an essential aminoacid , into kinurenine.
- Lymhocytes T are highly susceptible to tryptophan levels → when these are low, they stop proliferating.

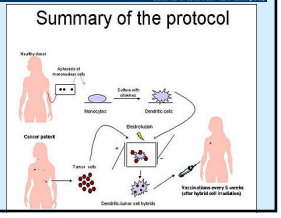


CELLULAR IMMUNOTHERAPIES OF CANCER



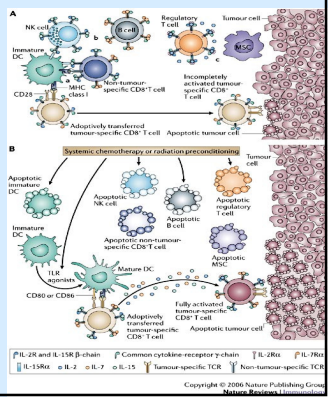
- *Ex vivo* selection and expansion of T lymphocytes isolated from tumor biopsies (TILs – Tumour Infiltrating Lymphocytes) → adoptive cell transfer/ adoptive immunotherapy.
- *In vitro* preparation of antigen-specific effector CD8$^+$ CTLs → adoptive cell transfer/adoptive immunotherapy.
- *In vitro* preparation of anti-tumor cellular vaccines based on DCs.
- *In vitro* preparation of autologous NK cells.

+ NONMYELOABLATIVE CONDITIONING
+ IMMUNE CHECKPOINT BLOCKADE!

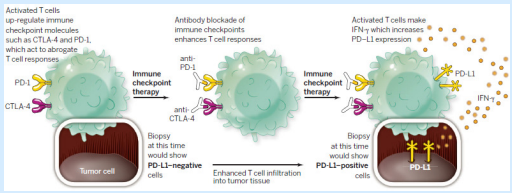


NONMYELOABLATIVE CONDITIONING OF PATIENTS PRIOR TO CELLULAR IMMUNOTHERAPY

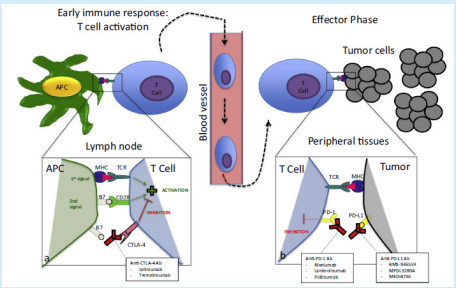
- **A:** in a non-pre-conditioned patient adoptive transfer of tumor-specific $CD8^+$ CTLs is ineffective → TA/TAA are presented by immature DCs lacking costimulatory elements → only very small amounts of proinflammatory cytokines are available and consumed by B, T and NK cells (cytokine sinks) → suppressive action of Treg, MDSCs and NK cells via IL-10, TGF-β, arginase, iNOS and direct intercellular contacts.
- **B:** in a pre-conditioned patient (chemotherapy, tumor irradiation) the numbers of lymphocytes, MDSCs, APCs (TAMs, DC2), therefore the pro-inflammatory conditions enable maturation of DCs into DC1 → more effective activation of T cells with TA/TAA and higher amounts of proinflammatory cytokines → apoptotic TCs are better targets for specific anti-tumor effector $CD8^+$ CTL clones.



THE IMMUNE CHECKPOINT BLOCKADE THERAPY

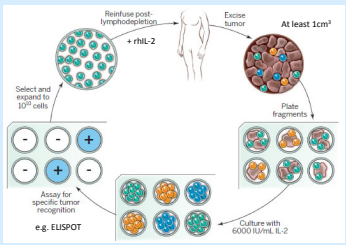


THE IMMUNE CHECKPOINT BLOCKADE



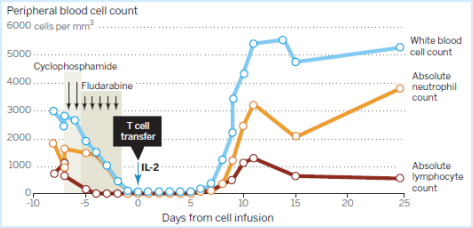
Kyi C, Postow MA. *FEBS Letters* 2014; 588:368-376

PREPARATION OF NATURALLY OCCURING AUTOLOGOUS TILs FOR ADOPTIVE CELL TRANSFER



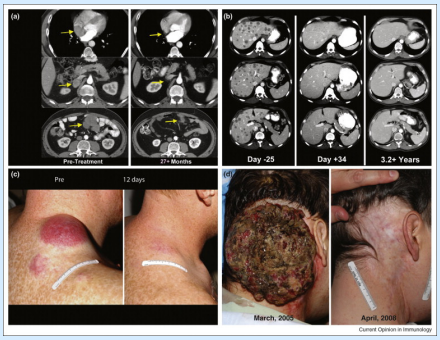
Rosenberg SA, Restifo NP. *Science* 2015; 348:62-68

LYMPHODEPLETION PRIOR TO T-CELL ADOPTIVE TRANSFER



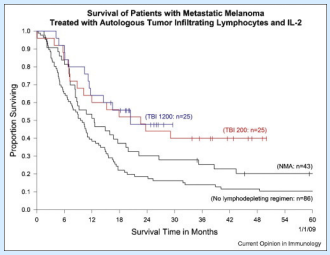
Rosenberg SA, Restifo NP. *Science* 2015; 348:62-68

CLINICAL RESPONSES TO ADOPTIVE TRANSFER OF AUTOLOGOUS TILs INTO METASTATIC MELANOMA PATIENTS



Rosenberg SA, Dudley ME. *Current Opinion in Immunology* 2009, 21:233–240

SURVIVAL OF TIL-TREATED METASTATIC MELANOMA PATIENTS DEPENDS ON THE INTENSITY OF THEIR PRE-CONDITIONING



Legend:

NMA – non-myeloablative chemotherapy (cyclophosphamide, fludarabine)

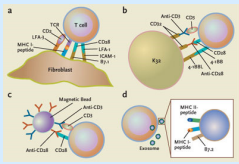
TBI – total body irradiation

Rosenberg SA, Dudley ME. *Current Opinion in Immunology* 2009, 21:233–240

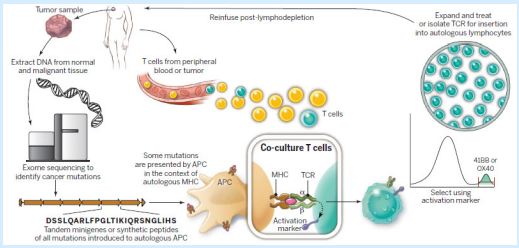
IN VITRO PREPARATION OF TA/TAA-SPECIFIC CD8⁺ CTL CLONES FOR ADOPTIVE CELL TRANSFER

- For *in vitro* stimulation of autologous lymphocytes we can use peripheral blood monocyte-derived DCs or artificial APCs, which express defined HLA class I molecules presenting specific TA/TAA peptides and costimulatory molecules.
- Effector CTLs are expanded following multiple antigen re-stimulations in appropriate cell culture medium containing proinflammatory cytokines (e.g. IL-2).
- In case of DCs, specific TA/TAA can be provided in different ways:
  - by incubating immature DCs with tumor cell (TC) lysates or apoptotic TCs;
  - by incubating them with defined TA/TAA-specific immunogenic peptides (considering HLA alleles);
  - by transfecting them with total tumor RNA, either native or amplified;
  - by preparing immunohybridomas → electrofusion of immature DCs and lethaly irradiated TCs.
- Finally antigen-loaded DCs are fully matured, using different activation factors and applied as stimulators of autologous lymphocytes.

Artificial APCs

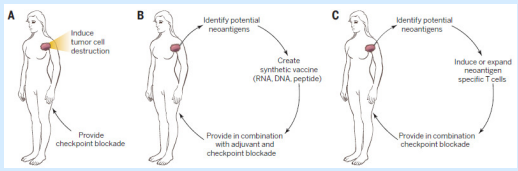


PREPARATION OF T-CELLS RECOGNIZING TUMOR-SPECIFIC MUTATIONS



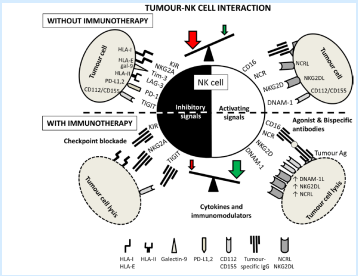
Rosenberg SA, Restifo NP. *Science* 2015; 348:62

STRATEGIES FOR TARGETTING PATIENT-SPECIFIC TUMOR NEOANTIGEN REPERTOIRE



Schumacher TN, Schreiber RD. *Science* 2015; 348:69-74

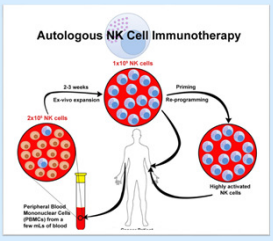
INTERACTIONS OF NK AND TUMOR CELLS



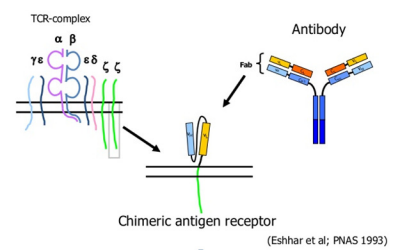
Tarazona R, et al. *Cancer Immunol Immunother* 2016; DOI 10.1007/s00262-016-1882-x

PREPARATION OF AUTOLOGOUS NK CELLS FOR IMMUNOTHERAPY

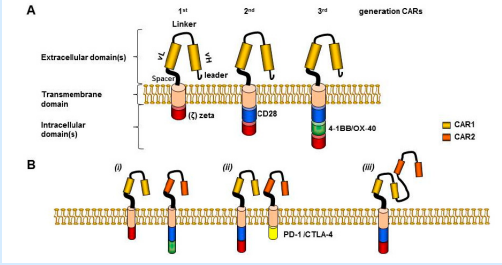
- NK cells are obtained from leukapheretic units after removal of lymphocytes and other cells.
- Then they are expanded and activated *in vitro*.
- This process lasts for 2-3 weeks while the cells are being cultivated in an appropriate medium containing NK-activating cytokines (e.g. IL-2).



T CELLS BEARING CHIMERIC ANTIGEN RECEPTORS (CARs)



DEVELOPMENT OF NEW GENERATIONS OF CARs

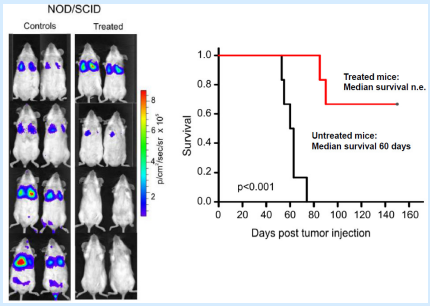


Minagawa K, et al. *Pharmaceuticals* 2015; 8:230-249

PROPERTIES OF CHIMERIC ANTIGEN RECEPTOR (CAR) ENGINEERED T CELLS

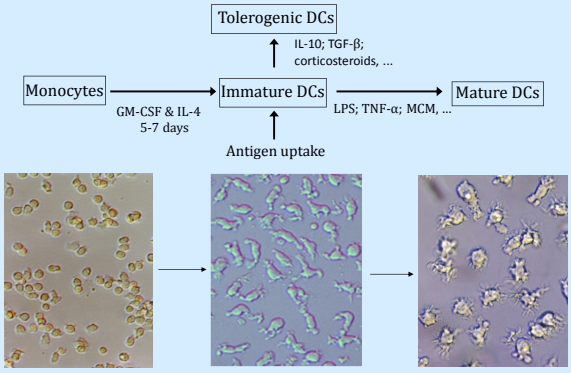
- Living drugs.
- Long-living cells (months/years).
- Traffic everywhere (including CNS).
- Make bioactive cytokines/engage other cells.
- Activity (on target and off target) occurs anywhere and at time of peak expansion.
- Multiple different CAR designs - new ones to reduce toxicity and extend to different tumors are on the horizon.

PSMA-SPECIFIC CAR- ENGINEERED T CELLS ERADICATE DISSEMINATED PC IN NOD/SCID MICE

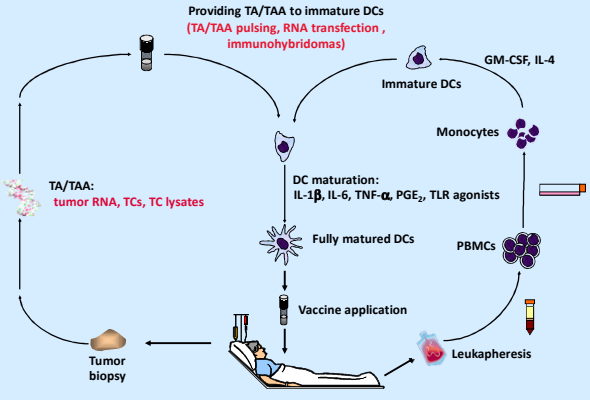


Zuccolotto G. et al. PLoS One. 2014; 9(10):e109427

IN VITRO PREPARATION OF DCs FROM HUMAN PERIPHERAL BLOOD MONOCYTES



PERSONALIZED CELLULAR IMMUNE THERAPY



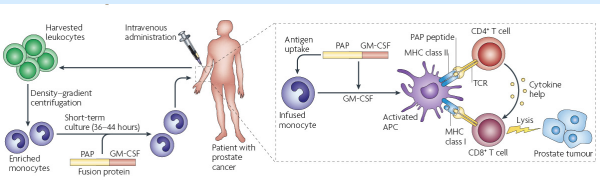
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JULY 29, 2010 VOL. 363 NO. 5

Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer

Philip W. Kantoff, M.D., Celestia S. Higano, M.D., Neal D. Shore, M.D., E. Roy Berger, M.D., Eric J. Small, M.D., David F. Penson, M.D., Charles H. Redfern, M.D., Anna C. Ferrari, M.D., Robert Dreicer, M.D., Robert B. Sims, M.D., Yi Xu, Ph.D., Mark W. Frohlich, M.D., and Paul F. Schellhammer, M.D., for the IMPACT Study Investigators*

SIPULEUCEL IMMUNOTHERAPY (FDA APPROVED)

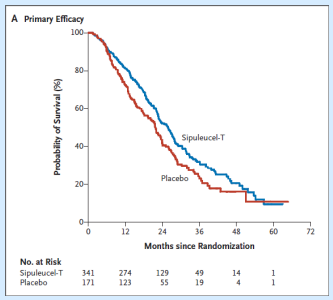


A minimum of 50 x 10^6 autologous CD54$^+$ cells (T-cells, B-cells, APCs, eosinophiles).

Legend: PAP – prostate acid phosphatase; GM-CSF – granulocyte and monocyte colonies stimulating factor

Drake et al, NatureReviews/Immunology, 2010

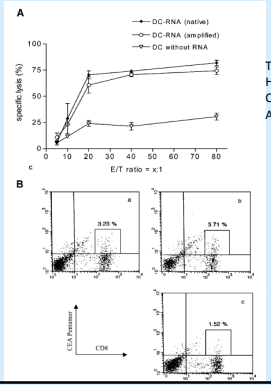
SIPULEUCEL-T: RESULTS OF THE "IMPACT" PHASE 3 TRIAL



4.1 month survival benefit
Reduction in risk of death: 22.5% **HR = 0,775 (95% CI: 0.614, 0.979)**
P=0.032

Kantoff MD. et al. N Engl J Med 2010; 363(5):411-422

ANTI-CEA EFFECTOR CTLs, GENERATED BY DCs TRANSFECTED WITH COLON CANCER RNA

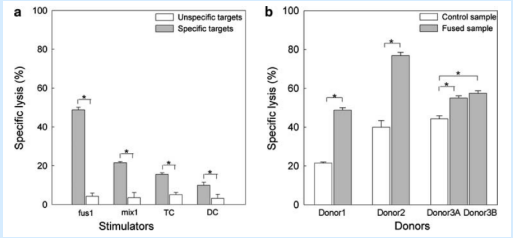


Target cells:
HLA A*02:01 positive T2 cells, pre-incubated with the CEA-specific peptide YLSGANLNL, that fits to HLA A*02:01 antigen-binding groove.

Percentages of anti-CEA specific CTL clones, assesed by using pentamers (HLA A*0201/ YLSGANLNL) and flow cytometry.

Immunobiology 2006; 211:179-189

Caco-2 SPECIFIC CTLs INDUCED *IN VITRO* BY USING IMMUNOHYBRIDOMAS MADE OF DCs AND Caco-2 CELLS



J Membrane Biol 2009; 229:11–18

IMMUNOHYBRIDOMA YIELDS ASSESSED WITH CONFOCAL MICROSCOPY AND FLOW CYTOMETRY

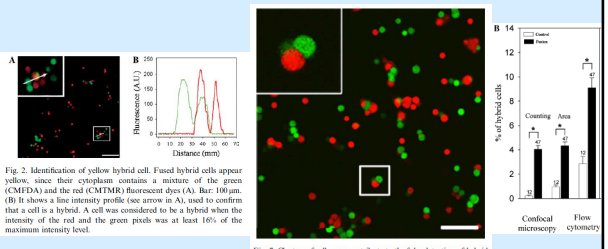
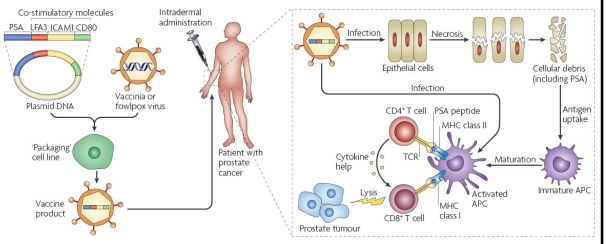


Fig. 2. Identification of yellow hybrid cell. Fused hybrid cells appear yellow, since their cytoplasm contains a mixture of the green (CMFDA) and the red (CMTMR) fluorescent dyes (A). Bar: 100 μm. (B) It shows a line intensity profile (see arrow in A), used to confirm that a cell is a hybrid. A cell was considered to be a hybrid when the intensity of the red and the green pixels was at least 16% of the maximum intensity level.

Fig. 7. Clusters of cells may contribute to the false detection of hybrid cells by flow cytometry. An aggregate of two unfused, red (CMTMR) and green (CMFDA) fluorescent cells, which may be detected as a faulty hybrid cell by flow cytometry. Bar: 100 μm.

BBRC 2004; 314:717-723

NON-CELLULAR PROSTVAC VF VACCINE: A PSA-SPECIFIC IMMUNOTHERAPY

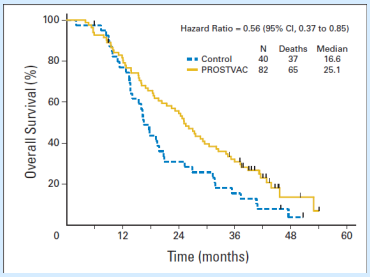


Viral vectors are injected intradermally to infect and destroy EC.

Legend: PSA – prostate-specific antigen; LFA3 and ICAM1 – adhesion molecules

Drake et al, NatureReviews/Immunology, 2010

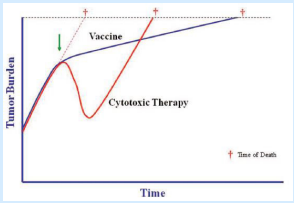
PROSTVAC EFFICACY IN PATIENTS WITH CRPC - A PHASE 2 CLINICAL STUDY



8.5 month survival benefit
Reduction in risk of death: 44%
HR: 0.56 (95% CL: 0.37-0.85)
P=0.0061

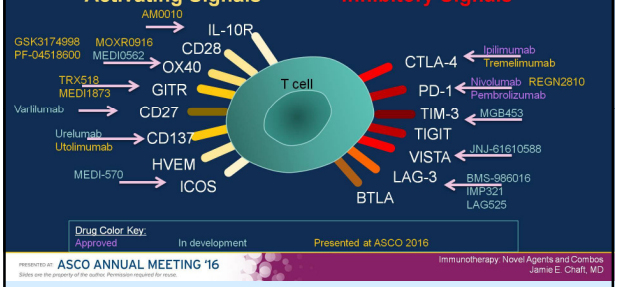
Kantoff MD. et al. J Clin Oncol 2010; 28(7):1099-1105

THERAPEUTIC ANTI-TUMOR VACCINES VS. CONVENTIONAL CYTOTOXIC THERAPY



	Conventional Therapy	Therapetic Vaccines
Target	Tumor or its microenvironment	Immune system
Pharmacodynamics	Often immediate action	Delayed
Memory response	No	Yes
Tumor Evolution / new mutations	Resistannce to therapy	New immunogenic targets
Limitations	Toxicity	Requires adequate immune system function (both systemically and at tumor site

T-cell complexities = more drug targets



Future:
Immunotherapies should be used early in the onset of malignant disease. Combinations of different therapies to be applied at the right time and in the right sequence.

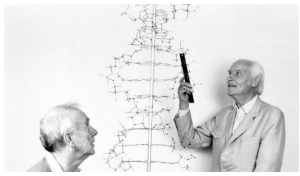
Life science research from a postdoc prospective

Janja Zupan
CEEPUS Summer school 2016
Ljubljana, 21.08.2016

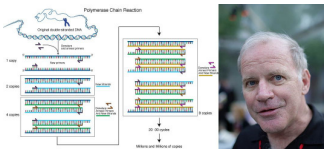
Outline

- Background: life science
- UK Arthritis research postdoc: my experience
- Conclusions: tips for applying for a postdoc

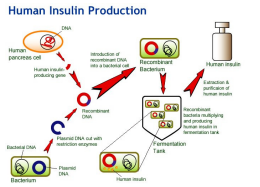
Alexander Fleming, **Penicillin discovery**, 1928



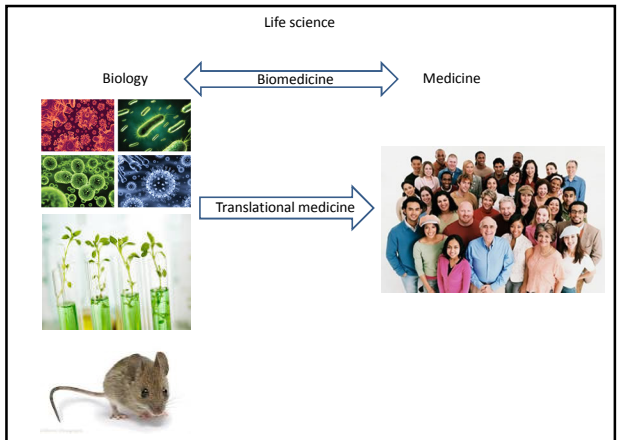
Francis Crick and James D. Watson, Nobel Prize for **DNA double helix structure**, 1962.



Kary Banks Mullis, Nobel Prize for the **PCR**, 1989

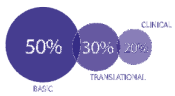


Johnson IS, **Human insulin from recombinant DNA technology,** Science 1983.



construction $150 billion

$5 billion per year



https://financeonline.com/10-worlds-most-expensive-science-experiments/
http://www.cancerresearchuk.org/funding-for-researchers/facts-and-figures-about-our-research-funding

CANCER RESEARCH UK
Arthritis Research UK
WE ARE MACMILLAN. CANCER SUPPORT
wellcome trust

Publicly funded research

Governmental sector
Research councils
National Institute for
Health Research Programme

Charitable sector
> 130 medical research charities

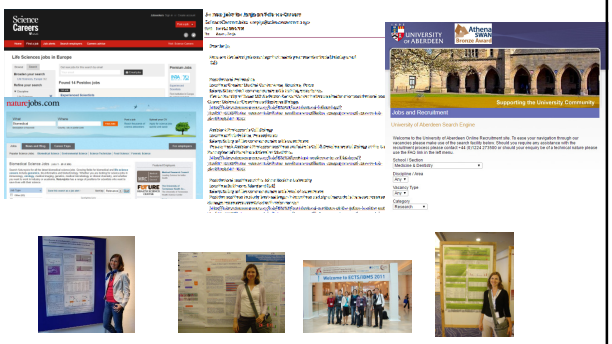
Life science in UK

Privately funded research

Elizabeth Klein, The state of the UK healthcare and life sciences sectors, 2016, http://www.euromedica.com/wp-content/uploads/2016/02/Final_Report_-_State_of_the_UK_Healthcare_Sector_.pdf

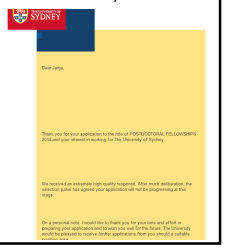
Working in life science

Searching for a postdoc



My experience in finding postdoc

- Started working as a researcher in 2007
- 2007-2012 Osteoimmunology and ECTS conferences: active participation
- 2011 ECTS PhD course Ljubljana
- 2011 ECTS PhD exchange scholarship grant at IGMM, University of Edinburgh
- Finished PhD in Clinical biochemistry and laboratory medicine University of Ljubljana 2012
- 2012-2014 applying for several postdoc positions



UNIVERSITY OF ABERDEEN

COLLEGE OF LIFE SCIENCES AND MEDICINE

SCHOOL OF MEDICINE AND DENTISTRY
DIVISION OF APPLIED MEDICINE

RESEARCH FELLOW

YMD033RX

FURTHER PARTICULARS FOR APPLICANTS

UNIVERSITY OF ABERDEEN

PERSON SPECIFICATION

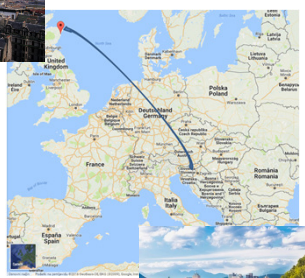
POST NO: YMD032RX

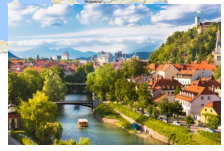
TITLE: Research Fellow

GRADE: 6

COLLEGE / SCHOOL: School of Medicine & Dentistry, Division of Applied Medicine

	ESSENTIAL	DESIRABLE
1. EDUCATION/QUALIFICATIONS Academic, technical and professional education and training	• PhD in a biological or medical science	• Experience in mouse genetics, molecular biology and skeletal biology • Experience in models of musculoskeletal disorders such as arthritis
2. WORK AND OTHER RELEVANT EXPERIENCE (INCLUDING TRAINING) e.g. Specialist knowledge, levels of experience, skills, supervisory experience, research.	• Experience in stem cell biology, developmental biology, immunohistochemistry and in vivo lineage tracing systems • Demonstrable ability to write and publish scientific papers in peer reviewed journals	• Experience of supervising undergraduate/ postgraduate students • Ability to write research grant
3. PERSONAL QUALITIES AND ABILITIES e.g. Initiative, leadership, ability to work on own or with others, communication skills.	• Good communication skills, both oral and written • Ability to work as part of a team • Demonstrable ability to be able to design and carry out studies independently	
4. OTHER eg Special circumstances (if any) appropriate to the job such as unsocial hours, travelling, physical requirements etc.		





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Dear Dr Zupan

Job Title: YMD033RX - Research Fellow 1394857

Area of University: Division of Applied Medicine

I am pleased to inform you, on behalf of the Selection Committee, that you are invited to attend for interview for the above position.

To book yourself for interview please login to the application centre using the link below.
https://jobs.ecs.co.uk/Company/aberdeen/Interest_applicant.html

Please use the links on the left hand menu to select an interview slot.

If you require any further information or assistance, please do not hesitate to contact me on

Regards

Natalie Reid
Human Resources Co-ordinator

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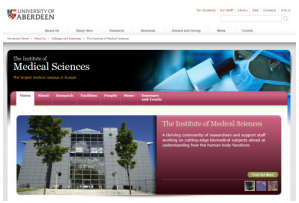

- Largest research institute at University of Aberdeen
- 411 researchers and support staff:
  - 84 principal investigators
  - 106 post-doctoral scientists and academics
  - 75 technicians
  - 140 PhD students
- £30 million in research funding raised in the last 12 months
- published 415 peer-reviewed papers

Iain Fraser Cytometry Centre
Multi-parameter analyses of cellular systems, and bio-contained high-speed sorting of single cells and particles
www.abdn.ac.uk/cytometry

Centre for Genome Enabled Biology and Medicine
Next generation sequencing, microarrays, bioinformatics & biostatistics, ancient DNA/single cell lab
www.abdn.ac.uk/genomics

Aberdeen Proteomics
Protein separation, exploration & identification
www.abdn.ac.uk/proteomics

Microscopy and Histology
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www.abdn.ac.uk/facilities/analytical

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Antibodies for research and commercialisation
www.abdn.ac.uk/sbf

Kosterlitz Centre
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www.abdn.ac.uk/kosterlitz

qPCR Facility
Rapid, high-throughput molecular quantification
www.abdn.ac.uk/facilities/qpcr

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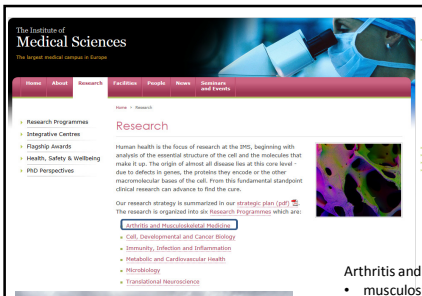


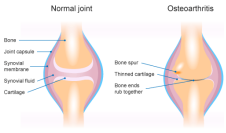
Arthritis and MSK research groups:
- musculoskeletal pharmacology
- bone and cartilage cell biology
- imaging and joint function
- musculoskeletal regenerative medicine and tissue engineering
- muscle physiology
- cohort studies and clinical trials
- nutrition
- genetics of bone diseases

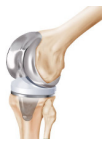
Regenerative medicine group

Arthritis Research UK

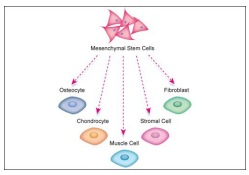
Understanding the ontogeny of mesenchymal stem cells in the joint to develop tools to study osteoarthritis

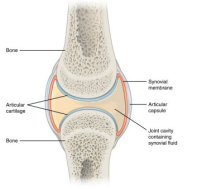
- a painful and debilitating disease that causes irreversible damage to cartilage and bones
- cartilage 'cushion' between the bones of the joint gradually erodes, leading to rubbing of bone on bone
- 1/6 people are affected by osteoarthritis in the UK
- the most commonly affected joints are knees, hips, spine and hands
- no cure or preventive treatment
- age, obesity and joint injury are known risk factors
- pain killers and joint replacement therapy

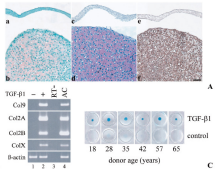


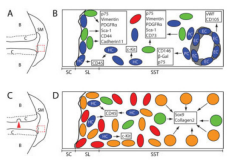


Mesenchymal stem cells (MSCs) in adult joint







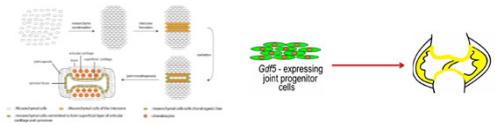


De Bari et al Arthritis Rheum. 2001 Aug;44(8):1928-42

Kurth et al Arthritis Rheum. 2011 May;63(5):1289-300

Ontogeny of MSCs in the adult knee joint

- developmental origin of MSCs in adult knee joint
- synovial and BM MSCs

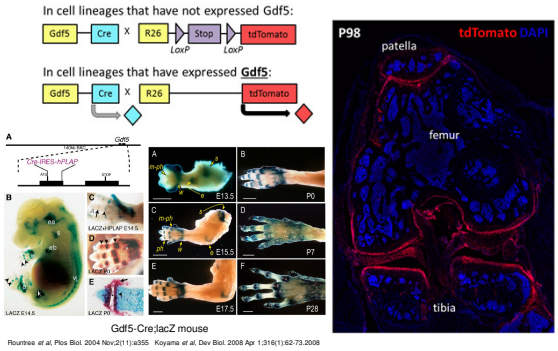


De Bari et al. Birth Defects Res C Embryo Today 2010 Dec;90(4):257-71

Hypothesis:
MSCs in the adult knee joint synovium descend from the Gdf5-expressing mesenchymal cells of the embryonic joint interzone.

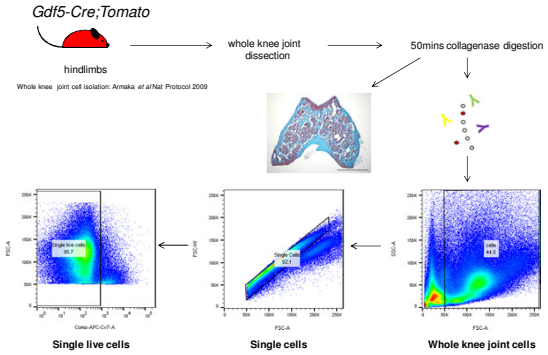
Aim:
fully characterize the subpopulations of the MSCs that are the progeny of Gdf5 expressing mesenchymal cells.

Gdf5-Cre;Tomato mouse model

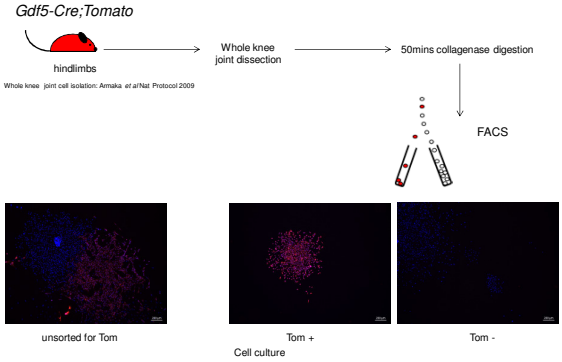


Rountree *et al*, Plos Biol. 2004 Nov;2(11):e355 Koyama *et al*, Dev Biol. 2008 Apr 1;316(1):62-73.2008

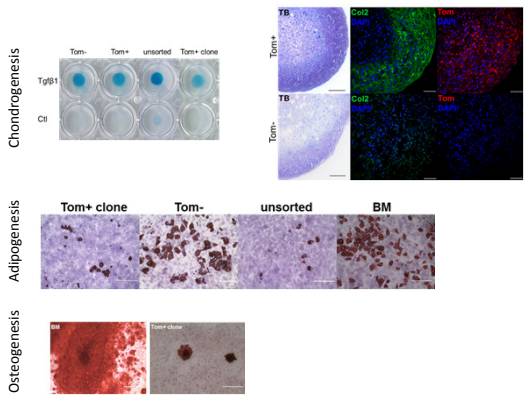
Immunophenotyping of freshly isolated Gdf5-Cre;Tomato mouse whole knee joint cells



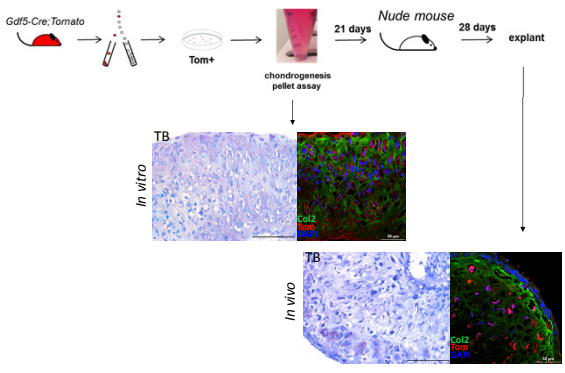
In vitro analysis of whole knee joint sorted cells



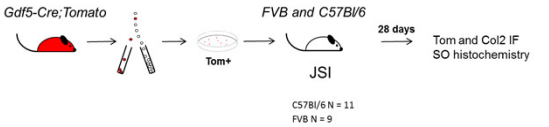
In vitro multipotency of Gdf5 progeny cells

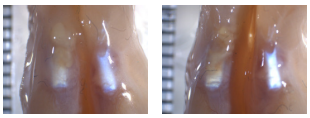


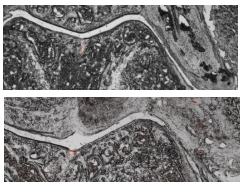
The fate of the Gdf5 progeny cartilage pellets *in vivo*



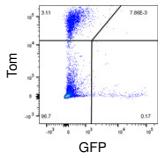
Transplantation of Gdf5 progeny cells after JSI

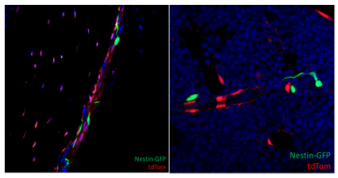


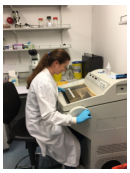


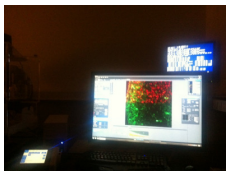


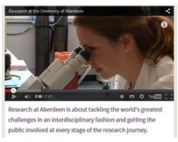
Triple transgenic mouse model Gdf5-Cre;Tom; Nes-GFP











Manuscript

Joint morphogenetic cells in the adult synovium

Anke J. Roelofs[1,2], Janja Zupan[1,2], Anna H.K. Riemen[1], Karolina Kania[1], Sharon Ansboro[1], Nathan White[1], Susan M. Clark[1], Cosimo De Bari[1]

[1]Arthritis & Regenerative Medicine Laboratory, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, United Kingdom

[2]Co-first author

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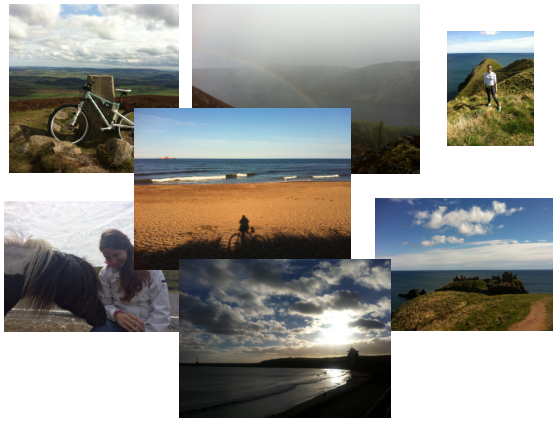

Tips for finding a postdoc

- Attend conferences and meetings

  ECTS European Calcified Tissue Society ESPT CEEPUS
- Professional networking

  ResearchGate LinkedIn
- CV, cover letter, PPT

Acknowledgements

Professor Janja Marc
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Professor Janez Prezelj
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Asist. Rihard Trebse
Simon Herman
Gregor Haring
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Dr Sharon Ansboro
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Dr Shahida Shahana
Denise Tosh
Ausra Lionikiene
Agathe Moraine

Iain Fraser Cytometry Centre
Microscopy and Histology Core facility

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