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Content

Review

Research article

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Нови агенти в лечението на множествения миелом

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Novel agents in the treatment of multiple myeloma

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РЕЗЮМЕ:

През последните две десетилетия беше постигнат значителен напредък в лечението на множествения миелом, особено с въвеждането на протеазомния инхибитор бортезомиб и имуномодулаторите талидомид и леналидомид. Независимо от терапевтичния напредък, мултиплениият миелом остава нелечимо заболяване и резултатите от лечението са хетерогенни. Крайните стадии на заболяването се характеризират с бързи рецидиви и рефрактерност на терапия. Докато тройните терапевтични комбинации, включващи имуномодулатори и бортезомиб, все още се препоръчват при високо-рискови и рецидивиращи пациенти, има голям интерес към разработването на нови агенти, които са ефективни при пациенти, резистентни към конвенционална терапия. Сред най-обещаващите от тези средства са новите протеазомни инхибитори и имуномодулатори, които надграждат постигнатото от техните предшественици. Някои от най-важните нови групи медикаменти в терапията на множествения миелом са моноклоналните антитела и хистон-деацетилазните инхибитори, които предлагат нови механизми за лечение на заболява-

ABSTRACT

During the past two decade significant progress in the treatment of multiple myeloma was achieved, particularly with the use of the proteasome inhibitor bortezomib and the immunomodulatory agents thalidomide and lenalidomide. Despite therapeutic advances, multiple myeloma remains an incurable disease and outcomes are heterogeneous. The end stages of the disease is characterized by rapid relapse and broad treatment refractoriness. While triplet therapeutic combinations, including immunomodulatory agents and bortezomib, are still recommended in high-risk risk and relapsed cases, there is great interest in developing new agents, which are effective in patients refractory to conventional therapy. Among the most promising of these agents are novel proteasome inhibitors and immunomodulators, which build upon the progress made by their predecessors. Some of the most important classes of new therapeutic agents in myeloma treatment are the monoclonal antibodies and the histone deacetylase inhibitors, which offer new mechanisms for therapy of the disease. Many other molecularly targeted therapies in multiple myeloma have also emerged. The optimal combinations of these agents are to be estab-

нето. Появиха се и много други молекулярни таргетни терапевтични агенти. Оптималните комбинации от тези медикаменти предстои да бъдат установени чрез включване на всички подходящи пациенти в клинични проучвания.

Ключови думи: множествен миелом, терапия, нови агенти.

lished though enrollment of all eligible patients in clinical trials.

Key words: multiple myeloma, anticancer therapy, novel agents.

INTRODUCTION

Multiple myeloma (MM) is the second most common hematologic malignancy and is characterized by the presence of a monoclonal protein detectable in the blood and/or urine, clonal plasma cell involvement of the bone marrow, and end-organ damage ("CRAB"): hypercalcemia,renal insufficiencyanemia and lytic bone lesions (12).

Big advances were made during the past two decadesin the treatment of symptomatic MM, particularly with the use of proteasome inhibitors (bortezomib) and immunomodulatory agents (thalidomide, lenalidomide) (20). One-third of the patients younger than 65 years now live beyond the decade. However despite therapeutic advances, outcomes in MM remain heterogeneous with an approximate 10% mortality rate within the first year following diagnosis (9).Newly symptomatic patients generally respond well to their first line of treatment and enter a period of remission characterized by stable and effective control of symptoms. Initial treatment strategies depend on the patient's ability to tolerate intensive treatment. Younger patients (up to 65 years of age) are treated with induction therapy, high-dose chemotherapy and autologous stem cell transplant (ASCT), whereas older patients and those with significant comorbidities receive more moderately dosed chemotherapy only. Induction therapy utilizing novel agents results in higher response rates post-induction and post-transplantation when compared with the previous standard of care VAD (vincristine, doxorubicin, dexamethasone) (37). As there is no curative treatment, MM inevitably relapses, and malignant plasma cell clones over time become increasingly aggressive and refractory to treatment, prompting relapse, progression, and death. It is during that phase of disease that novel investigational agents enter clinical use as part of clinical trials (24).

During the past few years of clinical research, novel proteasome inhibitors and immunomodulators have been developed, as well as new target therapies for myeloma – monoclonal antibodies and molecularly targeted agents.

PROTEASOME INHIBITORS

The proteasome is a key structure within the nucleus and cytoplasm of eukaryotic cells that degrades ubiquinated proteins and is involved in cell homeostasis. Proteasome inhibition results in accumulation of misfolded proteins, endoplasmic reticulum stress, and induction of apoptosis. **Bortezomib** (Velcade), a dipeptide boronic acid, is a first-in-class reversible proteasome inhibitor of the 20S proteasome primarily at the chymotrypsin-like and caspase-like active sites. It originally received accelerated approval by the US Food and Drug Administration (FDA) in 2003 based on the results of the phase II SUMMIT trial (28). In the international, randomised, phase III APEX study bortezomib demonstrated superior efficacy compared to high-dose dexamethasone in patients with relapsed MM (30).Combination approaches have been an area of active investigation and proved their efficacy for first-line treatment – triplet regmens, combining bortesomib and corticosteroids with liposomal doxorubicin, cyclophosphamide, melphalan, thalidomide or lenalidomide. Following the success of bortezomib, several other compounds with unique chemical characteristics have entered clinical testing in an attempt to overcome bortezomib resistance as well as to improve the safety profile of proteasome inhibition.

Carfilzomib (Kyprolis) is a second-generation proteasome inhibitor with distinct chemical properties from bortezomib with highly chymotrypsin-specific irreversible proteasome inhibition (12). In vitro, was found that carfilzomib have minimal neurotoxicity and minimal reaction to non-proteasomal proteases (11). Based on the encouraging single-agent activity and favorable toxicity profile, carfilzomib has been incorporated into several combination regimens. Carfilzomib, lenalidomide, and dexamethasone (KRd) were compared to Rd in the ASPIRE study and demonstrated a significant improvement in the progression-free survival (PFS) (26.3 vs. 17.6 months) and complete response(CR) rate (31.8 % vs. 9.3 %) in relapsed multiple myeloma (39).Based on this trial Kyprolis in combination with either lenalidomide and dexamethasone or dexamethasone alone is indicated for the treatment of adult patients with multiple myeloma who have received at least one prior therapy (11). The safety and efficacy of carfilzomib were evaluatedin a phaseIII, randomised, openabel, multicentrestudy (4) of Kyprolis plus dexamethasone (Kd) versus bortezomib plus dexamethasone (Vd). The study showed significant improvement in PFS for patients in the Kd arm over those in the Vd arm ($p<0.0001$). A statistically significant advantage in overall survival (OS) was observed inpatients in the Kd arm compared to patients in the Vd arm ($p=0.010$) (4).The most common adverse reactions (occurring in >20% of subjects) in carfilzomib trials were: anaemia, fatigue, thrombocytopenia,nausea,diarrhea, pyrexia, dyspnoea, respiratory tract infection,cough and neutropenia.In clinical studies with carfilzomib, cardiac toxicity and dyspnea typically occurred early in the course therapy(4,11,39).

Ixazomib (Ninlaro) is a second-generation, orally bioavailable 20S proteasome inhibitor with improved tissue penetration and antitumor activity. It is the first oral proteasome inhibitor to enter phase III testing.Ixazomib has significant single-agent activity even with prior bortezomib and carfilzomib exposure (12). The TOURMALINE-MM1 phase III trial, a randomized, double-blind, placebo controlled clinical study of 722 patients evaluating ixazomib plus lenalidomide and dexamethasone compared to placebo plus lenalidomide and dexamethasone in adult patients with relapsed and/or refractory MM. The study showed a PFS of 20.6 in the ixazomib arm versus 14.7 months in the control arm ($p=0.012$). Overall response rate (ORR) was 78.3 % in the ixazomib arm with median duration of response was 20.5 months, versus 71.5 % and 15 months in the control arm. The most common grade ≥3 adverse events in the ixazomib group included neutropenia, anemia, thrombocytopenia, and pneumonia (21). The FDA granted ixazomib approval in November 2015 based on the result of this study.Ninlaro in combination with lenalidomide and dexamethasone was indicated for the treatment of adult patients with multiple myeloma who have received at least one prior therapy (23).

Marizomib is an investigational proteasome inhibitor, which has been shown to irreversibly bind to all three catalytic subunits of the 20S proteasome (6). Marizomib's irreversible binding to the 20S proteasome and its significantly lower rate of efflux from malignant cells appear to account for its increased cytotoxicity and longer duration of action, as well as its vigorous activity in bortezomib-resistant cell lines. Phase I studies, though limited to date, have demonstrated relatively mild toxicities and no evidence of neuropathy or thrombocytopenia (22). Since marizomib and bortezomib are structurally dissimilar, and influence different apoptotic signaling pathways, there exists strong rationale for using the two agents in combination (6,22).

Oprozomib is a structural analog of the 26S proteasome inhibitor carfilzomib, which, unlike carfilzomib, is orally bioavailable. Phase I/II studies have demonstrated a tolerable safety profile with low incidence of neuropathy and encouraging preliminary response rates (7,22).

IMMUNOMODULATORS

Thalidomide was first reported to have benefit in MM in 1999 in patients with advanced relapsed disease, opening the door for investigating targeted therapies (36). The mechanism of the immunomodulatory drugs (IMiDs) has been elucidated following the identification of the cereblon protein (CRBN) as the target of thalidomide. IMiDs bind to

CRBN and cause degradation of certain transcription factors, resulting in downregulation of IRF4 and Myc and cytotoxicity to myeloma cells (12,43). Currently thalidomide-based regimens are recommended for the treatment of patients with newly diagnosed and relapsed MM, both in the transplant-eligible and non–transplant eligible populations.

Lenalidomideis an analog of thalidomide with more potent anti-MM activity and a more favourable toxicity profile. It was approved for the treatment of MM in 2006. A large Eastern Cooperative Oncology Group (ECOG) phase III study established the efficacy and safety of lenalidomide plus high-dose (RD) or low-dose (Rd) dexamethasone in newly diagnosed multiple myeloma (27). Lenalidomide with low-dose dexamethasone has since become astandard initial therapy. Thromboprophylaxis with aspirin, warfarin, or low-molecular-weight heparin is essential for all patients treated with IMiDs to reduce the near 25% incidence of thrombotic complications in patients without prophylaxis (12). A phase I/II trial evaluating the combination of lenalidomide, bortezomib, and dexamethasone (RVD) in newly diagnosed MM patients reported response rates of 100%, with 74% very good partial response (VGPR) or better (31). Additional trials have evaluated the role of maintenance lenalidomide following autologous stem cell transplantation, showing improvement in event-free survival (19).

Pomalidomide is the most potent IMiD and demonstrates significant activity even in lenalidomide-refractory patients. Similar to lenalidomide and thalidomide, pomalidomide binds to CRBN, and a potential mechanism of resistance is downregulation of the target. CRBN expression may be a potential predictive biomarker for the selection of patients most likely to respond to pomalidomide (34). Pomalidomide has broad immunomodulatory effects including T-cell stimulation, inhibition of T-regulatory cells, NK-cell activation, enhancement of antibody-dependent cell-mediated cytotoxicity, and osteoclast inhibition (1). The randomized IFM 2009-02 (13) and MM-003 (33) studies both demonstrated the efficacy of pomalidomide in combination with dexamethasone, although the median PFS was less than the initial reports. Pomalidomide was granted accelerated approval in 2013 for patients who have received at least two prior therapies with disease progression on or within 60 days of the previous therapy. The side effect profile is similar to other IMiDs with a low rate of sensory neuropathy (10 % all grades) however grade 3/4 neutropenia was observed in 47 % of patients (12). The safety and efficacy of pomalidomide in combination with carfilzomib and dexamethasone in patients with relapsed/refractory MM is a current area of investigation (NCT01665794 study).

MONOCLONAL ANTIBODIES

The next major breakthrough in the therapy of MM was the effective incorporation of monoclonal antibodies.

Daratumumab is a human IgG1k monoclonal antibody against CD38, a cell surface protein that is prominently expressed on myeloma cells and is involved in the messenger pathways, which regulate apoptosis, survival, and proliferation. Binding of daratumumab to CD38 has been shown to mediate phagocytosis of MM cells by macrophages (22). In November 2015 daratumumab was granted accelerated approval for the treatment of patients with myeloma who have received at least three prior lines of therapy, including a proteasome inhibitor and an IMiD, or who are double-refractory to a proteasome inhibitor and an IMiD. It was further approved by the FDA in November 2016 for the use in combination with bortezomib and dexamethasone, or lenalidomide and dexamethasone, for treatment of patients with multiple myeloma, who have received at least one prior therapy.Accelerated approval of daratumumab was based upon the multicenter, open-label, phase II SIRIUS trial (16), which enrolled 106 heavily pretreated patients with relapsed or refractory myeloma to receive daratumumab monotherapy at a dose of 16 mg/kg. The overall response rate demonstrated in the SIRIUS trial was 29.2% and median OS was 17.5 months. Of patients who responded to daratumumab, 25.8% had responses that deepened over time (16). The randomized, controlled, open-label phase III CASTOR trial enrolled 498 patients who had received one or more prior lines of therapy to receive a regimen of bortezomib and dexamethasone either alone or in combi-

nation with daratumumab (26). The CASTOR trial was halted due to an interim analysis showing significantly improved outcomes in the daratumumab group compared with the control group. The POLLUX trial was a randomized, controlled, open-label, phase III study that enrolled 569 patients who had received one or more prior lines of therapy to receive lenalidomide and dexamethasone either alone or in combination with daratumumab (5).As with the CASTOR trial, the POLLUX trial was halted early due to a protocol-specified interim analysis. The hazard ratio for disease progression or death in the daratumumab group vs. the control group was 0.37 ($p<0.001$), representing a 63% lower risk of disease progression or death in the daratumumab group (5).

Elotuzumabis a humanized IgG1 monoclonal antibody against CS1, a subunit of CD2, which is a cell surface glycoprotein and member of the signaling lymphocyte activation molecule (SLAM) family. CS1 is consistently expressed by MM cells and rarely expressed in other tissues, including hematopoietic elements (22). Elotuzumab in combination with bortezomib and dexamethasone was evaluated among 152 patients with relapsed/refractory MM. Patients were randomized to receive either bortezomib/dexamethasone alone or in combination with elotuzumab. The elotuzumab group demonstrated a median PFS of 9.7 months compared to 6.9 months among the control group. VGPR or better occurred in 36 % of patients in the elotuzumab group compared with 27 % in the control group. Addition of elotuzumab did not seem to add clinically significant toxicity to the treatment regimen (8). The first phase III trial of elotuzumab in MM, the ELOQUENT-2 trial, included 646 relapsed/refractory patients randomized to receive elotuzumab plus lenalidomide/dexamethasone versus lenalidomide/dexamethasone alone. Median PFS was 19.4 months in the elotuzumab group and 14.8 months in the control group. ORR was 79 % in the elotuzumab group and 66 % in the control group ($p<0.001$) (15). Accordingly, elotuzumab (Empliciti) was granted FDA approval in November 2015 for relapsed/refractory MM patients in combination with lenalidomide and dexamethasone. An additional phase III trial, ELOQUENT-1, comparing elotuzumab with lenalidomide/dexamethasone to lenalidomide/dexamethasone alone among patients with previously untreated MM is currently ongoing (22).

Siltuximab is a chimeric monoclonal antibody against IL-6. In a phase II randomized study of bortezomib-melphalan-prednisone (VMP) or VMP plus siltuximab followed by siltuximab maintenance in newly diagnosed MM patients, median PFS and 1-year OS were identical in both arms (17 months and 88%, respectively), while more hematologic events and infections occurred in the siltuximab group (32). In the relapsed setting, the addition of siltuximab to bortezomib did not significantly improve the response rate or PFS (12).

Indatuximab is a chimerized anti-CD138 monoclonal antibody conjugated to the maytansinoid cytotoxin DM4, a potent inhibitor of the microtubule assembly. CD138 is a relatively exclusive plasma cell marker, with minimal expression among other hematopoietic lineages. Conjugation of anti-CD138 to DM4 allows for the targeted delivery of cytotoxins to myeloma cells. Indatuximab was internalized at the cell surface, releasing DM4 into the cytoplasm where its anti-tubulin effects promote cell death (22). Preclinical studies have demonstrated considerable synergy between indatuximab and lenalidomide, prompting the design of a phase I/IIa trial investigating indatuximab-lenalidomide-dexamethasone among relapsed/refractory patients.The study showed the potentials of indatuximab in the treatment of refractory/relapsed MM patients (22).

SAR (SAR650984), like daratumumab, is a monoclonal antibody to CD38. It exerts antitumor activity via antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, direct apoptosis induction, and allosteric inhibition of CD38 enzymatic activity (22).

HISTONE DEASETYLASE (HDAC) INHIBITORS

Histone acetylation and deacetylation plays an important role in the regulation of gene

expression. In general, hyper-acetylated chromatin is transcriptionally active, and hypo-acetylated chromatin is transcriptionally silent. Altering the acetylation of chromatin may alter the expression of oncogenes and tumor suppressor genes and influence oncogenesis (22). Histone acetylation modulates gene expression, cellular differentiation, and survival and is regulated by histone acetyltransferases and histone deacetylases (HDACs). Inhibition of HDAC activity promotes differentiation, cell cycle arrest, and/or apoptosis of tumor cells. The effect of HDAC inhibitors on multiple pathways allows for good complementary activity with other antitumor agents (2).

Panobinostatis an oral pan-deacetylase inhibitor, which increases the acetylation of proteins involved in numerous oncogenic pathways.Panobinostat has been investigated in combination with other established agents (lenalidomide, melphalan, or bortezomib) for the treatment of relapsed/refractory MM. In a phase II trial, panobinostat was studied in combination with melphalan, thalidomide, and prednisone in relapsed/refractory MM patients; at least PR was observed in 38.5% of patients. However significant hematologic toxicities were reported: neutropenia in 71% and thrombocytopenia in 35.5% (25). In a phase III randomized double blinded study (PANORAMA 1 trial), patients with relapsed or refractory MM received bortezomib, dexamethasone, and either panobinostat or placebo (29). PFS was 12 months in the panobinostat group and 8.1 months in the placebo group (p<.0001). Panobinostatis the first HDACi to be approved by FDA in February 2015, in combination with bortezomib and dexamethasone, for treatment of MM patients who have received at least two prior standard therapies (including bortezomib and an immunomodulatory agent) (22).

Vorinostatis an orally bioavailable, non-specific histone deacetylase inhibitor (22).It causes apoptosis and molecular changes in MM cells and a reduction of IL-6 production by bone marrow stromal cells. Vorinostat was shown to enhance the activity of other pro-apoptotic agents, including dexamethasone and immunomodulatory drugs (2). The VANTAGE-008 study is a randomized, phase III study of bortezomib with or without vorinostat, which demonstrated no clinically meaningful improvement in median PFS (7.6 vs. 6.8 months; p=0.01) but an increased rate of nausea, vomiting, and fatigue in the vorinostat group (3). Vorinostat may be a salvage option in patients that are refractory to bortezomib and immunomodulators. HDAC6 selectivity may enhance the potency and reduce off-target effects compared to non-selective pan-HDAC inhibitors (vorinostat, panobinostat) (22).

Ricolinostatis an HDAC6-specific histone deacetylase inhibitor which shows synergistic effects with proteasome inhibitors (22). Murine models of MM have demonstrated significant delay in tumor growth and prolongation of survival when treated with combination of ricolinostat and bortezomib. The combination of ricolinostat with carfilzomib has demonstrated synergistic toxicity to myeloma cells resistant to bortezomib in the preclinical setting (22).

PI3K/AKT/mTOR PATHWAY INHIBITORS

The phosphoinositide 3-kinase (PI3K) family of enzymes are a group of lipid kinase signal transducers involved in a diverse spectrum of cellular functions including growth, proliferation, differentiation, and survival(22).The PI3K pathway mediates proliferative and antiapoptotic signals in MM and its activity was shown to increase with the progression of the disease. Activated AKT subsequently modulate the phosphorylation of several substrates involved in the regulation of cell survival, cell cycle progression, and cellulargrowth. The best-studied downstream substrate of AKT is the serine/threoninekinasemTOR(mammalian target of rapamycin) (2).

Among the various classes of PI3Ks, class 1 isoforms have proven to be the major target in drug designs. **Perifosine**is an oral bioactive alkylphospholipid that is thought to target cell membranes and modulate multiple signaling pathways, including inhibition of AKT and promotion of apoptosis in MM cells (2). Although it initially displayed promise in the treatment of relapsed/refractory MM, a phase III trial

comparing perifosine, bortezomib, and dexamethasone to bortezomib and dexamethasone alone was discontinued in 2013 after interim results showed that the addition of perifosine had not, and likely would not, significantly extend PFS (22). Another PI3K inhibitor, idelalisib, has proven more successful in the treatment of hematologic malignancies - chronic lymphocytic leukemia, small lymphocytic lymphoma, and follicular lymphoma. Numerous PI3K inhibitors are presently undergoing investigation for their potential use in MM. **BAY80-6946** and **GDC-0941** have demonstrated significant anti-tumor effects and induced cell cycle arrest and apoptosis in myeloma cell lines (22). **Buparlisib,** an oral PI3K inhibitor, has also demonstrated encouraging results in mouse models of myeloma and has been shown to mediate a reduction in osteolytic lesions via downregulation of osteoclasts and upregulation of osteoblasts (17). Based on the preclinical data to date, and their success in other malignancies, there seems to be a future for the use of PI3K inhibitorsin MM (22).

AKT is a protein kinase central to many of the signaling pathways, which guide cellular proliferation and apoptosis. Elevated expression of AKT has been demonstrated in myeloma cell lines as well as in bone marrow aspirates form patients with MM (22). The ATPcompetitive AKT inhibitor **afuresertib** has been tested in a phase 1 trial with the most frequent adverse events being nausea, diarrhea, and dyspepsia, and the dose-limiting toxicity proving to be liver function test abnormalities (38). Afuresertib has also demonstrated a favorable safety profile when combined with bortezomib and dexamethasone in patients with relapsed/refractory MM in a phase Ib trial with an ORR approaching 50% (42).

AKT indirectly activates mTOR, which consists of 2 distinct multimolecular complexes, mTOR complex 1 (mTORC1), and complex 2 (mTORC2). mTORC1 activity leads to increased mRNA translation, protein synthesis, and cellular proliferation. mTORC2 is involved in regulation of the cytoskeleton and is upstream from and directly to phosphorylates AKT. mTORC1 inhibition can lead to activation of the PI3K pathway due to mTORC2 negative feedback, resulting in phosphorylation of AKT (2). **Rapamycin** and its analogues **(temsirolimus and everolimus)** are inhibitors of mTOR and have shown preclinical potential as MM therapies and synergy in combination with other therapies. Their activity in MM however is limited, likely because of the lack of inhibition of mTORC2. **INK128** and **NVP-BEZ235** are dual mTORC1/2 inhibitors and new therapeutic agents against MM (2).

Bcl-2 INHIBITORS

Bcl-2 is known to be an essential anti-apoptotic protein and a rational target for novel chemotherapeutic agents. Small molecule inhibitors of the Bcl-2 family have been under development for over a decade.Peclinical results were particularly impressive with regard to hematologic malignancies (22). The Bcl-2-specific agent ABT-199 is the first inclass orally bioavailable Bcl-2-specific small molecule inhibitor to be developed. Preclinical investigations into its utility in MM have suggested therapeutic potential in certain subsets of patients. Myeloma cell lines particularly dependent on Bcl-2 for survival are sensitive to ABT-199, whereas cell lines dependent or co-dependent on other Bcl-2 family proteins are less sensitive to Bcl-2 inhibitors. ABT-199 has demonstrated considerable synergy with the novel proteasome inhibitor carfilzomib, maybe because among carfilzomib's manifold mechanisms of action is induction of Noxa, a proapoptotic member of the Bcl-2 family (18). ABT-199 has also been shown to be effective in cell lines of MMs with t(11;14), and accordingly, Bcl-2 may thus be a promising targeted therapy for this myeloma subtype (40).A phase 1 trial in relapsed MM is ongoing. The major dose-limiting toxicity in clinical trials in CLL patients was tumor lysis syndrome (TLS). Such rapid tumor lysis has not yet been observed in preclinical studies with MM, however measures toward TLS prophylaxis may be warranted in clinical studies (22).

BTK INHIBITORS

Bruton's tyrosine kinase (BTK) is an enzyme that plays a crucial role in B-cell survival and maturation. **Ibrutinib,** an orally adminis-

tered selective inhibitor of BTK, has demonstrated impressive efficacy in the treatment of B-cell malignancies and is EMA approved as a therapy for mantle cell lymphoma, chronic lymphocytic leukemia and Waldenström's macroglobulinaemia.Recent preclinical studies have demonstrated that BTK may also be involved in the propagation and maintenance of malignant plasma cell clones in MM (22). BTK expression has been shown to be markedly elevated and activated in dexamethasone-resistant MM cell lines, and patients with higher BTK expression in their malignant plasma cells have been shown to have a worse prognosis (14). Ibrutinib has been shown to be cytotoxic to malignant plasma cells in vitro, and treatment with ibrutinib has been shown to augment the cytotoxic activity of bortezomib and lenalidomide. Ibrutinib activity in myeloma cells seems to be mediated by its ability to interfere with the NF-kB-signaling pathway and thus promote apoptosis (22). Ibrutinib is presently being evaluated in a phase II dose escalation study, as a single agent or in combination with dexamethasone, in patients with relapsed/refractory MM, with preliminary results demonstratingevidence of anti-tumor activity in a heavily pretreated population (41).

CDK INHIBITORS

Dinaciclib is a novel small molecule inhibitor of cyclin-dependent kinases (CDKs), the ubiquitous protein kinases central to the regulation of the cell cycle. Dinaciclib primarily inhibits CDK1, CDK2, CDK5, and CDK9. It has proven to be relatively well tolerated in initial phase 1 trials and has demonstrated clinical efficacy in chronic lymphocytic leukemia and solid tumors (22). Dinaciclib was investigated in a dose escalation trial as a single agent for relapsed/refractory MM. Twenty-seven patients with a median of four prior therapies were treated with dinaciclib as a single agent with an ORR of 19 %(10). CDK5 inhibition has been shown to enhance the activity of proteasome inhibitors in vitro, suggesting that a trial combination of dinaciclib and bortezomib may have promising results in the future (22).

KINESIN SPINDLE PROTEIN INHIBITORS

Kinesin spindle protein (KSP), a constituent of the kinesin class of microtubule-based proteins, plays a key role in centrosome separation and bipolar spindle assembly during mitosis. KSP is also believed to have antiapoptotic properties via mediation of the cell survival protein myeloid leukemia sequence 1 (Mcl-1) (22). **Filanesib** is a KSP inhibitor with significant anti-tumor activity. Its most robust anti-myeloma properties seem to lie in its ability to promote apoptosis via the degradation of Mcl-1. Proliferating hematopoietic cells, including myeloma cells, are particularly dependent on Mcl-1 and thus particularly sensitive to inhibition of KSP (22). Phase I trials of filanesib in relapsed and refractory MM, both as monotherapy and in combination with various agents including dexamethasone, bortezomib, and carfilzomib, have demonstrated its toxicities to be largely hematologic (22). A phase II study, investigating filanesib both with and without low-dose dexamethasone in patients refractory to both bortezomib and lenalidomide, showed that among the 32 patients treated with filanesib alone, minor response was observed in 19 % and PR was observed in 16 %. Among the 18 patients treated with filanesib and low-dose dexamethasone, the ORR was 28 % (35).

CONCLUSION

The introduction of bortezomib and lenalidomide revolutionized the therapeutic approach to MM and established a new front line for treatment of the disease. Nevertheless, in spite of the progress achieved, the natural course of MM often remains one of a continuously relapsingrefractory disease. Although triplet therapeutic combinations, including IMIDs and proteasome inhibitors, are the backbone of treatment in relapsed disease, repeatedly relapsed or high-risk clones can and do become refractory to these agents. There is great interest in developing new agents, which remain effective in patients refractory to conventional therapy. Among the most promising of these agents are the novel proteasome inhibitors and immunomodulators, which build upon the progress made by

their predecessors. The development of novel monoclonal antibodies, two of which are now approved for use in relapsed/refractory disease, is another step forward in the treatment of MM. Histone deacetylase inhibitors offer a novel mechanism for MM therapy and many other molecularly targeted therapies have emerged. As the optimal combinations of these agents are not yet established, enrollment in clinical trials, where many of these agents are being evaluated as components of novel regimens, will be the future to pave the way to find the best effective treatment.

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1α,25$(OH)_2D_3$ и VDR в кожното възпаление и рак (допълнение)

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1α,25$(OH)_2D_3$ and VDR in skin inflammation and cancer (additional remarks)

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РЕЗЮМЕ:

Витамин Д е известен с това че е изключителна важност за здравето на костите. Това е група от мастно разтворими прохормони, които се превръщат в тялото в множество биологично активни метаболити, функциониращи като истински хормони, циркулиращи в кръвообръщението и регулиращи активността на различни клетъчни типове – с калцемични и некалцемични ефекти. Клетките таргетни за действието на 1,25$(OH)_2D_3$ експресират ВДР (Витамин Д Рецептор) в различни нива. Свързаните с лиганди ВДР формират хетеродимери с ретиноид Х рецепторът (retinoid X receptor - RXR) и свързват витамин D респонс елементи (VDRE), за да модулират генната експресия. Експресията на ВДР в клетки различни от тези на тънко черво, кости, бъбреци, и паратироидни жлези, води до установяването на т. нар. некалцемично действие на ВДР лигандите. ВДР протеинът се експресира в почти всички нормални човешки клетъчни типове и тъкани, и също в ракови клетъчни линии и тумори, произхождащи от няколко рган. Далнрегулиране на ВДР е установено в

ABSTRACT

Vitamin D is well known as being essential for bone health. It is a group of fat-soluble prohormones, which are converted in the body into a number of biologically active metabolites that function as true hormones, circulating in the blood and regulating the activities of various cell types - both calcemic and noncalcemic effects[1]. Cell responsiveness to 1,25$(OH)_2D_3$ mainly relays on VDR expression levels. The liganded VDR forms a heterodimer with retinoid X receptor (RXR) and binds to vitamin D response element (VDRE) to modulate the gene expression. The presence of VDR in cells other than those of the intestine, bone, kidney, and parathyroid gland led to the recognition of noncalcemic actions of VDR ligands. VDR protein is expressed in almost all normal human cell types and tissues, and also in cancer cell lines and tumors of several origins. VDR downregulation has been observed in a proportion of melanomas and colon, breast, lung, and ovarian tumors, which may jeopardize the response to therapy with vitamin D, 1,25$(OH)_2D_3$, or its analogs. 1,25$(OH)_2D_3$, and its synthetic analogs, is therapeutic efficacy in skin diseases involving

част от меланоми и дебело-чревен, на млечната жлеза, белодробен, и на яйчниците тумори, можещи да компроментират отговорът при терапия с витамин D, $1,25(OH)_2D_3$, или налозите му. $1,25(OH)_2D_3$, и синтетичните му аналози, е терапевтично ефикасен в кожни заболявани, включващи дефектна кератиноцитна диференциация, като псориазис, себореен дерматит, и ихтиоза. Промотиране на епидермалната диференциация от ВДР лигандите осигурява също механистична база за потенциалната им употреба при лечението на актинова кератоза, сквамозен клетъчен карцином в лицево-челюстната област (СЦКЛЧО), базален клетъчен карцином (БЦК) и меланом.

Така наречените не-геномни механизми на бърз витамин Д отговор бяха описани наскоро. Тези механизми не засягат пряко ВДР, неговата експресия, генната експресия на таргетни протеини или изисква допълнителен протеинов синтез.

Ключови думи: Витамин D_3, ВДР, Wnt/β-катенин, възпаление, БЦК, СЦК, меланом, Кожен рак, Рак в Лицево-Челюстаната област

defective keratinocyte differentiation, such as psoriasis, seborhheic dermatitis, and ichthyosis[2]. Promotion of epithelial differentiation by VDR ligands also provides a mechanistic basis for their potential use in the treatment of actinic keratosis, head and neck squamous cell carcinoma (HNSCC), basal cell carcinoma[3,4,5,6] (BCC) and melanomas.

So-called, non-genomic mechanism of rapid vitamin D response has been described recently. This mechanism does not directly affect VDR, its expression, gene expression of target proteins or require additional protein synthesis[7,5].

Keywords: Vitamin D_3, $1,25(OH)_2D_3$, VDR, Wnt/β-catenin, inflammation, BCC, SCC, melanoma, Skin cancer, Head and Neck cancer

Introduction:

A common feature of chronic inflammatory skin disorders such as psoriasis, atopic dermatitis, and allergic contact dermatitis, is epidermal hyperplasia and thickening, a phenomenon attributed to leukocyte-derived cytokines such as tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ), which are potent inducers of EGF (Epidermal Growth Factor) family growth factors and EGFR (Epidermal Growth Factor Receptor). In the course of T cell driven skin inflammatory diseases, activated Th1 lymphocytes infiltrating the dermis and the epidermis are the major source of IFN-γ and TNF-α. Among the various leukocyte subsets, Th1 lymphocytes dominate psoriatic and allergic contact dermatitis lesions, but they are present also in chronic atopic dermatitis. These cytokines initiate a program of increased keratinocyte expression of inflammatory mediators, including adhesion molecules, cytokines, and chemokines. In particular, prominent keratinocyte expression of CCL2 (monocytechemoattractant protein 1, MCP-1), CCL5 (RANTES), CXCL8 (IL-8), and CXCL10 (IFN-γ-induced protein of 10kd, IP-10) is a common finding in T cell-mediated skin diseases, and mediates the recruitment of T cells and other leukocyte populations in the skin [8,9].

Pastore S. *et al.* found that during their early response to TNF-α or IFN-γ (leukocyte and Th1 derived), keratinocytes release EGFR ligands including TGF-α, which induce EGFR autophosphorylation and as a consequence activate its signal transduction cascade. EGFR activation leads to the persistent induction of the classical MAPK pathway identified as ERK 1 and 2 (ERK1/2 - Extracellular signal Regulated Kinase 1/2), which plays a fundamental role in the EGFR-driven control of epidermal proliferation. In contrast, the other major subgroups of the MAPK (Mitogen

Activated Protein Kinases) family, namely p38α and β and the JNK/stress-activated protein kinase 1 and 2 (JNK1/2), are weakly activated by EGFR (HER1, ErbB1),whereas they are highly stimulated on exposure to TNF-α. EGFR modulates skin inflammation via an ERK1/2-dependent mechanism, by affecting some chemokine expression in keratinocytes. ERK1/2 is involved in the regulation of a number of potent proinflammatory chemokines in epidermal keratinocytes in vivo, and suggest that it is part of a homeostatic mechanism that tends to oppose skin inflammation [9].

EGFR ligands provide the main extracellular signals for high steady state ERK1/2 activity, which in turn drives intracellular proproliferation and prosurvival programs. Pastore S *et al.* found that ERK1/2 activity strictly depended on EGFR signaling in skin keratinocytes, with selective EGFR inhibition suppressing constitutive ERK1/2 and preventing its activation by TGF-α or the proinflammatory cytokines TNF-α and IFN-γ. Of note, the authors uncovered the existence of an EGFR-mediated differential regulation of cytokine-driven chemokine expression in these cells, with higher levels of EGFR activation associated with enhanced **CXCL8** (IL-8; interleukin-8), but suppressed CCL2, CCL5, and CXCL10 expression, whereas opposite events were registered when EGFR function was blocked. Impairment of ERK1/2 obtained either by EGFR or MEK1/2 inhibition was similarly associated with down-regulated CXCL8 (IL-8) and enhanced TNF-α-driven CCL2, CCL5, and CXCL10 in skin keratinocytes [9].

The same authors also found that ERK1/2, JNK1/2, or p38αβ selective inhibition each impaired AP-1 *trans*-activation, whereas JNK1/2 or p38αβ inhibition also reduced NF-κB *trans* activation through IκBα-independent mechanisms. This last finding could help to explain current and previous evidence that JNK1/2 or p38αβ inhibition decreases the promoter activity of proinflammatory genes known to strictly depend on NF-κB *trans* activation, such as CCL2 and CCL5. **Only CXCL8 promoter activity could be impaired by ERK1/2 inhibition,** confirming that ERK1/2-driven AP-1 trans activation plays a relevant role in its transcription [9].

Anti-inflammatory

Animal studies have linked the anti-cancer effects of 1α,25(OH)2D3 to its ability to regulate inflammation. In colon cancer cells, $1\alpha,25(OH)_2D_3$ can interrupt the Wnt-mediated crosstalk between tumor epithelial cells and macrophages in the tumor microenvironment by blocking the production of IL-1β, an inflammatory cytokine produced by tumor-associated macrophages. $1\alpha,25(OH)_2D_3$ up-regulated the expression of NAD+-dependent 15-hydroxy-prostaglandin dehydrogenase (15-PGDH - prostaglandin-degradating enzyme, potentiates tissue regeneration in multiple organs in mice) gene and down-regulated cyclooxygenase-2 (COX-2; PGHS-2 - Prostaglandin G/H Synthase) expression. Since prostaglandins are known to play a role in the development and progression of many cancers the ability of $1\alpha,25(OH)_2D_3$ to decrease prostaglandin concentration strongly suggests that one mechanism of anti-cancer effect of vitamin D may be mediated through its anti-inflammatory action [7,10].

$1,25(OH)_2D_3$ induced the expression of monocyte/macrophage differentiation marker CD14, and lipopolysaccharide binding protein CAP18, highlighting the role of keratinocytes in innate immunity. CD14 has previously been described as a vitamin D-responsive gene in HL-60 promyelocyticleukemic and SCC25 cells (spinocelllular carcinoma cell line). The expression of IL1 receptor like 1 (IL1RL1; T1/ST2) gene is induced by $1,25(OH)_2D_3$ in keratinocytes and SCC25 cells. As IL1RL1 gene disruption leads to a defect in T-helper type 2 (Th2) differentiation, vitamin D-mediated induction of its expression suggests that the shift of balance from pathogenic Th1 (IL-2-, IFN-γ-, and TNF-α secreting T cells [11]; IL-6 and IL-8 [12,3]), responsible for the exacerbation of the skin inflammation, to non-pathogenic (anti-inflammatory) Th2 (IL-4-, IL-5-, and IL-10-producing T cells) phenotype in psoriasis by VDR ligands may involve a direct role of keratinocytes in Th2 differentiation [11]. $1\alpha,25(OH)_2D_3$ the natural ligand of VDR enhances expression of anti-inflammatory cytokine, IL- 10, within the psoriatic lesions, as well as the expression of its receptors in keratinocytes [12,3].

Calcitriol (v.D_3, 1,25$(OH)_2D_3$) and its analogs attenuate epidermal inflammation and inhibit the hyperproliferation of keratinocytes associated with the inflammatory disorder, psoriasis. Since activation of extracellular signal-regulated kinase (ERK) promotes keratinocyte proliferation and mediates epidermal inflammation, the effect of calcitriol on ERK activation in HaCaT keratinocytes exposed to the ubiquitous inflammatory cytokine TNF was studied. Ziv E. *et al.* established that TNF activated ERK in an EGFR and Src dependent and an EGFR and Src independent modes. EGFR dependent activation resulted in the upregulation of the transcription factor, c-Fos, while the EGFR independent activation mode was of a shorter duration, did not affect c-Fos expression, but induced IL-8 mRNA expression. Calcitriol, enhanced TNF-induced EGFR-Src dependent ERK activation and tyrosine phosphorylation of the EGFR, but abolished the EGFR-Src independent ERK activation. These effects were mirrored by enhancement of c-Fos and inhibition of IL-8 induction by TNF. Treatment with calcitriol increased the rate of the de-phosphorylation of activated ERK, accounting for the inhibition of EGFR-Src independent ERK activation by TNF. It is possible that effects on the ERK cascade contribute to the effects of calcitriol and its synthetic analogs on cutaneous inflammation and keratinocyte proliferation [13,5].

Sustained activation of ERK signaling is involved in cell cycle progression, cellular transformation and differentiation. After ligands bind to the receptor tyrosine kinases (RTKs) and Ras is activated, members of the Raf family are recruited to membrane-associated activators. Raf then activates mitogen-activated protein kinase kinases 1 and 2 (MEK1/2), which in turn phosphorylate and dissociate from ERK1/2. Activated ERK1/2 phosphorylate targets at the membrane and in the cytoplasm, such as ribosomal S6 kinases (RSKs), and a portion of active ERK1/2 translocates into the nucleus. Active RSK1/2 also translocate into the nucleus where, together with nuclear RSK isoforms (RSK3/4, MSK1/2) and ERK1/2, they phosphorylate and activate several nuclear targets such as the transcriptional regulators Ets, STAT, CREB and histone H3. This results in the rapid induction of immediate early genes (IEGs) including c-fos. If signaling remains active after the IEG-encoded protein products are translated, the sustained signal can lead directly to phosphorylation of the IEG products and can prolong their expression and activity in the nucleus for several hours. If signaling is transient, the IEG products are unstable and are degraded by the proteasome [14]. In addition to increasing stability, the C-terminal modification of c-Fos (Ser362 and Ser374) by ERK and the ERK-regulated protein kinase RSK is established. In addition to stabilizing the c-Fos protein, these phosphorylations act as a priming event that permits the access of ERK to the DEF domain in c-Fos and leads to the phosphorylation of further sites (Thr325 and Thr331) which contribute to c-Fos transcriptional activity [15]. The IEG products (Fra-1, Fra-2, and c-Myc are other DEF domain-containing IEG products, which can act as sensors of ERK activity [15])) appear to be highly sensitive as they can detect relatively small changes in ERK signal strength, however, they may also act as gatekeepers by only allowing efficient DEF domain-dependent phosphorylation of transcription factors when ERK activity reaches a threshold strength or duration. It is proposed that these transcription factors may contribute to sustaining nuclear ERK activation by retaining the active ERK in the nucleus via DEF domain binding and by preventing the interaction of ERK with MAPK phosphatases [15]. On the basis of these observations, the authors propose that the DEF domain and phosphorylation of Thr325 and Thr331 are required to drive c-Fos into an activated state (during sustained ERK1/2 signaling) [14].

Crucially, the expression kinetics of IEG (Immediate Early Genes) -encoded protein products, such as c-Fos, is marked in response to agonists that induce sustained rather than transient ERK and RSK activation [14]. c-Fos protein is very unstable (half-life, t1/2≈30 min) and will accumulate only if its C terminus is phosphorylated under conditions of sustained ERK activation. Because c-Fos is an important component of the dimeric AP-1 transcription factor, an increase in its stability results in greater promoter occupancy and

expression of target genes (L.O. Murphy and J. Blenis, unpublished) and in cellular transformation. Accordingly, expression of the late-response gene Fra-1 (according other authors IEG product [15], connected with both v-rasHa keratinocytes driven keratinocytes proliferation and tumorogenesis [16] and Cholesterol sulfate (activate also PKCη [17] (Protein Kinase Cη)) induced involucrin expression [18]), a target of c-Fos, is sustained only after prolonged expression of c-Fos. Thus, the behavior of c-Fos after transient or sustained ERK signaling enables the cell to distinguish among agonists that induce different activation kinetics. Therefore, by its ability to exist in unstable and stabilized states, the c-Fos transcription factor can function as a 'sensor' for ERK activation dynamics [14]. And therefore upregulated c-Fos expression as a result of increased Vitamin D_3 concentrtation is a probable consequence of sustained ERK1/2 activity in keratinocytes.

It has been known for some time that the duration and strength of MAPK signaling can regulate distinct cell fate decisions. For example, in PC12 pheochromocytoma cells the sustained activation of ERK leads to differentiation while transient ERK activation promotes proliferation. Correlations between the duration of ERK signaling and cell behaviour have also been uncovered in other cell types. In fibroblasts sustained ERK activity is required for cell cycle re-entry and proliferation. This occurs via the expression of proteins required for cell cycle re-entry such as cyclin D1 and by the repression of anti-proliferative genes [15].

Further, in normal skin, Fos proteins were found to be abundant in the suprabasal regions with weak expression of Fos members in the basal layer [15]. c-fos expression is reduced in psoriatic lesional skin compared with normal epidermis [4]. Vitamin D-mediated regulation of c-fos expression was confirmed by Q-PCR, where 6 h treatment of KerTr (immortalized keratinocytes) and NHEK (normal human epidermal keratinocytes) with $1,25(OH)_2D_3$, showed 3–5-fold induction in its expression. Its expression was also induced at 24 and 48 h time points in KerTr cells [4]. AP-1factors consist of homo- or hetero-dimers of jun (c-jun,junB, junD) and fos (Fra-1, Fra-2, c-fos, fosB) family members, and, depending on the dimer composition, these factors can function as either activators or suppressors of transcription.The AP-1 binding sites of different promoters are not identical and may bind different AP-1 homo- and heterodimers,which in turn could account for their different regulation [19,16]. In addition to c-Fos and c-Jun, Fra-1, Fra-2, Jun B and Jun D are found in keratinocytes, and their distribution in the epidermis is both cell- and species-specific [20].

JunB, JunD, c-Fos and Fra1 are found to bind both of the proximal and distal AP-1 sites in the involucrin gene promoter region. Gel-shift analyses demonstrate a strong binding of the proximal AP1 site by JunB and Fra1 **in proliferating keratinocytes.** As keratinocytes differentiate, c-Fos replaces JunB and Fra1 to stimulate involucrin expression [21]. Induction of c-fos by $1,25(OH)_2D_3$ may help in normalization of differentiation observed by the treatment of psoriatic lesions by VDR ligands [4].

Fos staining was generally weak in chemically induced papillomas except for focal areas of intense nuclear staining that were seen in the basal and suprabasal regions. In the carcinomas that arose from the papillomas, intense nuclear Fos staining was seen throughout the tissue in all samples analyzed, demonstrating that Fos proteins are up-regulated *in vivo* during the process of skin carcinogenesis [15].

Rutberg SE *et al.* have shown that c-*fos* plays an obligate role in the development of skin cancers in mice, that c-*fos* is required for v-*ras*Ha keratinocytes to form tumors when grafted to the backs of nude mice, that v-*fos* can restore tumorigenic potential to c-*fos* null v-*ras*Ha keratinocytes, and that the expression of v-*fos* together with v-*ras*Ha is sufficient to transform primary mouse keratinocytes into cancer cells. In addition, the expression of Fos proteins increases during the malignant progression of skin tumors in mice. This may correlate with the induction of c-Fos, Fra-1, and DFos B seen in v-rasHa keratinocytes. They provide evidence that certain changes in marker gene expression that occur in v-*ras*Ha keratinocytes are mediated by AP-1 proteins. Use of a dominant-negative Fos construct pro-

vided evidence that AP-1 proteins suppress K1 (keratin 1) and K10 (keratin 10 – early differentiation spinous markers) in v-rasHa keratinocytes [16].

Inflammation and proliferation are regulated by a plethora of transcription factors, with nuclear factor-kB (NF-kB) considered to be a master regulator of these processes. NF-kB is also important in the development, prevention and therapy of cancer. NF-kB activity is stimulated by many pathways that converge on IkB kinases, including the signaling pathways activated by various cytokines, such as the proinflammatory cytokine IL-1, lipopolysaccharide (LPS) and tumor necrosis factor α (TNF-α). In mammals, the NF-kB family of proteins includes NF-kB1 (p105 processed to p50), NFkB2(p100 processed to p52), RelA (p65), RelB and cRel. A crucial negative regulatorthat controls NF-κB activation is the inhibitor of κB (IκB), which binds to p65 in the cytosol to block the nuclear translocation of the p65/p50 heterodimer. Phosphorylation of IκB by activated IκB kinase (IKK) [2] initiates the ubiquitylation and eventual proteasomal degradation of IκB, and a direct consequence of IκB degradation is nuclear entry of p65/p50 to transactivate specific gene expression. Thus, IKK plays an essential role in NF-κB activation. The kinase activity of IKK depends on the formation of the IKK complex by the IKKα, β, and γ subunits,which is activated upon phosphorylation by growth factors, proinflammatory cytokines (such as TNFα), and hormones through the TNF receptor or Toll-like receptor superfamily. IKK also phosphorylates p65 to promote its activity [22].

1,25-Dihydroxyvitamin D ($1,25(OH)_2D_3$) is known to suppress NF-κB activity, but the underlying mechanism remains poorly understood. Vitamin D receptor (VDR) physically interacts with IκB kinase β (IKKβ) to blockNF-κB activation. $1,25(OH)_2D_3$ rapidly attenuates TNFα-induced p65 nuclear translocation and NF-κB activity in a VDR-dependent manner. VDR overexpression inhibits IKKβ-induced NF-κB activity. GST pull-down assays and coimmunoprecipitation experiments demonstrated that VDR physically interacts with IKKβ and that this interactionis enhanced by $1,25(OH)_2D_3$. This interaction was not altered substantially by the presence of 1,25(OH)2D3, consistent with the above observation that, at high concentrations, VDR suppressed NF-κB even in the absence of 1,25(OH)2D3. Protein mapping reveals thatVDR-IKKβ interaction occurs between the C-terminal portions of the VDR and IKKβ proteins. Reconstitution of *VDR*$^{-/-}$ cells with the VDR C terminus restores the ability to block TNFα-induced NF-κB activation and IL-6 upregulation.VDR-IKKβ interaction disrupts the formation of the IKK complex and, thus, abrogates IKKβ phosphorylation at Ser-177 and abolishes IKK activity to phosphorylate IκBα. Consequently, stabilization of IκBα arrests p65/p50 nuclear translocation. Together, these data define a novel mechanism whereby $1,25(OH)_2D_3$-VDR inhibits NF-κB activation [22].

In HEK293 (human embryonic kidney; the cells contained Adenovirus 5 DNA, forms tumors in nude mice) and RAW264.7 (mouse; Abelson murine leukemia virus-induced tumor), $1,25(OH)_2D_3$, by rapidly inducing VDR-IKKβ association, blocks IKK complex formation and, hence, IKKβ phosphorylation, abolishing the IKK enzymatic activity to phosphorylate IκBβ and to activate NF-κB. VDR-/- cells with the C terminus of hVDR to a physiological level is sufficient to block TNFα induction of NF-κB activity and IL-6 expression [22].

Since nuclear factor-kB (NF-kB) plays a pivotal role in the regulation of cell proliferation, differentiation and apoptosis, Janjetovic Z *et al.* examined the capability of 20-hydroxycholecalciferol to modulate the activity of NF-kB, using 1,25-dihydroxycholecalciferol (calcitriol) as a positive control [23]. The side chain of vitamin D_3 is hydroxylated in a sequential manner by cytochrome P450scc (CYP11A1) to form 20-hydroxycholecalciferol, which can induce growth arrest and differentiation of both primary and immortalized epidermal keratinocytes. A new pathway for metabolism of vitamin D and pro-vitamin D that is catalyzed by cytochrome P450scc (CYP11A1), the enzyme catalyzing the conversionof cholesterol to pregnenolone for steroid hormone synthesis. The hydroxyl group of $20(OH)D_3$ is attached at the C20

position; which is interesting since the attachment at C1 is considered to be required for full biological activity and calcemic effects. $20(OH)D_3$ could have systemic effects when produced in organs expressing high levels of P450scc, such as adrenal cortex, corpus luteum, follicles and placenta, while in organs expressing low levels of P450scc, such as skin [23,32].

20-hydroxycholecalciferol inhibits the activation of NFkB DNA binding activity as well as NF-kB-driven reporter gene activity in keratinocytes. Also, 20-hydroxycholecalciferol induced significant increases in the mRNA and protein levels of the NF-kB inhibitor protein, IkBα, in a time dependent manner, while no changes in total NF-kB-p65 mRNA or protein levels were observed. Another measure of NF-kB activity, p65 translocation from the cytoplasm into the nucleus was also inhibited in extracts of 20-hydroxycholecalciferol treated keratinocytes. Increased IkBα was concomitantly observed in cytosolic extracts of 20-hydroxycholecalciferol treated keratinocytes, as determined by immunoblotting and immunofluorescent staining. In keratinocytes lacking vitamin D receptor (VDR), 20-hydroxycholecalciferol and $1,25(OH)_2D_3$ did not affect IkBα mRNA levels, indicating that it requires VDR for its action on NF-kB activity. Comparison of the effects of calcitrol, hormonally active form of vitamin D_3, with 20-hydrocholecalciferol show that both agents have a similar potency in inhibiting NF-kB and also increases IkBα protein levels through induction of IkBα mRNA expression (no statistically significant difference), forming transcriptionally inactive NF-kB/IkB complexes. Since NF-kB is a major transcription factor for the induction of inflammatory mediators, these findings indicate that 20-hydroxycholecalciferol, caltriol and its analogues may be an effective therapeutic agent for inflammatory and hyperproliferative skin diseases. Since recent studies demonstrate that activation of the alternative NF-kB pathway can also lead to the translocation of p65-containing dimers into the nucleus (including Protein Kinase D1 (PKD1), PKD is a mediator of NF-κB induction in a variety of cells exposed to GPCR agonists except the oxidative stress), the authors data cannot exclude the possibility that $20(OH)D_3$ also blocks this signaling pathway as well. Moreover, $20(OH)D_3$ has significant biological activity in human keratinocytes, as it inhibits their proliferation and stimulates their differentiation [23]. Vitamin D analogues are now widely used drugs for the treatment of psoriasis, an inflammatory hyperproliferative dermatoses and cancer [23,5].

NF-kB regulated genes play important roles in inflammation, immunity, cell growth, cell survival and oncogenesis [23,24,25]. NF-kB plays an important role in protecting keratinocytes against apoptosis during programmed cornification. In normal human keratinocytes, $1,25(OH)_2D_3$ reduces NFkB DNA binding activity by increasing IkBα protein levels, which inhibits IL-8 production. A similar effect is also seen in murine macrophages. Effects of $1,25(OH)_2D_3$ on NF-kB that are not mediated by the VDR have also been reported for fibroblasts lacking the VDR [23].

Inhibitors targeting the NF-kB signaling pathway effectively suppress NF-kB activity, protect and relieve inflammatory symptoms, and induce apoptosis of tumor cells. NFkB represents an attractive drug target for therapy of inflammatory and autoimmune disorders, as well as for cancer. Thus, $20(OH)D_3$ is a new powerfull analog of vitamin D_3, could exert beneficial effects in inflammatory, autoimmune disorders and cancer [23,10,5].

Interleukin 8 signaling is angiogenic factor, interrupt of which by the hormone (vitamin D_3, $1,25(OH)_2D_3$) can also leads to the inhibition of endothelial cell migration and tube formation. It has been proposed that the inhibitory effect of $1α,25(OH)_2D_3$ on metastasis observed in the prostate and lung murine models may partially depend on its anti-angiogenic property. It should be noted that in vascular smooth muscle cells, $1α,25(OH)_2D_3$ was shown to upregulate VEGF mRNA [7], although in other cell types it represess its expression; in the bovine aortic endothelial cells; in wild type human cancer cells, but failed to suppress VEGF expression in HIF-1α knockout human colon cancer cells. Taken together, the anti-angiogenesis effect of $1α,25(OH)_2D_3$ in cancer cells is likely mediated by HIF-1/VEGF pathway. $1α,25(OH)_2D_3$

can also up-regulate mRNA levels of the potent anti angiogenic factor thrombospondin 1 in human colon tumor cells [7,5].

Tumor cells induce angiogenesis through a multistep process, called the „angiogenic switch", which ultimately tips the balance toward pro-angiogenic factors. HIFs can directly activate the expression of a number of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), VEGF receptors FLT-1 and FLK-1, plasminogen activator inhibitor-1 (PAI-1), angiopoietins, platelet-derived growth factor β, and matrix metalloproteinases MMP-2 and -9. There is considerable evidence that VEGF is a major tumor angiogenesis factor. Many tumor cell lines secrete VEGF *in vitro.* Several *in situ* hybridization studies have demonstrated that the VEGF mRNA is expressed in a vast majority of human tumors so far examined. Anti-VEGF monoclonal antibodies exert a potent inhibitory effect on the growth of many tumor cell lines in nude mice. Several inhibitors of the VEGF pathway have been approved by FDA for cancer treatment, which is a significant advance in the therapy of cancer. In addition, platelet derived growth factor (PDGF), fibroblast growth factors (FGFs) and Notch Delta-like ligand 4 (DLL4) have been reported to promote angiogenesis independent of VEGF. Other than the hypoxia-induced pathological angiogenesis, it has been proposed that deficiency in MMP-19 may contribute to an earlier onset of tumoral angiogenesis, in contrast to most MMPs that promote tumor progression [7].

Pro-differentiation role

Expression profiling and QPCR results could be distilled into a vitamin D-mediated keratinocyte/epidermal differentiation network, wherein many of the components were identified for the first time as vitamin D-responsive genes. The network involves seven major nodes or components that are directly regulated by the VDR ligand. These components are: (1) crosslinked proteins that give rise to the cornified envelope; (2) PADI family of enzymes (a family of Ca^{2+}-dependent enzymes that catalyze the post-translational deimination of arginine residues to citrullines. The degree of modification of arginines to citrulline residues directly correlates with the structural order of substrate. The differentiated cornified layer of the epidermis contains deiminated keratins (K1, K10, K14, and K5) and filaggrin4, thus suggesting a role for protein deimination during the final stages of epidermal differentiation.); (3) TGase I, the enzyme that crosslinks substrates to form the cornified envelope (late marker of keratinocytyes differentiation, expressed in stratum granulosum); (4) KLK family of serine proteases; (5) SERPIN B family members (serine proteinase inhibitor); (6) KLF4; and (7) c-fos (transcriptional factors). One of the most studied are: involucrin, cystatin EM, and SPRR1B (small-prolinerich protein 1B), TGase I, Kruppel-like factor 4 (KLF4), and c-fos [4].

Epithelial enriched transcription factor, KLF4, is highly expressed in the differentiating layers of epidermis and it is required for establishing epidermal permeability barrier. KLF4 showed $1,25(OH)_2D_3$-dependent induction in its expression in KerTr (immortalized keratinocytes) and NHEK (normal human epidermal keratinocytes) cells [4].

In microarrays, the expression of fos gene was induced 10-, 10-, and 4-fold, respectively, after 6, 24, and 48 h treatment with $1,25(OH)_2D_3$. Vitamin D-mediated regulation of c-fos expression was confirmed by Q-PCR, where 6 h treatment of KerTr and NHEK showed 3–5-fold induction in its expression. Its expression was also induced at 24 and 48 h time points in KerTr cells [4]. AP-1 factors consist of homo- or hetero-dimers of jun (c-jun, junB, junD) and fos (Fra-1, Fra-2, c-fos, fosB) family members, and, depending on the dimer composition, these factors can function as either activators or suppressors of transcription. The AP-1 binding sites of different promoters are not identical and may bind different AP-1 homo- and heterodimers, which in turn could account for their different regulation [19]. Further, c-fos expression is reduced in psoriatic lesional skin compared with normal epidermis. Therefore, induction of c-fos by $1,25(OH)_2D_3$ may help in normalization of differentiation observed by the treatment of psoriatic lesions by VDR ligands [4].

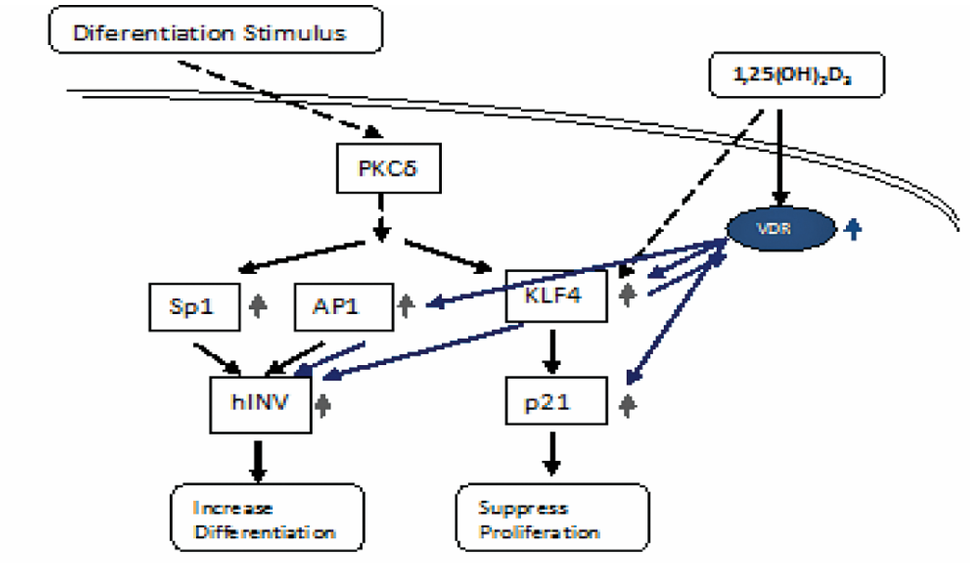

Figure 1: ***PKCδ and VDR, induced human involucrin (hINV) promoter activity, through specific binding elements of AP-1 and KLF4 promoter.***

Overexpression of KLF4 augments the PKCδ (Protein Kinase Cδ)-dependent increase in Iinvolucrin expression, whereas KLF4 knockdown attenuates this response [26]. Increased KLF4 expression significantly upregulated VDR (Vitamin D Receptor) expression and sensitized the cells to the inhibitory effects of 1,25(OH)$_2$D$_3$ (v.D3) [27].

Protein kinase C activation appears to be essential for the calcium-dependent induction of keratinocyte differentiation, whereas a protein-kinase-C-independent activation of activator protein 1 (AP1) DNA binding and keratinocyte differentiation is responsible for the 1α,25(OH)$_2$D$_3$-induced effects [28].

The cyclin-dependent kinase inhibitor p21waf1/cip1 (and p27 kip1) were suggested as a VDR target genes [7,27,PhD work in preparation].

Additionally, PKCδ coordinate regulation of keratinocytes differentiation and proliferation. PKCδ initiates a cascade the coordinately increases keratinocyte differentiation and suppresses keratinocyte proliferation. Active PKCδ increases AP1 and Sp1 transcription factor level and nuclear accumulation. These factors bind to the distal regulatory region of the hINV promoter to drive transcription and increase involucrin expression (i.e. differentiation). Active PKCδ also increases KLF4 levels and binding to GC-rich elements in the p21Cip1 proximal promoter where it acts to increase p21Cip1 expression to suppress proliferation [29; (modification of Chew YC et al., 2011 29)]. Additional: Figure 4 from Ivanova P and Dimov I, 2018 [10].

Now, we know that in cultured cells, administration of 1α,25(OH)$_2$D$_3$ or its analogs can regulate the expression of numerous genes that are associated with the differentiated cell of origin, and thus inhibit the processes critical for tumor growth and metastases. In VDR expressing SW 480-ADH human colon carcinoma cell line, 1α,25(OH)$_2$D$_3$ induces differentiation by promoting the expression of proteins implicated in adherent junction formation, including differentiation marker E-cadherin, and other adhesion proteins, such as occludin and vinculin. This process is mediated by the upregulation of Id1gene and down-regulation of Id2 gene in response to 1α,25(OH)$_2$D$_3$. In breast cancer cell lines, the induction of differentiation markers, such as E-cadherin, casein, lipid droplets, was also observed following 1α,25(OH)$_2$D$_3$ -induced growth arrest. E-cadherin is a member of the cadherin family of cell membrane adhesion glycoproteins that play an important role during cell migration. E-cadherin is expressed on the epithelial cells and forms the cell-cell tight junction. Decreased expression of this protein will cause a decrease of homotypic cell adhesion and increased cell migration and invasion, suggesting that E-cadherin may act as a tumor suppressor. In MCF-7 and HEC-1B endometrial cancer cells, Icb-1, a human gene product involved in differentiation processes

of cancer cells, has been shown to be a mediator of the 1α,25$(OH)_2D_3$ -induced up-regulation of E-cadherin expression. Hsu et al. demonstrated that 1α,25$(OH)_2D_3$ promoted prostate cancer cell aggregation by up-regulating E-cadherin expression, and therefore interfering their adhesion to microvascular endothelial cells and reducing their metastatic potential. In addition to colon cancer, E-cadherin is induced by 1,25$(OH)_2D_3$ or analogs in normal mammary and bronchial epithelial cells and in tumor cell lines derived from breast, prostate, non-small cell lung, and squamous cell carcinomas, usually associated with an increase in epithelial differentiation, a reduction in cell migration and invasion, and the inhibition of Wnt/β-catenin signaling. The mechanism of E-cadherin induction by 1,25$(OH)_2D_3$in human colon cancer cells is transcriptional indirect and requires the transient activation of the RhoA-ROCK-p38MAPK-MSK1 signaling pathway [30]. Thus, 1α,25$(OH)_2D_3$ -induced differentiation may be one mechanism responsible for inhibiting tumor growth and metastases [7].

The Wnt/β-catenin pathway is an evolutionarily conserved signaling cascade that plays key roles in development and adult tissue homeostasis and is aberrantly activated in many tumors. β-Catenin, one of the downstream signaling molecules of Wnt signaling pathway, can be found in three cell compartments: the plasma membrane, the cytoplasm, and the nucleus. The membrane-localized β-catenin is sequestered by the epithelial cell-cell adhesion protein E-cadherin to maintain cell-cell adhesion (inhibiting nuclear translocation), and the cytoplasmic accumulation of β-catenin and its subsequent nuclear translocation eventually leads to activation of Wnt target genes such as c-Jun, c-Myc, fibronectin, and cyclin D1 and TCF1 (transcription factor 1), CD44, MMP-7 (matrix metalloproteinase-7) [31] and and MT1-MMP (MMP-14) [65]. In the absence of Wnt ligands, cytoplasmic of β-catenin is phosphorylated by the destruction complex, consisting of axin, adenomatous polyposis coli (APC), glycogen synthase kinase-3β (GSK-3β) and casein kinase (CKI), recognized by E3 ubiquitin ligase β-Trcp for proteasomal degradation. In the presence of Wnt, GSK-3β is inactivated and β-catenin is stabilized, resulting in translocation into the nucleus and regulates the transcription of Wnt target genes, such as Snail and Slug [42]. Activation of canonical Wnt/ β-catenin signaling seems to play a role in SCC, specific histological BCC subtypes and melanoma. These subtypes include early stages of superficial BCC, pilomatricoma (a tumor of the hair follicle) as well as infiltrative BCC variants [32].

Four mechanism of influencing Wnt/ β-catenin activity by VDR have been established: 1/ liganded VDR binds to β-catenin protein to inhibit its nuclear translocation in colon cancer cells, thus blocking the transduction of the oncogenic signal of β-catenin to the nuclei. Detailed mapping studies revealed that the interaction between VDR and β-catenin occurs between the VDR activator function 2 (AF-2) domain of the VDR and the β-catenin C terminus [22].

2/ As it was mentioned in our previous paper studies in breast and colon cancer cells found that VDR, β-catenin, and Snail are interrelated. When VDR is activated, it will compete with β-catenin to combine with transcription factor 4 (TCF-4), thus inhibiting the activity of β-catenin in colon cancer. The molecular mechanism of the induction by 1α,25$(OH)_2D_3$ was further studied in LS180 colon cancer cells using chromatin immunoprecipitation-seq and gene expression analyses. It was found that VDR and RXR co-localized to VDRE sites in a ligand-dependent manner near a set of genes that included c-FOS and c-MYC. The expression of both c-FOS and c-MYC was modulated by 1α,25$(OH)_2D_3$. At the c-FOS gene, both VDR/RXR and TCF4/β-catenin bound to a single distal enhancer located 24kb upstream of the transcriptional start site. At the c-MYC locus, binding was at a cluster of sites between - 139 and -165 kb and at a site located -335 kb upstream (down-regulation), where both VDR and β-catenin activation was interlinked to basal and 1α,25$(OH)_2D_3$-inducible activities [26,10].

3/ 1α,25$(OH)_2D_3$ is known to regulate two genes encoding two extracellular Wnt inhibitors, DICKKOPF-1(DKK-1) and DICKKOPF-4 (DKK-4), in opposite directions; 1α,25$(OH)_2D_3$ up-regulates DKK-1 which

acts as a tumor suppressor in human colon cancer cells, whereas 1α,25$(OH)_2D_3$ down-regulates DKK-4, an oncogenic protein and a target of the Wnt/β-catenin pathway. Taken together, these data reveal complex models of action in the regulation of target genes from 1α,25$(OH)_2D_3$ [26,10].

4/ Many epidermal genes induced by WNT/β-catenin contain VDR response elements and were activated independently of TCF/LEF, implying that it is part of a TCF/LEF-independent aspect of WNT signaling. Likewise, depletion of follicular keratinocyte populations in VDR-null mice was linked to aberration of the canonical WNT pathway [33].

Additionally, 1,25$(OH)_2D_3$ reduces the expression of the mesenchymal marker N-cadherin and the myoepithelial proteins P-cadherin, integrin α6, integrin β4, and α-smooth muscle actin (α-SMA). Thus, 1,25$(OH)_2D_3$ reverts the myoepithelial features that are associated with more aggressive and lethal forms of human breast cancer. Likewise, N-cadherin expression is strongly suppressed by 1,25$(OH)_2D_3$ in mouse osteoblast-like cells. In line with these data, 1,25$(OH)_2D_3$ treatment blocks the EMT-associated cadherin switch (from E-cadherin to N-cadherin) in pancreatic cancer cells [30].

VDR, which activates CDH1/E-cadherin expression upon ligand binding, is repressed by Snail but induced by ZEB1. As ligand-activated VDR induces epithelial differentiation and the expression of CDH1 and other intercellular adhesion genes, VDR repression by Snail1 and Snail2 (Slug) guarantees the induction of EMT, even in the presence of 1,25$(OH)_2D_3$. This effect seems to be specific to the Snail family of transcription factors, since other EMT inducers such as ZEB1, ZEB2, E47, and Twist1 do not inhibit the human VDR gene promoter [27].

The hedgehog (Hh) gene was initially identified in fruit fly Drosophila and subsequently three homologs, Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh), were identified in vertebrates. In humans, the genes were found to be an essential developmental signaling pathway in maintaining tissue polarity and stem cell population. Inactivation or hyperactivation of this pathway can cause serious health problems, including developmental defects such as holoprosencephaly, and different forms of cancer including basal cell carcinomas (BCCs), medulloblastomas, leukemia, gastrointestinal, prostate, ovarian, breast and lung cancers. The receptor for Hhs is a transmembrane protein called Patched (PTC). In the absence of Hh ligands, PTC is bound to another transmembrane protein, smoothened (SMO), and functions as an inhibitor of SMO. The binding of Hh ligands to PTC releases SMO from the inhibitory effect of PTC and allows SMO to transduce signals leading to the activation of transcription factor, called glioma associated (Gli), and the expression of genes involved in regulating embryonic and postnatal development, and the transformation of cancer- and metastasis-initiating cells [7,32]. In our previous paper, it was mentioned studies indicate a cross-talk between vitamin D_3 and Hh (hedgehog) signaling mediated by at least two mechanisms [10].

First, PTC has been shown to stimulate the secretion of a vitamin D_3-related compound, which is likely responsible for the inhibitory action of PTC on SMO. Second, 1α,25$(OH)_2D_3$ can down regulate the expression of some members of the Hh pathway genes, including PTC, SMO and Gli in an epidermal explants culture system, suggesting a direct regulation by 1α,25$(OH)_2D_3$, that in turn decreases Hh signaling, leading to enhanced p21 and p27 expression and other actions, including rapid nongenomic responses. These results are in agreement with the increased expression of Shh in the keratinocytes of the VDR-null animal and hyperactivation of the Hh pathway, predisposing the skin to the development of both malignant and benign epidermal neoplasms [7,34].

The work by Uhmann et al. who demonstrated that 1α,25$(OH)_2D_3$ was capable of inhibiting Hh signaling at the level of SMO in the absence of VDR, and by Tang *et al.* who also found that 1α,25$(OH)_2D_3$-induced Gli expression in murine BCC cells was independent of VDR. Thus, in addition to the report by Wali et al, these two studies provide strong evidence of the non-genomic and rapid non-

VDR action of 1α,25$(OH)_2D_3$ on cell growth and differentiation [7,34].

Tang *et al.* who studied murine basal cell carcinomas (BCC) in vitro and in vivo, found that the effect of 1α,25$(OH)_2D_3$ on Gli expression is likely independent of VDR. The results provide strong evidence of the non-genomic action of 1α,25$(OH)_2D_3$ on cell growth and differentiation mediated by Hh/Gli signaling pathway [7,34].

We could not find data concerning Cystatin D expression in human epidermis (keratinocytes), influencing E-cadherin expression and EMT [10,5]. Another differentiation-specific protein, is cystatin EM, also a component of the cornified envelope that is highly expressed in differentiated epidermis [35], showed a vitamin D-dependent induction in its expression. Expression profiling of an HNSCC cell line also showed induction of cystatin EM by a synthetic vitamin D analog EB1089 [36,11,5]. EB1089 driving SCC25 cells toward a less malignant phenotype, similar to that of basal keratinocytes, performing three key functions of a cancer chemoprevention agent: antiproliferative, induces cellular differentiation, and has potential genoprotective effects [36].

Vitamin D signaling can suppress expression of genes regulated by c-MYC, a transcription factor that controls epidermal differentiation and cell proliferation and whose activity is frequently elevated in cancer. Salehi-Tabar R *et al.* show through cell- and animal-based studies and mathematical modeling that hormonal 1,25-dihydroxyvitamin D (1,25D) and the vitamin D receptor (VDR) profoundly alter, through multiple mechanisms, the balance in function of c-MYC and its antagonist the transcriptional repressor MAD1/MXD1. 1,25D inhibited transcription of c-MYC–regulated genes in vitro, and topical 1,25D suppressed expression of c-MYC and its target setd8 in mouse skin, whereas MXD1 levels increased. 1,25D inhibited MYC gene expression and accelerated its protein turnover. In contrast, it enhanced MXD1 expression and stability, dramatically altering ratios of DNA-bound c-MYC and MXD1. Remarkably, F-box protein FBW7, an E3-ubiquitin ligase, controlled stability of both arms of the c-MYC/MXD1 push–pull network, and FBW7 ablation attenuated 1,25D regulation of c-MYC and MXD1 turnover. Additionally, c-MYC expression increased upon VDR knockdown, an effect abrogated by ablation of MYC regulator β-catenin. c-MYC levels were widely elevated in *vdr*$^{-/-}$ mice, including in intestinal epithelium, where hyperproliferation has been reported, and in skin epithelia, where phenotypes of VDR-deficient mice and those overexpressing epidermal c-MYC are similar. Thus, 1,25D and the VDR regulate the c-MYC/MXD1 network to suppress c- MYC function, providing a molecular basis for cancer preventive actions of vitamin D [6].

Microarray results indicated downregulation of small proline-rich protein, SPRR1B expression. The expression of SPRR1B protein is increased significantly in psoriasis (Koizumi *et al.,* 1996) and it may contribute to abnormal keratinocyte differentiation in psoriatic lesional skin. Therefore, potent inhibition of SPRRIB expression by 1,25$(OH)_2D_3$ may contribute toward normalization of keratinocyte differentiation observed by VDR ligands in psoriatic skin more in: [4].

Discussion:

Vitamin D is well known as being essential for bone health. It is a group of fat-soluble prohormones, which are converted in the body into a number of biologically active metabolites [34] that function as true hormones, circulating in the blood and regulating the activities of various cell types - both calcemic and noncalcemic effects. Their known important action is the maintenance of plasma Ca^{2+} concentration by increasing Ca^{2+} absorption in the intestine, lowering its renal excretion and storage (deposit) of Ca^{2+} into the bones.

Cell responsiveness to 1,25$(OH)_2D_3$ mainly relays on VDR expression levels. The presence of VDR in cells other than those of the intestine, bone, kidney, and parathyroid gland led to the recognition of noncalcemic actions of VDR ligands [2]. Vitamin D_3 has a wide variety of actions in autoimmune diseases and cancer, as well as on bone physiology and blood pressure, keratinocytes and ovarian cells, improves muscle performance, neuroprotective role – VDR and CYP27B1 presented

in the hypothalamus and the dopaminergic neurons of the substantia nigra, improving Parkinson's disease patients. Recent study also suggests potential use of high-doses of vitamin D in prevention and treatment of preeclampsia in pregnancy [23,3]. $1,25(OH)_2D_3$ and its synthetic analogs therapeutic efficacy in skin diseases involving defective keratinocyte differentiation, such as psoriasis, seborhheic dermatitis, and ichthyosis [2]. Promotion of epithelial differentiation by VDR ligands also provides a mechanistic basis for their potential use in the treatment of actinic keratosis, head and neck squamous cell carcinoma (HNSCC), and basal cell carcinoma [4,5].

VDR protein is expressed in almost all normal human cell types and tissues, and also in cancer cell lines and tumors of several origins. Remarkably, elevated VDR expression is associated with high tumor differentiation, absence of node involvement, and good prognosis in colon cancer, with lower tumor grade, late development of lymph node metastases, and longer disease-free survival in breast cancer, and with improved overall survival in prostate and non-small cell lung cancer and melanoma. However, certain cancer cell lines do not express VDR and are unresponsive to $1,25(OH)_2D_3$. Accordingly, VDR downregulation has been observed in a proportion of melanomas and colon, breast, lung, and ovarian tumors, which may jeopardize the response to therapy with vitaminD, $1,25(OH)_2D_3$, or its analogs. These lines of evidence prompted the scientist to study the mechanisms responsible for VDR downregulation in cancer. It was found that SNAIL1 (a key transcriptional factor-TF in Epithelial to Mesenchimal Transition - EMT) represses the expression of VDR by binding to three E-boxes in the human VDR gene promoter [30].

VDR knock-out animals showed that the cells were completely resistant to $1\alpha,25(OH)_2D_3$-mediated *growth arrest* and *apoptosis* over the range of 0.01-100 nM $1\alpha,25(OH)_2D_3$. The expression of VEGF (a major tumor angiogenesis factor) related protein has been shown to be down-regulated by EB1089, a less calcemic analog of $1\alpha,25(OH)_2D_3$, function as a potent *anti-angiogenesis factor.* $1\alpha,25(OH)_2D_3$ induces *differentiation* by promoting the expression of proteins implicated in *adherent junction formation,* including differentiation marker E-cadherin, following $1\alpha,25(OH)_2D_3$ -induced growth arrest. Recently, effects of $1,25(OH)_2D_3$ on the expression of several EMT-TFs have been described. $1,25(OH)_2D_3$ inhibits SNAIL1 and ZEB1 expression in non-small cell lung carcinoma cells, accompanied by an increase in E-cadherin expression, vimentin downregulation, maintenance of the epithelial morphology, and inhibition of cell migration [32]. SNAIL1 and SNAIL2 (Slug) repress the expression of VDR by binding to three E-boxes in the human VDR gene promoter [30].

Consistently with its inhibitory effect on EMT, $1,25(OH)_2D_3$ downregulates the secretion of MMP-2, MMP-9, and MMP-13 in prostate, breast, pancreatic, and squamous cell carcinoma cells and increases TIMP1 and TIMP2 activity in prostate and breast cancer cells. In addition, $1,25(OH)_2D_3$ reduces the increase in MMP-2 and MMP-9 induced by TGF-β1 in human bronchial epithelial cells. Through these mechanisms, $1,25(OH)_2D_3$ inhibits the capacity of cancer cells to degrade the extracellular matrix and invade the surrounding tissue and may thus reduce tumor cell metastatic potential. Remarkably, several studies from Pŕerez-Fernŕandez's group have demonstrated that $1,25(OH)_2D_3$ represses the expression of the gene encoding the pituitary transcription factor 1 (PIT1) in breast cancer cells and that *PIT1* silencing downregulates SNAIL1, MMP-1, and MMP-13 proteins. In agreement with this, high PIT1 protein expression correlates with elevated MMP-1 and MMP-13 levels, SNAIL1 protein expression, and presence of distant metastasis in invasive ductal breast carcinoma [30].

Tokar *et al.* found that vitamin D3 exerted its anti-invasive effects by upregulating VDR and decreasing matrix metallopeptidase 9 (MMP-9) and matrix metallopeptidase 2 (MMP-2) activity [27], degrades type IV colagen in the Basal membranes [37,38,39] and major proteinases associated with increase invasive activity [40,41,42] (increased in both SCCs and BCCs [43,32]). Production of

MMP-9 and MMP-13 markedly increased when a human squamous cell carcinoma (SCC) cell line (DJM cells) were treated with TNF-α. Calcipotriol suppressed the production of MMP-9 and MMP-13 mRNA and proteins significantly, in a dose-dependent manner. Induction of MMP-9 by TNF-α was suppressed by an extracellular signal-regulated kinase (ERK) inhibitor but not by a p38 inhibitor, whereas induction of MMP-13 was inhibited by a p38 inhibitor but not by an ERK inhibitor. Calcipotriol inhibited the phosphorylation of both ERK and p38, showen by western blotting [44].

It has been demonstrated that invasive melanoma cell lines show higher MMP-9 expression and higher activity when compared to non-invasive cell lines. In primary melanomas, MMP-9 is variably expressed in radial but not in the vertical growth phase and the *de novo* expression seems associated with early invasion. MMP-2 was evident by immunohistochemistry in malignant melanoma lesions, but not in benign and atypical nevi. Corte *et al.* found an association between MMP-13 (collagenase-3) expression with mitotic index ($p = 0.002$) in cutaneous malignant melanoma (CMM) [45,46,5,10]. Benign lesions were consistently negative for MMP-13, whereas three of the ten in situ melanomas (30%) and 23 of the 51 invasive CMMs (45%) showed positive immunostaining for MMP-13 (couls be used as a cancer marker). However, these results did not show any significant association between tumoral MMP-13 expression and relapse-free survival in patients with invasive CMM [45].

Overexpression of VDR caused also the downregulation of stem cell markers, including c-Met and CD44 (stem cells marker, induced by β-catenine during EMT [32])), in PDAC (pancreatic ductal adeno-carcinoma) cells [27].

VDR (Vitamin D Receptor) ligands inhibit expression of pro-inflammatory cytokines produced by T lympho¬cytes, such as IL-2, IFN-γ, IL-6 and IL-8 (NF-kB binding sequence), which are re¬sponsible for the exacerbation of the skin inflammation. Apart from that, $1α,25(OH)_2D_3$ enhances expression of anti-inflammatory cytokine, IL-10, within the psoriatic lesions, as well as the expression of its receptor in keratinocytes [3].

In other tissues $1,25(OH)_2D_3$ down-regulates also a variety of genes, including IL-12, IL-8 (induced by MMP-8 in breast cancer), MCP-1 (CCL2), PAI-1, angiotensinogen, and microRNA-155 by blocking NF-κB activation. Therefore $1,25(OH)_2D_3$ suppression of NF-κB activation has great biological and pathological relevance [22], and could be used in the treatment not only of skin inflammatory and premalignant diseases, but also in the treatment of skin and Head and Neck cancer (BCC [47-50,44], SCC [11,6,35,10,5] and melanoma [51-55])(see below).

The skin is the major source of Vitamin D3 (cholecalciferol), and ultraviolet light (UV) is critical for its formation. Keratinocytes, the major cell in the epidermis, can further convert Vitamin D_3 to its hormonal form, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$, calcitriol]. $1,25(OH)_2D_3$ in turn stimulates the differentiation of keratinocytes, raising the hope that $1,25(OH)_2D_3$ may prevent the development of malignancies in these cells. Skin cancers (basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanomas) are the most common cancers afflicting humans. UV exposure is linked to the incidence of these cancers-UV is thus good and bad for epidermal health.

The effect of UVR on human basal cell carcinoma (BCC) epidemiology is complex-the incidence rises until approximately 30,000 hours of lifetime sunlight exposure and then plateaus. Makarova A *et al.*hypothesize that UVR has opposing effects on BCC carcinogenesis-stimulatory via mutagenesis and inhibitory via production of hedgehog-inhibiting vitamin D_3 (D_3). The authors found that UVR exposure of ionizing radiation-treated *Ptch1*$^{+/-}$ mice accelerates BCC carcinogenesis in male mice, in which UVR does not produce D_3. By contrast, in female mice, in which UVR does produce D_3, UVR fails to accelerate BCC carcinogenesis, thus mirroring the plateauing in humans. However, if D_3 production is attenuated in female mice by deletion of keratinocyte lathosterol 5-desaturase, then UVR accelerates ionizing radiation-induced BCC carcinogenesis. Congruently, chronic topical

application of D_3 inhibits ionizing radiation-induced BCC tumorigenesis. These findings confirm that UVR-induced production of D_3 in keratinocytes significantly restrains murine BCC tumorigenesis and demonstrate the counterintuitive conclusion that UVR has anti-BCC carcinogenic effects that can explain, at least in part, the complex relationship between exposure to UVR and BCC incidence [47].

One of the key molecular characteristics of BCC is the sustained activation of hedgehog signaling through inactivating mutations in the tumor suppressor gene patch (Ptch) or activating mutations in Smoothened [57,58]. Recent studies indicate that BCC progression involves a crosstalk between Hh signaling, vitamin D derivatives and the vitamin D receptor (VDR) signaling pathway. This has been demonstrated also in BCC cell lines, in which both vitamin D_3 and its active metabolite calcitriol (1alpha-25$(OH)_2D_3$) exert antitumor effects. Interestingly, the antitumor effects are mainly ascribed to an inhibition of Hh signaling. Furthermore, as evident from studies in VDR deficient mice, calcitriol may also repress the activity of Hh signaling in a VDR-independent fashion thereby establishing an additional inhibitory feedback on Hh signaling activity [58].

The active metabolite of vitamin D 1α,25-dihydroxycholecalciferol ($1,25D_3$) has exhibited broad-spectrum antitumor activity in xenograft animal models. Squamous cell carcinoma (SCC) or $1,25D_3$ -resistant variant SCC-DR cells were treated with $1,25D_3$. SCC cellular morphology and actin organization were altered by 10 nM $1,25D_3$. $1,25D_3$ inhibited SCC cell motility and invasion, which were associated with reduced expression and secretion of MMP-2 and MMP-9, and $1,25D_3$ promoted the expression of E-cadherin. These findings were not observed in SCC-DR cells. Knock down of E-cadherin rescued $1,25D_3$ -inhibited cell migration. Intravenous injection of SCC or SCC-DR cells resulted in the establishment of extensive pulmonary lesions in saline-treated C3H mice. Treatment with $1,25D_3$ resulted in a marked reduction in the formation of lung tumor colonies in mice that were injected with SCC cells, but not in mice that were injected with SCC-DR cells [44].

Skin cancers producing $1,25(OH)_2D_3$, containing ample amounts of the Vitamin D receptor (VDR), and responding to $1,25(OH)_2D_3$ with respect to induction of the 24-hydroxylase, but failing to differentiate in response to $1,25(OH)_2D_3$, have rised the question: Why? Another possible explanation may lie in the overexpression of the DRIP complex, which by interfering with the normal transition from DRIP to SRC as coactivators of the VDR during differentiation, block the induction of genes required for $1,25(OH)_2D_3$ -induced differentiation [56,10,34].

The incidence of malignant melanoma worldwide continues to grow despite the enormous advances in topical and systemic therapy. The well-known risk factors for malignant melanoma include sunburns and occasional sunbathing, whereas regular sunbathing is associated with a lower incidence. Besides DNA damage, exposure to the sun also results in the synthesis of vitamin D (cholecalciferol) in the skin, which contributes to over 90% of circulating Calcidiol (25(OH)D) in serum. Calcitriol inhibited human melanoma proliferation at 10 nM (evaluated in three melanoma cell lines(human and hamster)), while only calcidiol inhibited proliferation of hamster lines at 10 and 100 nM doses [51]. In a randomized study effect of vitamin D supplementation, as an adjuvant therapy, in the follow-up period after diagnosis and surgical resection of the primary tumor, has a protective effect on relapse of cutaneous malignant melanoma (CMM) was studied [54,55,64]. Supplementation with vitamin D or alternatively UV exposure may be regarded as an adjuvant for the treatment of many types of tumors (e.g. tumors of the colon, prostate, and breast) [53].

Reduced level or absence of VDR is associated with melanoma progression (melanogenesis can suppress the expression of the receptor (bad prognostic marker)), resulting in deteriorated survival of melanoma patients. Vitamin D suppresses a key tumour pathway in BCCs development — Hedgehog signalling pathway. $1α,25(OH)_2D_3$ also inhibits the growth of SCCs *in vivo* as well as *in vitro,* inhibiting Wnt/β-catenin signaling and inter-

fering with ERK1/2 signaling [5,59], connected also with development of melanoma. Although the *in vitro* and animal studies suggested that vitamin D may prevent development of BCCs and SCCs, additional studies on humans are needed to assess the suitability of topical or oral vitamin D_3 supplementa¬tion in chemoprevention of head and neck skin cancers. Administration of $1,25(OH)_2D_3$ to cancer patients is restricted by its hypercalcemic effects at the therapeutic doses, enforcing the development of several analogs that maintain the antitumoral properties but have less calcemic actions, including pancreatic cancer patients, organ which is thought not to be target for vitamin D action (https://www.clinicaltrials.gov/) [30,10,1].

The aim of the present work was to review the scientific data connected with the use of Vitamin D_3 and its analogues in treatment of inflammatory skin diseases, especially their potential use in treatment of Skin Head and Neck Cancers (HNC). VDR downregulation has been observed in a proportion of melanomas and colon, breast, lung, and ovarian tumors, which may jeopardize the response to therapy with vitaminD, $1,25(OH)_2D_3$, or its analogs. The major hurdle is the hypercalcemic side effect induced by administering a high dose of $1\alpha,25(OH)_2D_3$, that is necessary to exert the anti-tumor effects of vitamin D in humans. The potential of using less calcemic analogs of vitamin D with much higher potency than $1\alpha,25(OH)_2D_3$ for treating cancers still exists (Seocalcitol was with positive opinion from EMA for treatment of hepatocellular carcinoma and recommended from the producer for treatment also of pancreatic cancer, have showen cardiotoxic activities), especially in combination with other anti-cancer agents or immunomodulatory drugs [10]. Reports estimating, on molecular level, the effects of $1\alpha,25(OH)_2D_3$ or its analogues in treatment of HNC – BCC [47], SCC [3,33,36,44,48-50,60-62], melanoma [51-53] are published and additional studis in phase II and III are ongoing (BCC [47,63,57], SCC [44] and melanoma [55,64]).

Conflict of Interest Statement:
The author declare no conflicts of interests.

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Личност, благополучие и психично здраве

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Personality, well-being and mental health

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РЕЗЮМЕ:

Днес в психологичната наука здравето се разглежда като мултидименсионален конструкт, който обхваща не само негативните страни на човешкото съществуване, а и позитивното психично функциониране и благополучието на човека. Това обуславя важността на проблема за психичното благополучие, който проблем изразява една нова перспектива в психологията, насочена към изучаването на позитивния опит и разгръщането на човешкия потенциал. В този контекст в настоящата статия се търси комплексен подход за обяснение на предикторите и изясняване на механизмите, водещи до поддържането на високо психично благополучие при съвременния човек. Фокусът е върху отчитането на позитивите в човешките преживявания и възможностите за неспирно личностно развитие през цялостния жизнен път. Тези акценти очертават основното ни теоретично и емпирично допускане за развитийното съотношение между рисковите за психичното здраве фактори и протективните психологични ресурси на личността с оглед повишаването на благоприятните изходи от справянето с всекидневния стрес и неблагоприятните житейски обстоятелства. В този смисъл нашето намерение се заключава във верифицирането на необходимостта от преосмисляне на доминиращите в клиничната психология концепции за психопатологията, но също така и в изследване на конкретни възможности и начини за оптимизиране на стратегиите за психопрофилактика и психотерапевтична грижа.

Ключови думи: благополучие, психично здраве, личност, темперамент, характер

ABSTRACT

Today, in psychological science, health is seen as a multidimensional construct which includes not only the negative aspects of human existence but also the positive human mental functioning and well-being. This determines the importance of a problem of mental well-being that presents a new perspective in psychology directed at studying the positive experience and the development of human potential. In this context, this article searches for a comprehensive approach to explain the predictors and to clarify the mechanisms leading to the maintenance of high mental well-being in modern human. The focus is on registering the positives in human experiences and the opportunities for endless personal development through the whole life-time. These highlights outline our main theoretical and empirical assumption of the developmental ratio between risk factors for mental health and the protective psychological resources of the personality in order to increase the favorable outcomes of coping with everyday stress and unfavorable life circumstances. In this sense, our intention is limited to verifying the need to rethink the concepts of psychopathology that dominate clinical psychology, but also to examine specific possibilities and ways to optimize strategies for psycho-prophylaxis and psychotherapeutic care.

Keywords: well-being, mental health, personality, temperament, character

Introduction

The modern perspective is directed to an integrated approach to explain the predictors and to clarify the mechanisms leading to the maintenance of high mental well-being. The focus of attention is interactions on person-environment and the influence of various variables. We have chosen the perspective of subjective mental well-being (SMWB), which examines two components - cognitive (life satisfaction) and affective (feeling of happiness, positive and negative affects). Both components are subjective and represent the global cognitive and affective assessment of past, present and future (Diener et al., 1999).

Nature of mental health and subjective well-being

An examination in science of well-being has recently suggested the need to test outdated traditional concepts of healthy personality by recognizing character traits that support adaptation to current challenges to the survival of humanity (Cloninger and Zohar, 2011, Cloninger and Kedia, 2011). As I have described in previous work, Menssana Monographs (MSM), it is a miserable fact that war, greed and divisive propaganda dominate the world stage at the moment despite the incredible human ability for sympathy, generosity, and self-awareness (Cloninger, 2008). While people are able to treat the world as an unrestricted source that is indiscriminately used, it was necessary to consider self-directed and cooperative people as healthy, even at a low level of self-transcendence (Cloninger and Kedia, 2011). For example, Freud suggests that healthy people are those who can work and love; he considers spirituality as an immature, willing thinking (Freud, 1927). This concept of a well-organized character with a low level of self-transcendence is still the preferred social norm in western cultures (Josephson, 2012). Organized character is even offered as a description of a healthy person in the Diagnostic and Statistical Manual of Mental Disorders - 5 (Cloninger, 2010).

But, since 1986, human resource exploitation has exceeded the planet's ability to recover (Vakernagel, 2002). Therefore, the characteristic for healthy people needs to be reviewed to meet the need for people to live normally in line with the needs of humanity as a whole and the ability of the environment to support these needs. The changing world conditions reveal important advantages that the creative character with a high level of self-transcendence has over the organized character with a low level of self-transcendence.

Over the last few decades, there has been a strong revival in happiness research (or more generally referred to as subjective well-being, as is commonly referred to in the literature). Numerous theories have been proposed in an attempt to identify the reasons for this seemingly elusive state (for reference, see Argyle, 1987, Diener, 1984, Eysenck, 1990, Friedman, 1978, Meyers, 1992 Meyers and Diener, 1995, Veenhoven, 1994). The Economic forces (Juster and Stafford, 1985), the levels of activity (Cummings and Henry, 1961, Lehmann and associates, 1972, see also Csikszentmihalyi, 1975, 1990), levels of adaptation (Brickman and Cambell, 1971, see also Mihalos, 1985, Parduchi, 1984), the goals (Emons, 1986, Omoday and Wearing, 1990), the life events (Heidi and Wearing, 1989) and the mood factors (Costa and McCrae, 1980, 1984) - all of them were considered as determinants of happiness. Although most studies have examined how certain objective variables affect well-being, almost a century of research shows that objective circumstances, demographic variables, and life events are less closely related to happiness than intuition, or daily experience suggests that it has to be so (see Diener, 1984; Lubomirski and Ross, 1997a). For example, naturalistic studies show that even extreme events have a surprisingly low impact on subjective well-being (Brickman and associates, 1978). Such findings direct us to consider the significance of subjective processes in happiness. So, within the subjectivist tradition, scientists are not surprised that some people find themselves happy despite their personal obstacles, tragedies, or the lack of great love or fortune, and others are seen as unhappy although they are surrounded by all the comforts and benefits of life.

The current indicators of subjective well-being either evaluates one of its two compo-

nents (affective or cognitive), or they are one-component global evaluation tools that do not contribute to the study of psychometric characteristics. Therefore, respondents are asked to determine the levels of their positive and negative affects over a given period of time, or to assess the quality of their life in general. What is lacking in literature is an indicator of the overall „subjective happiness" – i.e. a global, subjective assessment of whether a person is happy or unhappy. Such an indicator will reflect a wider and more moral category of well-being and will affect more global psychological phenomena (Diener, 1994).

In a comparison of subjective criteria of well-being among different peoples and cultures, the situation is more complex. The data show that some forces can increase subjective well-being on a cultural level; these include gross national product (GNP), political freedom, social equality, social security, adequate citizen-official relationships, high levels of trust and effective public institutions. However, some forces can reduce subjective well-being on a cultural level: civil and international conflicts (military), the suppression of political opposition and the nondemocratic government (Triandis, 2000). According to the „Economist Intelligence unit" report (2005), more than 80% of variations in national well-being levels can be explained by nine determinants: GNP per person, life expectancy, political stability, divorce rate, social life, climate, unemployment rate, political freedom and gender equality. Of all these forces, most often studied in relation to subjective well-being is GNP. There are many studies comparing the GNP with the satisfaction of life or happiness in different countries. Although the results are controversial, most of these studies often find that there is no linear relationship between these two indicators for „national well-being", despite how high the value of correlation is. The typical picture of such interconnection shows an almost linear increase in subjective well-being with a rise in GDP at the bottom of the scale, but a drop is reported in the economic scale. Engelhardt and Klingeman (2000) compared GNP with happiness and life satisfaction (measured in the World Value Study 1997) in 65 countries. They found that at a GNP of 13,000 USD per capita, there is no significant connection between fortune and subjective well-being. Similar results have been repeated in some of the studies presented in the works of Boarini, Johansson and D'Ercolle (2006).) After analyzing this interconnection, Engelhardt and Klingeman (2000) concluded that the different levels of well-being are more closely connected with political institutions in society than with its economic development.

It is clear that people's well-being does not solely depend on economic prosperity, and therefore some authors argue that political decisions at organizational, corporate, and governmental levels should be more strongly influenced by the problems of people's assessing and feeling about their lives (Diener and Seligman, 2004). Since monitoring of subjective well-being has become a standard procedure in most developed countries, in 2003 the European Foundation for the Improvement of Living and Working Conditions launched a project to monitor the quality of life in Europe. The project included - 12 member states + Austria, Finland and Sweden (joined in 1995): EU-15 (Austria, Belgium, Denmark, Finland, France, Germany, Spain, Sweden, and Great Britain), 9 member states + Greece (joined in 1981): EU-10 (Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Malta, Poland, Slovakia and Slovenia) and countries candidates for accession: CK-3 (Turkey, Romania and Bulgaria). The monitoring project is directed toward a comparative analysis of trends in quality of life, detecting emerging issues and problem areas within an enlarged Europe, and providing a solid basis for EU politicians to promote improvements in the coming years (Feichi, Nolan and Wellen, 2003).

Despite the poor dynamics in life satisfaction and the intensity of positive affects, most studies show that mental well-being is preserved regardless of age (Veenhoven, 1984; Diener et al., 1985; Inglehart, 1990; Diener & Suh, 1998). A tendency has been reported that the intensity of positive affects decreases with age, with a slight increase in life satisfaction (Diener et al., 1999). In the conducted research, the age range varies widely and usually covers the life cycle of 20-25 years until the end of life and no effect of age is

reported. Variations are observed when comparing little age intervals of several years, as in teenage age the younger the age group is, the higher the values of life satisfaction and happiness are (Silgidjiyan, 1998; Diener & Suh, 1998, 2000).

The results of a number of studies show that gender does not influence subjective mental well-being as a whole and the cognitive and affective component separately (Diener, 1984, Diener et al., 1997, Diener & Oishi, 2000, Diener & Biswas-Dener 2001). For life satisfaction in various studies, only minor gender differences have been identified or not found at all (Ingenerhart, 1990, Diener et al., 1997, Diener et al., 1999). Concerning the affective component of mental well-being, close levels of emotional reactions have been found in both genders. The more expressed intensity of experiencing unpleasant emotions in women is compensated by the more intense joy, experienced in pleasant situations.

The impact of individual income on subjective mental well-being is traced in two lines - influence when comparing with others and influence when changing income. Income is related to subjective mental well-being to the extent that it meets the basic needs for food, shelter and clothing, and results show that it is only relevant in poor countries and in poor people in wealthy countries. Above a certain income threshold, the psychological well-being of people does not differ (Veenhoven, 1991; Diener & Oishi, 2000). In terms of income changes, the increase of the income does not automatically lead to an increase in subjective mental well-being, but the decrease in income leads to increased dissatisfaction (Diener et al, 1993; Diener, & Biswas-Diener, 2000, 2001).

The basic psychological theories, directed to the study of health as a multidimensional construct, which covers not only the negative aspects of human existence but also the positive mental functioning and well-being. These models seek to shift the traditional focus of psychological science to examine and study of deficit and the negative to positive in human experiences. The development of subjective well-being theories, where the research interest seeks to answer the question of what makes people happy focuses on finding comprehensive indicators of quality of life and developing recommendations for improving social policy. The empirical research that has been done (Diener, 2000) prove that people highly evaluate their subjective well-being or experiencing happiness when they experience many positive emotions and when they are engaged in interesting activities, they feel great pleasure and little pain and are satisfied with the lives they have. These results, in turn, lead to the acceptance of the three-component structure of the subjective well-being: life satisfaction; a positive affect and a negative one. What follows is registering the influence of positive and negative affect on the experiencing of subjective well-being and happiness and the consideration of interactive effects of the three components of subjective well-being. Particular attention has been paid to the research of the determinants of subjective well-being.

Other studies present the other major branch in the study of positive human health - the eudemonic tradition, which main purpose is to offer a clear operational definition of positive human functioning. A particular attention has been paid to the theoretical principles for defining positive human health derived from modern philosophical views and psychological concepts of human well-being. The role of emotions is also registered in understanding the interconnections between mind and body. The new moment in this dualistic tradition consists of the attempts of the representatives of the eudemonic paradigm to carry out the necessary reorientation according to them towards a study of the physiological substrates of the „positive states of the psyche", as most of the previous studies focus on the negative influences of the mind on the body and vise verse - the negative states of the body on the mind. At the next stage the detailed six-component model of the psychological well-being of K. Ryff is presented. His development (Ryff, 1989) begins with the revision of the classical concepts of human well-being and is based on a number of psychological theories of optimal aging, positive functioning and normal human development. Thus, as indicators of positive human health in

this orientation, the following indicators are outlined: autonomy, dealing with the environment, personal growth, positive relations with others, and purpose in life and self-acceptance. A key point in defining mental well-being is that it is examined as a dynamic multidimensional process of the overall life-time and not as a final condition. This process covers a number of intellectual, social, emotional and physical indicators that characterize the possibilities for realization of human potentials.

In this regard, one of the main hypotheses in the study of positive health concerns the relation between well-being and the optimal functioning of a number of physiological systems (Ryff& Singer, 1998; Singer & Ryff, 2001).

The results of research in this area confirm the expectation of the relationship between the experience of well-being and better physical health (Pressman & Cohen, 2003). Contemporary formulation of the problem of well-being - health interconnection presents a counterpoint to traditional medical health models, focusing mainly on disease and dysfunctions. Such results are in support of the thesis that interventions towards optimization of the experiencing of well-being, and are not only orientated to treat dysfunctions and illnesses that have already occurred, could contribute to maintaining a good overall health condition.

The last subpart in the well-being therapy (Fava & Tomba, 2009) - a modern clinical method, developed on the basis of Ryff's model for mental well-being, which shows good results in the treatment of depressive and anxiety conditions in combination with classical cognitive behavioral therapy. Researches of psychological endurance include focusing on the definitions and descriptions of this concept, the variables associated with it, its biological correlates and the possibilities for various interventions. In determining whether or not a person reveals a resilience profile and to what extent the two mandatory components to be taken into account are: (1) misfortune (i.e. high risk situation or threat) and (2) successful adaptation/competence. The misfortune is defined by assessing the negative circumstances in the individual's life, and adaptation - as a successful coping with the tasks of age growth. Thus, the general definition considers psychological endurance as an adaptation and effective coping despite the crash with trauma, difficulty or misfortune. The fact is that misfortune can occur at any moment of development and can have consequences that can potentially change its course both in the near and in the long term. This makes the life-time development perspective essential to a complete understanding of resilience (Masten & Wright, 2010). These grounds lead to the contemporary conceptualization of resilience, according to which individual qualities, family aspects and the surrounding social environment (as well as culture) together play a role in resilience, which means that it is a multidimensional construct. Therefore, in recent decades, within the framework of clinical psychology of health, personal resilience is seen as a predictor of: 1) mental and physical health; 2) coping with stress and recovery from life's misfortunes, and 3) successful adaptation to changes and experiencing well-being. In literature is accepted (Fredrickson, 1998, 2001; Tugade & Fredrickson, 2004; Demos, 1989; Werner & Smith, 1992; Kumpfer, 1999) that the necessary condition for the development of this psychological resource is the positive emotionality as a personal predictor of resilience. The fourth subpart of this paragraph attempts to present a psychological profile of the resilient people. The following factors have been identified, which are considered interactive, derived from different theories and resilience related frameworks (Inke et al., 2006):

- Good relations with other people and the capacity to obtain social support;
- Good cognitive and communicative skills;
- Talents or achievements that are valuable to a particular person or appreciated by others;
- Self-sufficiency (total expectation of competence), self-respect and hope;
- Sense of purpose;
- Religion or sense of belonging;
- Contribution to public life;
- Self-encouragement;
- Adapting ways of coping so that they fit the situation and the person;

• Positive emotion and humor;
• Active problem solving skills;
• Belief that stress can have a boosted effect;
• Flexibility: ability to adapt to change;
• Accepting negative feelings, growing through negative experiences;
• Dealing with stress, treating stress as a challenge;
• Access to and use of protection resources and processes;

The destructive indicators of mental health deal with the problem of depression - a clinical picture, consequences for society, for the individual and for his immediate environment. According to us, depressive symptoms do not always lead to the development of mental illness, but they disrupt the person's overall emotional tonus and impede the experience of subjective and mental well-being. We consider depression as a consequence of difficult life circumstances and specific interpretation or perception of these stressful changes and events in life. We assume that one of the important life circumstances to increase depression is chronic failure to deal with daily stress, which in turn means that one of the necessary conditions for improving mental health is coping with chronic stress and using strategies for successful adaptation. In this connection, we analyze in detail the Lazarus' psychological model for adopted stress, after briefly presenting the different definitions of the stress. Experiencing stress is as a challenge for the adaptability of the individual and the experience of prosperity). In the basis of Lazarus' theory lies the transaction model, which examines the relationship between the individual and the environment as part of a dynamic reciprocal system. Interactions between these two components of the system provoke changes in the individual's emotional condition. Cognitive assessments and coping are examined as transactional variables, meaning that they are not derived solely from the personality or just from the situation, but from their mutual influence. Cognitive assessment, along with the mobilization of resources and coping strategies, presents the main elements of the psychological stress model. A distinctive feature of this model is that emphasis is placed not on objective stressors but on cognitive and motivational processes, perception and assessment of the situation - if and how we deal with stressful events depends on the cognitive assessment as it depends on the essence and the intensity of the emotions we experience (Lazarus & Folkman, 1984). In turn, evaluation has direct effects on individual variations of mental health and self-assessment of physical health, on mental well-being, and life satisfaction, as it includes changing assessments of the significance and meaning of the life events that one experiences. The basic idea here is that coping evolves over time and involves multiple situations, behavioral, emotional and cognitive processes. It is the change and activation of the personality and his psychological and social resources that make it possible to personal development and maintenance of the usual level of well-being and life satisfaction (Lazarus & Folkman, 1984).

There has long been a heated debate on the quality of society and calls for social reform. During the last centuries, political philosophers have introduced in this debate a system that examines different standards for assessing the quality of society.

Different standards

One of the standards of good society is the happiness of its citizens. This principle is at the center of the „utilitarian" moral philosophy, and more precisely, of the „utilitarianism of the rules", which states that politicians should strive for a society that will bring the greatest happiness to the greatest number of citizens. This criterion is applied in practice in empirical studies of happiness, and in particular in surveys that compare the average level of happiness among different peoples, and try to discover the characteristics of the society behind the observed differences (Veenhoven, 1997, 2004).

Another standard used to assess the quality of a society is the degree of inequality among its citizens. This principle is the basis of a tradition of „egalitarian" moral philosophy that claims politicians should try to reduce inequality as much as possible. This criterion also applies to empirical social research, espe-

cially in international comparisons of equality of rights and income inequality.

Such given principles may come into conflict. Promoting happiness can be at the expense of social equality, and in this context, a standard objection to utilitarianism is that it legitimizes the suppression of minorities. Similarly, social equality can be to the detriment of happiness, and the failed communist experiment has shown that this it is so. As there is broad support for both principles, politicians need to look for alternatives to satisfy each of the above-mentioned traditions.

The need for a comprehensive indicator

This requires appropriate social indicators; politicians need to know which interventions will most likely serve both principles. It needs an indicator that combines happiness and equality in the field of research.

A similar problem exists in public health. A guiding principle in this area is to keep life as long as possible, and the successful realization of this principle is usually measured with average life expectancy. Another moral stimulus is to promote good health, which is usually measured by respondent disability research. These goals can also come into conflict because longevity can be attained at the cost of good health. People can be maintained alive but with poor quality of life, which is reflected in the need to fight health problems too long. Good health can, in some cases, be achievable, but at the expense of longevity, as its maintenance requires life-shortening therapies. How to find a balance between short, but in good health and long-lasting, but with health problems life? Politicians in this area needed a health indicator which to reflect an acceptable mix of these goals. In response, the World Health Organization (WHO) proposed a combined indicator called „Disability-adjusted life year", abbreviated DALY, which was first used as a criterion of health outcome when compared to national health systems worldwide (WHO 2002).

Personality, temperament and character as the basis of subjective well-being

In order to more accurately reveal the personality in the context of subjective well-being, we must answer the question: What is the temperament and character of a person, and do they have a relation to the matter under consideration?

The temperament is a common system of procedure and coexistence of individual psychic properties that define the mental activity of a person. The types of temperament are determined by the psychological characteristics of the properties: Sensitivity, Reactivity, Activity, Ratio Reactivity-Activity, Reaction Rate, Plasticity and Rigidity. The classical typology of temperaments is based on an ancient Hippocratic classification that describes four main human types according to „the predominant fluid in their body": sanguine person (blood), choleric (yellow bile), phlegmatic (lymph), and melancholic (black bile).

Most psychologists determine the temperament with an inherited genotype characterized by specific, characteristic features typical for the individual. In their study, Thomas and Chess describe individual differences in infants according to nine behavioral physiologically-reactive categories as part of overall rhythm, intensity of reactions, persistence and activity levels (10). According to the authors, from very early age, individual behavioral patterns and styles have been degraded and highlighted, influencing the environment in which they are situated (10).

There are different understandings, interpretations and studies in the field of temperament. Witkin and associates measure and define in a wide range of psychological systems such as temperamental differences - perceptive, cognitive, emotional and social. On the other hand, Shapiro describes several neurotic styles, according to experience and perception (10).

The temperament is a hereditary and more stable characteristic, while the character is a more adaptable and changing personality characteristic. According to Cloninger [4], the temperament as the basic essence of the personality includes four basic early-forming emotions - fear, aggression, affection, and persistence of behavior. These emotions are formed in response to external stimuli - threat (danger): novelty, reward, frustrative nonreward, respectively. According to him, the 4

temperament traits are changing during the life-time, due to their hereditary character. They also do not only reflect the temperament nature but also the structure of personality disorders diagnosed on the basis of immaturity [4,5,6,7,8,9].

The character is determined
by the social reactivity of the personality.

A number of researchers, including Freud, Jung, From, Adler and Olport, give different interpretations of character typology. Adler and Olport are opposed to the final typology, considering that the uniqueness of the personality cannot be determined by several meaningful expressions, but it is possible to be specified if all these subtle nuances of our spirituality are inferred and terminologically stated (10).

According to Bowbek, the character is the regulator of the ego and impulses, through concrete values - affection to others, emotional dedication to clear purposes, emotional control. He believes that in the presence of a characteristic disorder, we notice a deficit of these three characteristics (10).

The characteristic peculiarities of the personality in mutual commitment are called an integral character portrait. This portrait is based on information related to: age, social background and status, communication, relationships; peculiarities of the environment where the person has been educated and grown up (physical and mental aspect, parents).

Currently, in specialized literature, is examined that the healthcare sector, divided into the respective professional groups, is one of the most affected by the burnout syndrome. At the same time, there is no standardized model for detecting vulnerability to professional burnout. Existing standardized methods, such as Christina Maslach's test, only record the already irreversible changes in the functioning of the individual. Based on this ground, the high levels of distress and professional burnout among medical specialties at budgetary support are a cause for concern and research interest. The personality in its entity and in the context of the syndrome implies the clarification of the dynamic nature of future prevention programs. It is well-known that the personality is built in the process of our constant socialization; it is a dynamic structure, a combination of knowledge, skills and specific experience.

The creation of an eventual working model for a comprehensive assessment of the psycho-climate in healthcare organizations, which to be directed to early diagnosis of the burnout syndrome and its adequate prevention, according to the personality, will create a necessary discussion to change the existing conservative system. In our opinion, this model should consist of three dimensions:

- individual measurements of the organizational socio-psychological climate, which are a subject of another study;
- decrease of self-actualization as a key marker of increased vulnerability to professional burnout;
- functional adjustment to working environment according to the Katz model.

Connection between well-being and character profiles

Physical, mental and social well-being depends heavily on the profile of the peculiarities of the character of self-directedness (SD), cooperativeness (CO) and self-transcendence (ST), measured by the Temperament and Character Inventory (Cloninger, 2004; Cloninger 2010; Cloninger and Zohar, 2011; Josephson, 2011). Clinical characteristics and dynamics of development course for all possible combinations of these personality traits are described elsewhere (Cloninger, 2004; Josephson, 2012). Here, only the most and the least healthy configurations will be examined because of their crucial importance in determining what constitutes a healthy person under the current world conditions. When high and low extremes of these three traits of character are taken into consideration, the healthiest people have a high level of both self-directedness and cooperativeness, and the unhealthiest and the most immature are those with a low level in these two traits (Cloninger, 1993). Among these relatively healthy people, two types of profiles can be distinguished:

- „Organized" character means that people

have a high level of self-directedness (SD), a high level of cooperativeness (C) and a low level of self-transcendence (ST).

- „Creative" character means that people have a high level of self-directedness (SD), cooperativeness (C) and self-transcendence (ST).

„Creative" characters are typically happier than „organized" characters, but both have similar physical and social health in modern western societies (Cloninger & Zohar, 2011, Josephson, 2011).

The type „organized" character is typical for leaders and other successful people in western societies. People with an organized character are highly self-confident, creative, purposeful and responsible (in other words, with a high level of self-directedness). In addition, they are highly tolerant, helpful and forgiving (in other words, with a high level of cooperativeness). Finally, they have a low level of self-transcendence and therefore they are mainly concerned about their own interests and those who consider friends or partners with common goals and interests. They are often quite traditional, material and practical than being contemplative, intuitive or spiritual. As a result, organized character is a highly willfully practical and purposeful leader motivated by achieving personal goals. Paradoxically, a highly willfully „organized person" is often a social conformist who diligently manages his or her reputation because behind confidence there is an isolation that puts him or her in inconsistency with local social norms. Hence, the organized character resists radical changes and drastic alterations in the notion of life, preferring to maintain dogmatic beliefs and the status quo rather than challenging the faithfulness of the assumptions they relied on for their success in the past, thus confirming the description of „organized" (Cloninger, 2004).

On the other hand, the „creative" character is typical for positive philosophers and leaders of civilizations during the Renaissance and Enlightenment (Cloninger, 2004). People with creative character have the same opportunity for ingenious productivity and useful cooperativeness, such as those with an organized character, but they are also more intuitive and more contemplative, they are recognized in nature, humanity and probably in the divine or in the universe as a spiritual whole. The creative character is guided by a coordinated interest and is guided by its intuition to express their potential through self-awareness in harmony with others and nature. They are not eccentric, because of eccentricity itself, because they have developed harmony and unification. They are more tolerant to the ambiguity and uncertainty of organized characters, and are more receptive to radical changes in society when there is a realistic and innovative basis to be made, thus confirming the description of „creative" (Cloninger, 2004).

Self-transcendence is a necessary but not sufficient feature of well-being. It is remarkable that people are often unhealthy, unhappy and insincere when they notice that they have a high level of self-transcendence (Cloninger, 2010, Cloninger and Zohar, 2011). Such individuals are schizotypal with often dreaming thinking than with spiritually mature characteristics typical for the creative character. Therefore, this is the combination of a strong development of these three character traits that depict people who are healthy, happy and satisfied (Cloninger, 2004).

The importance of these three character traits is further proved by the findings of the „third wave in psychotherapy", which aims to address the limitations of earlier „behavioral" and „cognitive-behavioral" approaches (Cloninger, 2004, Cloninger, 2006; Cloninger, 2010). Cognitive-behavioral therapies are effective in helping self-transcendence and cooperativeness, but do not turn to self-transcendence. In contrast, psychotherapies from the third wave, such as Attentiveness-based cognitive therapy, Dialectical-behavioral therapy, and acceptance and liberation therapy, reduce weakness and improve physical, mental and social health by adding self-knowledge and related spiritual practices that support self-transcendence (Cloninger, 2004, Cloninger, 2006, Cloninger, 2010).

There have been established positive relationships between the high levels of life satisfaction and characteristics such as extraversion (Pavot, Diener and Fujita, 1990), self-assessment and optimism (Cha, 2003), pur-

posefulness and sense of control and more. At the other end of the spectrum, neuroticism is negatively related to life satisfaction (Diener et al., 1999). Consequently, a great part of the personality characteristics, influencing life satisfaction (such as extraversion, neuroticism, etc.) are part of the so-called a five-factor model for the personality. Heidegger notes that one of the common misconceptions of people is that the world exists for them and that they can use it and can destroy it in the name of their vital well-being. „Mitwelt" is the common or social „co-existence" of man, which includes the attitude towards his „place among the people" and the rules of „living like humans" with all the resulting human problems and emotions. Here are the social comparisons of delay, competition, and supremacy over the others. „Eigenwelt" is the inner world of everyone: self-awareness, self-acceptance, pursuit of self-understanding, and the intentions of personal development and change. Or said in another way, the vector directed inwards (Heidegger, M., 2005).

Subjective well-being is the center of many studies related to health, morbidity and life expectancy. The great research interest in this area is dictated by two reasons. Firstly, the positive psychological functioning at an early stage of life explains the successful old age and life expectancy. Secondly, the assessment of one's own well-being determines subsequent outcomes and the development of life regardless of objective variables such as income and absence of disease (Schmitt & Juchtern, 2001). Optimism and negative expectations are relatively stable variables that support or interfere with mental and somatic health. Optimism can be related to health via health habits and behaviors. Higher levels of optimism and life satisfaction are related to the orientation towards a healthier lifestyle (Steptoe, 2006; Ylostalo et al., 2002). On the other hand, subjective well-being is determined by optimism (Turkum, 2005). Taking control also contributes to health-related behaviors. Research has shown that it is a strong predictor of both healthy lifestyle and psychosocial adaptation toward chronic illness, which is connected to positive and optimistic attitudes (Lin & Tsay, 2005). The external localization of control leads to the use of passive strategies for coping with stress (Masters & Wallston, 2005) and is negatively related to social support. The latest is positively connected to internal control localization (Chen et al., 2001).

Well-being is a diverse subject and object of study for many disciplines such as psychology, philosophy, sociology and theology. The subject of well-being studies is, on the one hand, the concept of subjective well-being and, on the other hand, psychological well-being. In this book the subject is subjective well-being. It is part of the positive psychology, whose founder is Martin E. P. Seligman (11). As a starting point, he mentions three main tasks of psychology in the period before World War II: „To treat mental illnesses, to organize more productive and more fulfilling life for all people, to recognize and promote talent" (12). After World War II, the focus has shifted primarily towards mental illness and their treatment; the latest two tasks have fallen into oblivion (12). In the recent years, resources, strengths respectively skills and values, as well as quality of life, are once again the focus of attention and disease prevention is becoming increasingly important. „The goal of positive psychology is to begin to catalyze the change in the focus of psychology by dealing only with correcting the worst things in life so as to build positive qualities." (Page 5) Positive psychology is divided into three areas (12): prosperity and satisfaction (in the past), hope and optimism (for the future), ebulliency (Flow) and happiness (in the present). On individual level, it is about positive characteristics, such as the ability to love, talents, courage, ability for interpersonal communication, aesthetics, perseverance and tenacity, forgiveness, self-confidence, future mindedness, gifts, spirituality and wisdom. On a group level, it is about public values and institutions, individuals to be better physical citizens, to which the following is related; responsibility, caring, compassion and nuturance, altruism, politeness, moderation, tolerance and professional ethics. In many definitions of health, well-being is a key component. As stated in the WHO definition: „Health is a state of complete physical, mental and social well-

being, and not only the absence of disease or disability The best health condition is one of the fundamental rights of every human being, regardless of race, religion, political beliefs, economic or social conditions." (WHO, 1946)

This definition does not remain without criticism, since it based on the lasting state of perfect prosperity that is more bound to utopia than to real life. The important thing in this definition, however, is the examination of well-being in several aspects: physical, psychological and social. Wydra describes this as a multidimensionality of socially oriented health consciousness that stands out from a physiologically oriented medical concept and finds expression through the WHO definition.

Some authors divide well-being into a psychic and subjective (14,15); others see mental and subjective well-being as two aspects of the same construct (16), respectively examine the subjective as part of the mental well-being (17, 15).

According Keyes and colleagues (2002) psychological well-being refers to the ceaseless solution of existential life tasks and challenges, in other words, the pursuit and achievement of important goals such as growth and development of personality and the creation of qualities for connection, attitude, commitment, linking with others.

Carol Ryff (18) distinguishes six dimensions or qualities of mental well-being: self-acceptance (Selbstakzeptanz), positive relationships with others, autonomy, control of the environment, life with goals and personal growth.

The idea of subjective well-being stems from Greek hedonism (19). There are many definitions of subjective well-being in the literature. On the one hand, they are partly used as synonyms of well-being, happiness and satisfaction; and on the other hand the need for a strict distinction between them is emphasized.

Already in 1967, based on his studies of happiness, Wilson defines the happy person as „young, healthy, well-educated, earning well, extrovert, optimistic, without problems, religious, married with high self-confidence, moral work, with modest aspiration to the opposite gender and very intelligent" (20).

Based on this definition, a number of studies have been conducted. Although many of Wilson's conclusions are outdated - youthful and modest expectations are no longer seen as a prerequisite for well-being and happiness - yet some of his conclusions have been repeatedly examined and researched (20).

Diener and colleagues (20) define subjective well-being as: „... a wide category of phenomena that includes people's emotional reactions, domain satisfaction and global life satisfaction solutions." „Diener and Tov (19) indicate that subjective well-being is identified with happiness. Even Wydra (22) speaks about the use of the terms happiness and well-being as synonyms. It is important to note that the use of the concept should be based on the subjective assessment (21).

Mayring (21.) defines happiness as a positive, long-term emotional and cognitive factor of well-being.

The life satisfaction component should be defined as a lack of complaints, burden and worries, and the availability of certain opportunities and a certain standard of living, as well as the individual quality of life, although not only the objective living conditions matter, but also their subjective assessment (22). On the one hand, it is about the satisfaction of life as a whole, and on the other hand, it is about satisfaction with individual areas of life such as partnership, marriage, family life, and profession (23).

Well-being has a central place concerning life quality. In his book „Feeling Good: The Science of Well-Being," Cloninger [24] presents a complete concept of subjective well-being and shows ways of existence full of value. Own well-being is crucial for how other areas of quality of life will be assessed, such as social integration, social and material living conditions, etc.

Conclusion

Subjective well-being is an important indicator not only for a person's quality of life and health, but also for the quality of a whole society. According to Diener, Oishi and Lucas (20) the good life, the happy man and the happy people will create a happy and good society. Although the well-being of people

does not itself create a good society, it is an essential and necessary part. Both the individual and the politics seem to always seek for a good and happy life (25). Well-being, happiness and satisfaction are essential goals in people's lives, and specific strategies for their implementation and achievement are increasingly being used in therapy and consulting.

Numerous studies indicate the positive circumstances of a happy life: strengthening physical and mental health, more active attitude towards life, a high level of awareness, sensitivity and openness to reality, empathetic social orientation towards others and an integrative and supportive impact on the person (21).

Well-being, happiness and satisfaction have their significance in many different areas (21): in philosophy that deals mainly with happiness, in Christian theology, in literature, in economic and social sciences (well-being as a social factor as an indicator of quality of life, as a subject of research in national and international studies), in gerontology (happiness as an indicator of successful aging), in psychology (for example psychology of emotions and psychology of health care) and in physiology (physical foundations of well-being).

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Съвременен подход при изучаване на метастатични мозъчни тумори

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Contemporary approach in the study of metastatic brain tumors

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РЕЗЮМЕ:

Авторите проучват МТМ, чиято честота и значение в невропатологията нарастват значително. Въз основа на изследвани 748 МТМ е посочено тяхното разпределение по пол, възраст и локализация, както и доминиращите злокачествени тумори, метастазиращи в мозъка. Изтъкната е ролята на ИХХ, която е ключов инструмент не само за разграничаване на първични от метастатични МТ, но и за определяне на органния произход на първичния тумор, неговата субтипизация и имунофенотипизация. Акцент е поставен на МТМ с неуточнен първоизточник, които представляват близо една трета от пациентите, постъпващи с клинични данни за първичен МТ. Приложеният алгоритъм с подходящ подбор на ИХХ панел се отличава с надеждност и достоверност- само 46 от 273 тумори /16,84%/ с неясен преди операцията екстрацеребрален тумор са останали неуточнени на базата на ИХХ изследвания. В контекста на съвременните изисквания е показано и значението на някои прогнозни и предиктивни биомаркери при МТМ, които могат да са различни от първичния тумор.

Ключови думи: метастатични мозъчни тумори (МТТ), биомаркери, алгоритъм за диагноза на ММТ, ММТ с неизяснен произход.

ABSTRACT

The authors examine MBTs, whose frequency and importance in neuropathology are increasing significantly. On the basis of 748 MBTs studied, we indicate their distribution by sex, age and localization, as well as the dominant malignant tumors metastasized in the brain. The role of IHC is emphasized, which is a key tool not only to distinguish primary from metastatic BT, b _ПШПºр ut also to determine the organ origin of the primary tumor, its subtype and immunophenotype. Emphasis is placed on MBT with an unknown origin, which accounts for nearly one third of patients receiving clinical data on primary BT. The applied algorithm with an appropriate selection of the IHC-panel is distinguished by reliability and credibility - only 46 of 273 tumors (16.84%) with unknown pre-surgery primary extracerebral tumor remained undetected based on IHC examination. In the context of current requirements, is also shown the importance of some prognostic and predictive biomarkers in MBT, which may be different from the primary tumor.

Key words: metastatic brain tumor (MBT), biomarkers, algorithm of diagnosis of MBT, MBT with unknown origine.

Introduction

Metastatic brain tumors (MBT) are among the most common intracranial tumors, accounting about 30% of them (1,21,26) exceeding the incidence of glioblastomas (13,16). Each year in the United States, more than 100,000 patients with cancer develop metastases. At least 53% of MBTs are solid and are subjected to surgical intervention (29). Epidemiological studies of their frequency and gender distribution, age, and localization in the brain vary widely because of the differense of investigated patients - by specialized neurosurgery clinics, oncology hospitals, or the general population (8, 4, 29). Regarding to the most common type of extracranial tumor metastasing in the brain, the publications are pooled around the belief that most often metastasize lung cancer (about 50%), followed by breast cancer (about 15%), melanoma (11%), renal cell cancer, and all other malignant tumors are below 1% (1,8,21). An important feature of MBT is that it can precede the clinical manifestations of the primary tumor, which creates clinical, diagnostic and therapeutic problems (20, 29).

The aim of the present study is to investigate the frequency and distribution of MBT, as well as to define a practical and appropriate algorithm for the diagnosis of tumors with an unknown primary source.

Materials and methods:

Examined retrospectively, 748 metastatic brain tumors (MBT) were operated in the neurosurgical clinic at Pirogov for a 16-year period (2001-2016). They represent 31.88% of all brain tumors from the same period (BT) – 2 346 cases. Routine histologic, histochemical and immunohistochemical (IHC) studies were performed with the following antibodies: AE1 / AE3, CK7, CK8, CK20, EMA, S-100 protein, GFAP, TTF-1, estrogen and progesterone receptor, chromogranine A, synaptophysin, E-cadherin,mammoglobin, GCDFP , HER-2, PSA, AMACR, HMB-45, Melan A. Antibodies are products of DAKO. IHC studies were performed on paraffin blocks with conventional PAP technique and additional staining with DAB **(Table 1).**

Table 1. ***Used biomarkers in IHC study for determining the cytogenesis of metastatic tumors***

1. Non small- cell lung carcinomas	CK 7 (+); CK 20 (-); TTF-1 (+); AE 1/AE 3 (±)
2.Small-cell lung carcinomas	CK 7 (-);CK 20 (-); CД 56 (+); TTF-1 (+) или AE 1/AE 3
3. Squamous cell carcinomas of lung and others organs	AE 1/AE3 (+); CK 5/CK 6 (+); CK 20 (-); CK 7 (-);TTF-1 (-)
4. Colorectal carcinomas	CK 20 (+); CДX 2 (+); CK 7 (-);TTF-1 (-)
5. Renal cell carcinomas	TTF-1 (-); CK 7 (-); CK 20 (-); CД 10 (+); Vim (+); RCCM (+); AE 1/AE 3 (±)
6. Breast carcinoma	TTF-1(-); CK 20 (-); CK 7 (+); mammoglobin (+) ER (+); Ca 125 (+); GCDFP (+)
7. Endometrial carcinomas	TTF-1 (-); CK 7 (+); CK 20 (-); GCDFP (-); ER (±); Ca 125 (+)
8. Biliopancratic carcinomas	CK 7 (+);CK 20 (-)
9.Ovarial carcinomas	CK 7 (+);CK 20 (-)
10. Prostate gland carcinomas	AE 1/AE 3 (-); TTF-1 (-); CK 7 (-); CK 20 (-); S 100 (+); HBM 45 (+)
11. Malignant melanomas	Melan A (+); PSA (+)
12. Lymphomas	CД 20; CД 3 and others

Results

Gender distribution: The males are 468 (62.56%), female-280 /37.43%/. Men's ratio: women = 1.67.

Distribution by age is shown on fig. 1. The peak of MBT is in the 6th decade of life(38.63%). The youngest patient is 19 years of age, the oldest - 97 years old (Gaussian distribution)under 30 years old - 14 cases /1.87%/, between 31 and 40 y.o. - 45 /6.01%/, 41-50 y.o. -108 /14.43%/, 51-60 y.o. - 289 /38.63%/, 61-70 y.o. - 206 /27.54%/, over 70 y.o. - 86 /11.49%/.?).

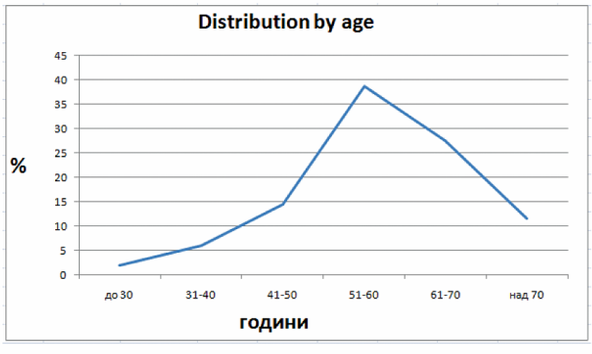


Fig.1

Distribution by localization: Solitary metastasis predominate, i.e. single tumor nodules in the brain without metastasis in the internal organs - 573 /76.60%/. Multiple metastases were observed in 175 cases (23.39%). In brain, solitary metastases were localized mainly in cerebral hemispheres - 422 /73.64%/, followed by cerebellum hemispheres - 137 /23.90%/, stem - 11/1.91%/, pituitary gland and selar area – 3 /0.52%/.

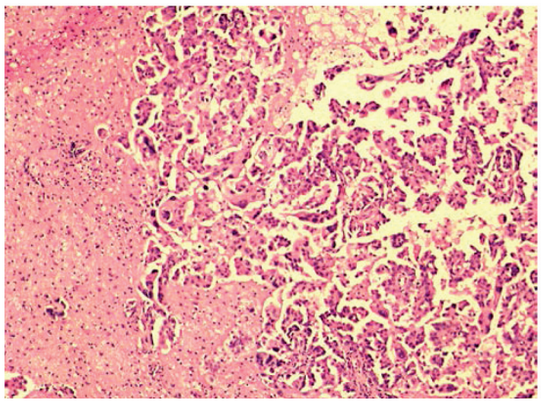


Fig2. ***Brain metastasis from lung adenocarcinoma. H&E staining.***

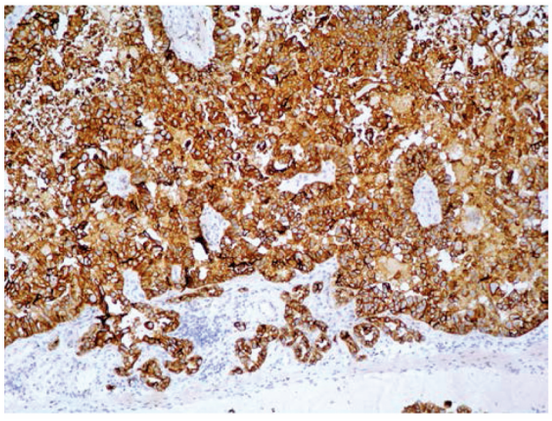


Fig.3. ***Strong positivity for CK-7 in the same brain metastatic tumor from adenocarcinoma of the lung.IHC staining.***

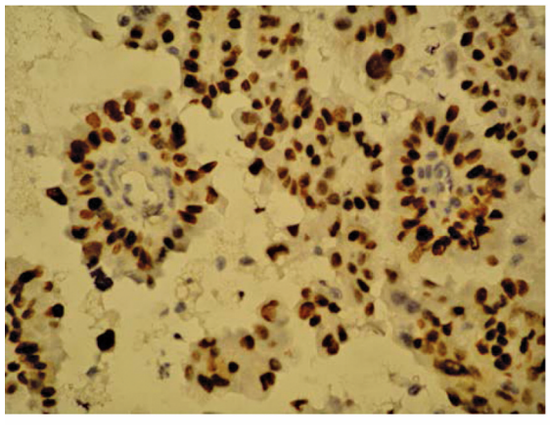


Fig.4. ***TTF-1 positive staining of the same metastatic brain tumor from lung adenocarcinoma.***

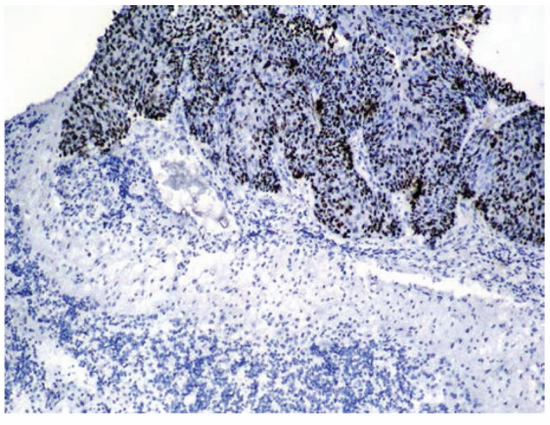


Fig.5. ***Immunohistochemical nuclear positivity for TTF-1 in cerebellar metastasis from undifferentiated small cell carcinoma of the lung.***

MBT with unknown primary tumor.

Primary extracranial tumor prior to surgery was diagnosed in 475 cases (63.50%), while the remaining 273 (36.49%) patients

were admitted with clinical data of primary MT, including diagnostic imaging results. Determination of the primary tumor is a major task for the pathologist and requires precise methods, experience and professionalism. The final decision is based on a comparison of the histological phenotype with imaging results and other clinical data. Most cases rely on IHC research, which is a powerful tool for specifying the origin of metastases. Pathomorphological exact diagnosis of primary tumor was determined in 227 patients, 46 patients remained with an unspecified extra-cranial tumor (16.84%) and were targeted to specialized oncology hospitals.

Proposed algorithm for examination of MBT with unknown primal tumor.

The first step in the algorithm is the determination of the basic structure or cellular characteristics of the tumor and histological phenotype of the tumor.

The second step is the determination of IHC panels, according to the phenotype (Table 1). IHC-markers were selected according to the histological diagnosis /structure / of the tumors and proposed IHC algorytm (Scheme 1,2). Among most used antibodies were cytokeratins AE1/AE3 and CK 7, and TTF-1.(See fig. 2-5)

Final distribution of MBT, according to original tumor site /total of 702 cases/was shown on **Fig. 6.** Unknown tumor site were 46 (6,14%) of total of 748 cases .

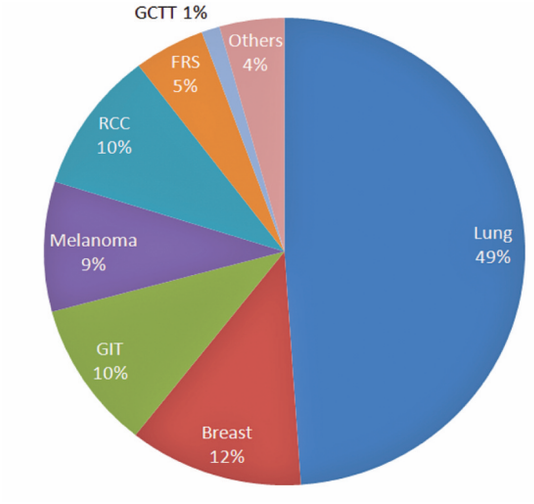


Fig. ***6. Distribution of the metastatic brain tumors: GIT – Gastrointestinal tract; RCC – Renal cell carcinoma; FRS – Female reproductive system; GCTT – Germ Cell Tumors of the testis.***

32 metastases origin from rarely-metastazing tumors in the brain, which consist below 1% of MBT (4.55% of all MBT). MBT have their origin from prostate gland cancer (5 cases), Thyroid gland (3 cases), two cases from Neuroendocrine tumors of the gastrointestinal tract, Urothelial carcinoma of the bladder, Chorionepithelioma, peripherial PNET, Liposarcoma, Epipharyngeal carcinomas, Salivary gland carcinoma, and Sinonasal carcinoma. By one case of MBT are leiomyosarcoma, mesothelioma, laryngeal carcinoma, basal cell carcinoma, alveolar soft tissue sarcoma, ganglioneuroblastoma, pulmoblastoma, olfactor neuroblastoma.

From 343 MBT from lung carcinoma - 137 (39.94%) are adenocarcinomas, 93 (27.11) - squamous carcinomas, 84 (24.48%) undifferentiated , 13 (3.79%) adenosquamous and 16 (4.66%) others.

Discussion

Our results confirm the increase in the incidence of MBT (8,14,16,26). There are single reports about reduction in the incidence of breast cancer metastases (21). According to Weseling et al (29) the frequency is set to 11/100 000 but is probably underestimated (25). Autopsy showed metastases in 25% of patients who died of cancer (4). Literature sources indicate a frequency of MBT of 3.5 to 41% of primary BT (29). Our own observations conducted only in neurosurgical clinics show a strong increase within 45 years. For the period between 1958-1972 years they accounted for only 5.07% of all operating MT (31), and in the last decade we have seen 31.88%.

The distribution of MBT by gender and age according to our study coincided with most of the publications (1, 25). It is unanimous to claim that they are more common in men, due to the high incidence of metastatic lung carcinoma prevalent in males, the ratio 1.64: 1 after his study. Over 40 years of age begins a stepwise increasing as the most common are in the 6th decade. Their incidence in patients under 25 years is 1/100,000 and increases to 30/100,000 in patients over 60 years of age (25).

Practically, important is the fact that a large number of MBTs appear as primary brain tumors without clinical symptomatic from the

extracerebral site. This number reaches 30% of MBT (20,29). Different IHC approaches were proposed to determine the primary tumor (6, 19, 20). The application of IHC panel for establishing the primary tumor is a key position in our behavior and is based on the histological structure. The algorithm we apply is in accordance with IHC rules for diagnosis of malignant tumors (29). An alternative algorithm begins with an initial IHC study with biomarkers characterizing major types of tumors, e.g. epithelial and glial cells, followed by a panel expressed in corresponding groups of tumors and a consecutive expansion of the panel for histologically specific tumor types. (Table 1). But sometimes too extensive examination can also involve coexpression, complicate DD and expensive unnecessary the procedure. The proposed algorithm used to determine MBT with an unknown primary site according to the basic histological structure is practical and economical, devoid of polypragmatism and gives effective and reliable results. This algorithm is recommended for diagnostic practice and will facilitate pathologists in this complex case - identifying an unknown primary tumor for MBT.

Lung cancer dominates among MBT not only because of its high frequency, but also because of the affinity to metastasize to the brain. Colorectal carcinoma and prostate cancer are not among the frequent MBTs, although their incidence is high in population. Good blood supply to the brain can not explain also the preference of lung carcinoma to brain dissemination. Only after finding the molecular basis of the metastatic process began to be clarified why lung cancer prefer to metastazing to the brain. Our results affirm the published data-lung cancer is the most common MBT, predominating in males - the most common are adenocarcinoma followed by squamous cell, often manifested primarily by MBT (1,21,25). In most publications on second place is undifferentiated cancer, which ranks third in our study. This may be related to the higher relative frequency of squamous cell cancer among the Bulgarian population.

DD difficulties often occurs in determining the origin of adenocarcinoma from the lung or from other organs. The application of TTF-1 to a ICH panel is of great importance for the diagnosis, because it is expressed predominantly in lung and thyroid cancer, the last is easily recognized by the characteristic phenotype (15, 24). Keep in mind that TTF-1 is not positive in 100% of tumors, so a negative result does not exclude lung as a primary organ. Papillary adenocarcinomas also cause major difficulties in DD with carcinoma of plexus choroideus. Even IHC cannot help in all cases. It remains the W.Willis' old rule from 1942 year - excluding papillary carcinoma with non-brain localization by clinical resources.

Another difficult problem of DD arises with the development of small cell carcinoma in the posterior cranial fossa in young people - MBT from undifferentiated lung carcinoma has a similar histological and immune phenotype with medulloblastoma which might occur in the brain up to 40 years of age, although very rare. The MRI (magnetic resonance imaging) of the lung is necessary for exact diagnosis.

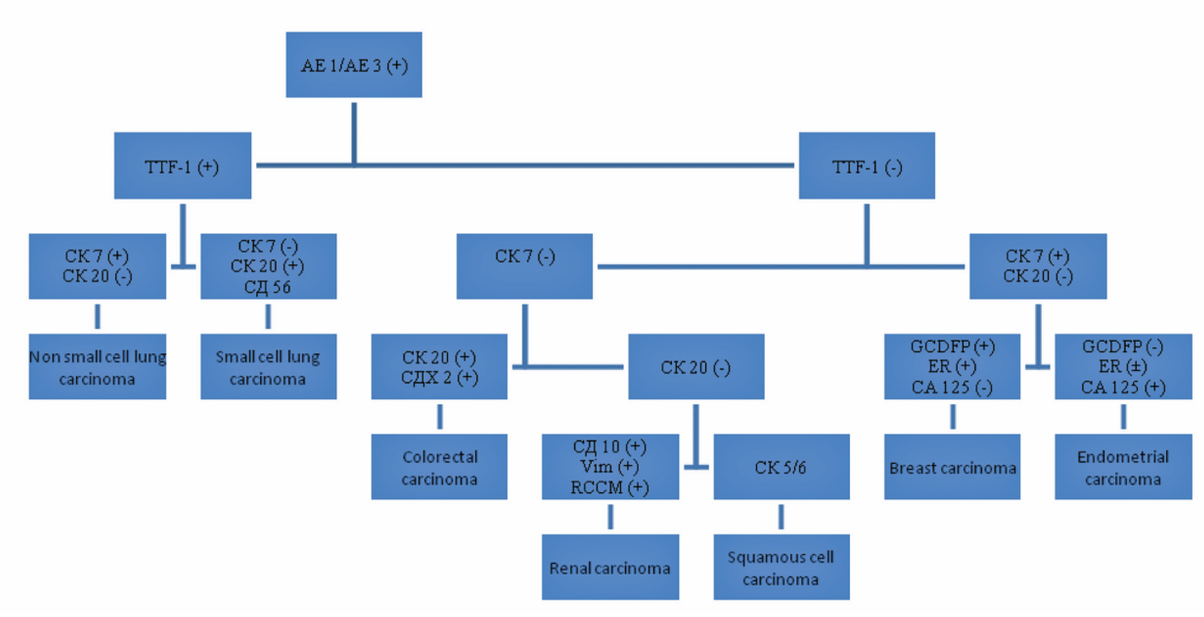
Recall that MBT from lung cancer is very often its primary manifestation. Lung cancer is sometimes difficult to detect, even after a purposeful imaging study after IHC-verification of the lungc origin of metastasis. There is still a percentage of undetected primary lung cancer –from 16 to 48% of the cases (4,13).

Breast cancer ranks second among MBT, and first among women. The frequence of breast cancer in CNS is 5.1%based on population-study (1), in cases series- 10-16% (Lin et al). Often there is a history of verified and treated carcinoma. Well-taken history, histology and mammography reveal the primary tumor if it has not been manifest before surgery. The period between the onset of primary tumor and metastasis varies widely. In our biopsies, the latest recurrence is after 15 years. Late onset of metastasis more often gives rise to doubt that it is possible to develop a second metastatic brain tumor. This, as well as the possibility of changing the immune profile in the metastasis (10), requires a detailed IHC study and comparison with the primary tumor. Molecular-pathological studies on breast cancer metastasis have shown relationship between the immune phenotype of cancer and the incidence of metastasis (27). Triple negative tumors (7, 22), those with overexpression of HER-2 (9, 12, 17) show a higher tendency to brain dissemination, specially HER-2 (+) second place among risk factors after lung metastases (18).

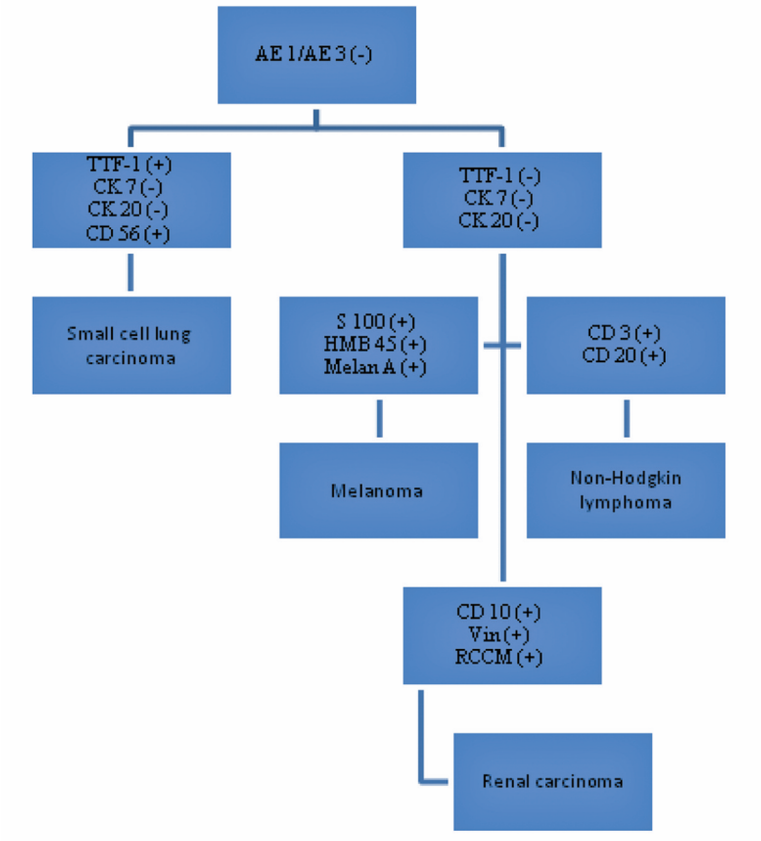
These data target therapy with trastuzumab and give a chance to predict the metastasing in the brain of different tumor subtypes and to undertake specific prevention about them (2,11,18,20,23).

Malignant melanoma is a tumor with proven encephalophilia. Melanoma metastases, if they contain melanin, should first be distinguished from primary melanocytic intracranial tumors. They only account for about 1% of all malignant melanomas, and only 0.07 of all brain tumors (28). An IHC study involving glial markers also aids the diagnosis, whose final decision is leptomening localization and exclusion of an extra-cranial tumor. In the achromatic variants of melanoma DD includes a wide arsenal of metastatic epithelial tumors and sarcomas and from anaplastic lymphomas (primary or secondary in the brain). The high tendency of melanoma to spread in the brain requires us to include it in the DD of MBT with an unknown primary tumor and in all malignant tumors with solid non-cohesive structures without formation of tubular or glandular structures.

In the last decade, significant processes regulating the molecular basis of metastasis and the participation of vascular modeling mediators have been revealed (3, 5, 30). Molecular pathology is expected to solve a number of theoretical clauses, for example, to what extent is the genotypic and immune phenotypic characteristic of MBT changed in comparison to the primary tumor? What spontaneous or therapeutically modified gene alterations can activate the metastatic ability of the tumor? Which biomarkers would predict the risk of developing MBT and how can they be inhibited? Some of these questions have an answer about breast and lung cancer (3), others expect their answer to improve the dark predictions of MBT **(Scheme 1 and 2).**



Scheme N 1. ***IHC algorhytm, AE 1/AE 3 positive(19,29)***



Scheme N 2. ***IHC algorhytm , AE 1/AE 3 negative (19,29)***

CONCLUSION

- **The study shows that MBT account for 31.88% of all brain tumors (BT) and their proportion among all BTs is significantly increasing.**
- **One third of MBT start as primary BT with an unknown primary origin, the finding of which is crucial for subsequent therapeutic behavior.**
- **The application of IHC provides the opportunity for a safe differentiation of primary and metastatic BT, determines in many cases the organ origin of the primary tumor and provides prognostic and predictive information.**
- **The algorithm, used by us for determining the origin of MBT is highly reliable and credible. Only 46 (16.84%) of 273 tumors with unknown primary tumor before surgery remained unspecified.**
- **The use of appropriate biomarkers in MBT allows for the application not only of diagnosis but also of target therapy.**

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Остра миелобластна левкемия след малария – клиничен случай

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Interesting Clinical Case of Acute Myeloid Leukemia after Malaria

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РЕЗЮМЕ:

Представяме клиничен случай на 34-годишна пациентка, с анамнеза за чести пневмонии с усложнения, флуктуиращи анемия и левкопения, хепатоспленомегалия. Хистологичните промени в костния мозък се приемат за реактивни, без да може да се изключи начало на миелодиспластичен синдром. При последната пневмония и информация за посещения на маларийни райони без профилактика, се диагностицира малария квартана, причинена от Pl.malariae и е проведено лечение. Два месеца по-късно пациентката е хоспитализирана в Клиниката по хематология на ВМА-София, с фебрилно-интоксикационен синдром, рентгенови данни за белодробен инфилтрат, спленомегалия, панцитопения и бласти в ДКК. Изследването за малария е негативно. Костномозъчната аспирационна биопсия е с инфилтрация от 75% бласти. Диагностицира се остра миелобластна левкемия, флоуцитометрични данни за М2 по FAB. Дали маларията протича на фона на развиващо се хематологично заболяване, и възможна ли е някаква патогенетична връзка с последващата

ABSTRACT

We present a 34-year-old woman, treated several times with complicated pneumonia. Besides fever, she had hepatosplenomegaly, fluctuating anemia and leukopenia. The core biopsy showed mostly reactive changes, but initial MDS could have not been excluded. During subsequent pneumonia, she was diagnosed also malaria quartana caused by Pl.malariae after information of visiting malaria regions without prophylaxes and treatment was provided. Two months later the patient was hospitalized in Hematology clinic with fever, intoxication, X-Ray of lung infiltrate, pancytopenia and blast cells in peripheral blood. The malaria test was negative. Bone marrow examination showed 75% of blast infiltration and acute myeloid leukemia was diagnosed, AML - M2 (FAB). Weather malaria co-exists with an initial hematological disease or a possible pathogenic relationship between the infection and the subsequent hematologic malignancy presents is difficult to differentiate. Despite significant advances in characterizing the effects of infection and inflammation on bone marrow and hemopoiesis, there are no

малигнена хемопатия, е трудно е да бъде определено. Въпреки разширяващите се познания за влиянието на възпалителния процес върху костномозъчната функция, няма описана връзка между малария и миелодиспластичен синдром или остра миелобластна левкемия. Взаймодействията между секретираните в резултат на инфекциозния процес биологично активни молекули и клетъчната хемопоетична микросреда, както и възможността те да създават условия за малигнена транформация, все още не са напълно изяснени.

Ключови думи: остра миелобластна левкемия, малария, миелодисплазия

established data about a relation between malaria and acute leukemia or myelodysplastic syndrome. The interactions between inflammation derived biologically active molecules and hematopoietic cells, that might probably provide conditions for malignant transformation, are still not fully appreciated.

Key words: acute myeloid leukemia, malaria, myelodysplasia

INTRODUCTION

Certain infectious diseases are of hematological interest due to clinical symptoms that resemble hematological malignancies, hematopoietic or lymphatic tissue involvement as a specific target of the infection, or anemia and peripheral blood count disorders they cause directly or indirectly. Some infectious pathogens have been linked to the disease etiology of malignant hematopathies, especially of lymphoproliferative. These organisms include viruses such as Epstein-Barr virus, human lymphotropic virus type 1, hepatitis C virus, human herpes viruses 8 and 6, and HIV, the last is associated with immunosuppression and not with oncogenesis. Bacteria associated with lymphomas of malt lymphoma type and Sezary syndrome are Helicobacter pylori, Campylobacter jejuni, Chlamydia pneumonia and psittaci, Borrelia burgdorferi and afzelii [1,2]

The bone marrow is the place of erythropoiesis and immune cells development and provides a niche for plasma cells and memory T-lymphocytes. It participates in immune homeostasis as well as in response to infections by proliferation and mobilization of immune cells, e.g. increased granulocytopoiesis and mobilization of neutrophils from the bone marrow are a resistance mechanism to many pathogens. Similarly, the increased erythropoiesis may represent a physiological response to acute inflammation, but some infections lead to reduction and depletion of precursors with subsequent anemia. Severely and prolonged chronic infections result in changes in both peripheral blood – as impaired cell survival and impaired immune response, and in the bone marrow microenvironment of normal haematopoietic proliferation and differentiation, induced primarily by activation of immunocompetent cells and secretion of inflammatory cytokines [3,4].

Malaria is an infectious disease caused by an intracellular parasite of the genus Plasmodium, with a mosquito-transmitting vector of the genus Anopheles, and is typical for the tropical and subtropical regions. Five species of the genus Plasmodiums are responsible for infections in humans: Pl.falciparum, Pl.vivax, Pl.ovale, Pl.malariae and Pl.knowlesi [5]. They are characterized by different geographical distribution and diverse disease course. The main clinical presentation is malaria paroxysm, and main hematological symptom - hemolytic anemia, the most severe in Pl.falciparum [6]. Malaria in endemic areas is considered a possible cause and complicating factor of febrile state with hepatosplenomegaly in patients with malignancies. Associations with hematologic neoplasms have been reported only for the endemic form of Burkitt's lymphoma and Pl.falciparum infection in children [7,8], which results in polyclonal B cell stimulation and T-cell immune disorder with subsequent uncontrolled proliferation of the Epstein-Barr virus, associated with endemic form of Burkitt's

lymphoma [7,9]. Malaria may lead to complications in the clinical course of acute leukemia, and leukemia may contribute to more severe and recurrent malaria paroxysms when these two diseases coexist, but no pathogenic connection between them is described.

CLINICAL CASE

The patient is a 34-year-old woman treated several times in the last eight months for complicated pneumonia with pleural effusions and sepsis. At hospitalizations the patient presented with febrile-intoxication syndrome - fever to 39.6C, chills, asthenia, X-ray evidence of inflammatory lung infiltrates. Anemia was fluctuating with decreased hemoglobin levels - 80 g/l, leukopenia - 1.8G/l with neutropenia - 0.6G/l, elevated ESR and CRP, CT finding of mild hepatosplenomegaly and enlarged portal lymph nodes. She was treated with prolonged antibiotic courses that leaded to clinical improvement, resorption of pulmonary infiltrates, elevation of hemoglobin and leukocyte count, but still not reaching the reference values. Rheumatic, cardiovascular and renal pathology were excluded in the diagnostic work-up of febrile condition. Patient had no pre-existing history of cytopenia. Hematologic malignancy was excluded as flow cytometry did not detect blast cells or a monoclonal lymphocyte population in peripheral blood and bone marrow aspiration. Granulocyte colony stimulating factor (G-CSF) was administered for one time because of life-threatening infection, but leukocyte count response was temporary. Three months after disease start bone marrow biopsy was performed due to persistent bicytopenia episodes. The histological finding was of hypercellular bone marrow, with proliferation of the three hematopoietic lines. The granulocyte and erythroblast populations were more prominent with ratio of 1:1, and peritrabecular erythroblast groups. Granulocyte lineage maturation was preserved. Megakaryocytes were polymorphic, with dysplastic microforms, hypolobulated nuclei and scarce cytoplasm; some had peritrabecular location **(Figure 1).** The bone marrow fibrosis was MF1. Finding was interpreted as reactive changes, but as biopsy was performed one month after G-CSF administration, it was not possible to exclude the onset of myelodysplastic syndrome.

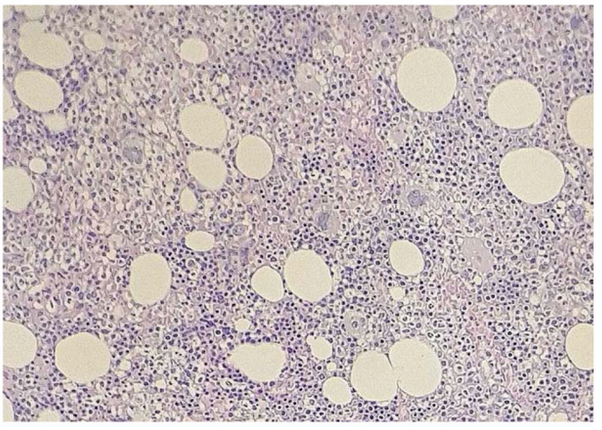

Figure 1. ***Bone marrow biopsy. Erythroblast proliferation with morpho-atypical, partially grouped megakaryocytopoiesis (Giemsa x10)***

When the patient was hospitalized for the fourth time for pneumonia she presented again with fever 39.50C, pulmonary infiltrate, bicytopenia - Hb 91g/l; WBC 1.8G/l, persistent hepatosplenomegaly. She reported frequent trips in tropical and subtropical regions without conducting antimalarial prophylaxis. Parasitological studies were performed and malaria quartana caused by Pl.malariae was diagnosed. Antimalarial and antibiotic treatment leaded to clinical improvement, hemoglobin and leukocytes increased to lower limit values. Control tests for malaria after treatment were negative.

Two months later the patient was hospitalized in our clinic with fever, weakness and pancytopenia. The laboratory tests revealed Hb 102g/l; WBC 8.34x109/l, with blasts - 30%; Mc - 1%; J - 1%; St - 7%; Sg - 18%; Ly - 40%; Mo - 3%; erythroblasts - 7/100 cells; Plt 61x109/l. Biochemical parameters of hepatic and renal function were within reference ranges. Bone marrow aspiration biopsy showed normal to hypercellular bone marrow, with infiltration of 75% blast cells and reduced residual hematopoiesis **(Figure 2).**

Flow cytometry analysis of bone marrow was performed with a result: 71% myeloblasts with phenotype CD45+low/ CD34-/+/ CD117+/ CD13+/ CD33+/ CD11b-/ CD14-/ CD15+/-/ CD16-/ MPO+. FISH analysis for 11q23, t(16;16) inv16, t(8;21) were negative. The chest X-ray showed left pulmonary infiltrate, and the ultrasound showed enlarged liver and spleen. Diagnosis was acute myeloid leukemia with differentia-

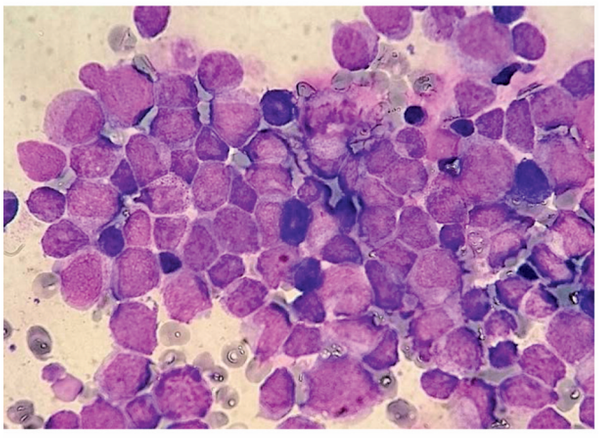

Figure 2. ***Bone marrow aspiration. Myeloblast infiltration, reduced residual hematopoiesis (Giemsa x100)***

tion, AML-M2 (FAB). Parasitological examinations of the peripheral blood for malaria and of the bone marrow for leishmaniasis were negative. Induction chemotherapy was recommended, but the patient continued her treatment in another hospital.

DISCUSSION

Diagnosis of acute myeloid leukemia in our patient raises the question of whether malaria is on the background of a developing hematological disease, and is it possible that the infection has some pathogenic relationship with the subsequent malignancy, which is difficult to tell in this case. During diagnostic work-up septic condition, toxic-drug bone marrow suppression, secondary hematological changes due to prolonged malarial infection were discussed as a cause of blood count changes.

Malaria quartana infection is caused by Pl.malariae, which is most common in areas of North Africa, but also encountered in Central and South America and Middle East - areas that the patient has visited. It is characterized by low grade parasitaemia, unlike infections with other parasites, and affects only mature erythrocytes, but not reticulocytes, which explains milder clinical symptoms. Besides malaria attacks, infection with Pl.malariae may present with chronical course causing recrudescence with remote years exacerbations. This is the least severe infection among the four types of malaria. Pl. malariae has no quiescent liver stage forms and cannot cause a late relapse from persistent liver stage parasites. However it is able to persist in the blood with low level parasitaemia for extremely long periods, perhaps for the life of the human host [5,10].

Pulmonary involvement in malaria is not atypical, but there are no reliable clinical or radiographic signs to confirm or rule out malaria. Negative microbiology of our patient during pulmonary inflammation is susceptible to malaria, but this is most typical of Pl.falciparum and Pl.vivax. They have often severe course with respiratory distress syndrome [11,12], and such is not found in the patient. The immune response to malaria involves CD4+ T-lymphocytes, NK cells and macrophages. The underlying cause is the imbalance between Th-1 and Th-2 lymphocytes that affects the cytokine network [13]. Severe Pl.falciparum malaria is characterized by an increase in pro-inflammatory cytokines - tumor necrosis factor-α (TNF-α), interleukins-1, -6, and -8 (IL-1, IL-6, IL-8), which are also involved in the pathogenesis of respiratory distress syndrome, by changing the ratio with anti-inflammatory cytokines - interleukin -4 and -10 (IL-4, IL-10) [14]. A few severe cases of Pl.malariae infection are described with multiple organ dysfunction syndrome, incl. pulmonary dysfunction, and genetic polymorphism involvement with a tendency of patients to develop severe sepsis is discussed [15,16]. The patient we describe lacks multiple organ failure, incl. renal impairment of immune-complex type, which is typical for Pl.malariae infection [5,10]. Suppression of adaptive immune mechanisms is also observed in malaria infection. An altered function of dendritic cells is described, with a consequent disorder in T-cell activation, follicular migration and absence of a B-cell antigen response, which may explain the connection observed in epidemiological studies between endemic malaria and secondary infections [17]. This is another possible explanation for recurrent pneumonia in our patient, although the results from humoral and cellular immunity tests did not show any immune deficiency and the imbalance was typical for immune response to inflammation.

The hematological changes in the patient - anemia and leucopenia, are severe during

pneumonia, possibly related to malarial infection. Hematological abnormalities are the most common complications encountered in malaria - anemia, thrombocytopenia, splenomegaly, atypical lymphocytosis, coagulation changes, leucopenia or leukocytosis [18,19]. Anemia in malaria infection is caused by hemolysis of intracellular parasites, hypersplenism, suppressed and ineffective erythropoiesis and anemia in chronic diseases. Leukocyte count in malaria may be increased, when associated with secondary bacterial infection or decreased.

These changes in leukocytes are best described in Pl.falciparum and Pl.vivax. They are associated with severe clinical course and go together with anemia and thrombocytopenia [20,21]. Decreased to normal leukocyte count is more closely related to leukocyte localization in peripheral circulation and spleen, rather than stasis or destruction [22,23]. Platelet count in our patient is within the reference range, despite splenomegaly, which makes hypersplenism a less probable cause of cytopenia.

Histological examination of bone marrow was performed after the onset of fever attacks and pulmonary inflammation to explain bicytopenia and before the diagnosis of malaria infection. The bone marrow is hypercellular, with erythroblast hyperplasia and a tendency to atypical peritrabecular location. Megakaryocytes also show dysplastic morphological features and atypical localization of some cells peritrabelicularly. Considering the clinical symptom and G-CSF administration a month before core biopsy, the changes resemble reactive, and it was not possible to exclude the onset of myelodysplastic syndrome [24,25]. Bone marrow examination was not performed when malaria was diagnosed and bicytopenia was explained by the infection as there were no atypical or blast cells in the peripheral blood.

Increased production of proinflammatory cytokines, such as TNF-α, IL-1, IL-6, as well as decrease in erythropoietin level, parasite products, hyperplasia and macrophage dysfunction contribute to bone marrow suppression and hematological abnormalities with cytopenias in malaria ifections [14,26-28]. Hematological changes and hematopoietic suppression, along with severe anemia, are typical and more pronounced for Pl.falciparum and Pl.vivax infections, in which parasitemia is high and involves reticulocytes [5]. Even erythroid precursor cells - orthochromatic erythroblasts - may be invaded and serve for development of stages of Pl.falciparum [29,30] and Pl.vivax [31]. Despite the possibility of suppressed and ineffective erythropoiesis with dyserythropoietic features in malaria infection with severe anemia, no cases of myelodysplastic syndrome have been reported.

Normal hemopoiesis is regulated by complex interactions between stimulating and inhibiting cytokines, growth and migration factors of the cell microenvironment, and hemopoiesis changes in both infections and hematological diseases such as myelodysplastic syndrome and acute leukemia [4,32]. Hematopoietic stem cells have common signal pathways and receptors with leukemic stem cell, and activation and signal mechanisms of the immune system in response to infection may be a disadvantage in leukemia [32]. Whether the malarial infection in our patient develops on a hematological condition background as an initial myelodysplastic syndrome has not been elucidated. Difficult diagnostic cases, especially in the absence of increased blast cells in the bone marrow, are recommended a second bone marrow biopsy with cytogenetic examination after several months [24].

There are cases of malaria in patients with hematological neoplasms described in medical literature - most often lymphomas and less commonly leukemias [33-35]. A relationship is reported for the endemic form of Burkitt's lymphoma and Pl.falciparum infection in infancy [7-9,36,37]. Other hematologic malignancies are commented mainly for complications that are more severe when malaria infection coexists. Complications include febrile neutropenia after chemotherapy in lymphomas and acute lymphoblastic leukemia [33], hepatosplenomegaly, fever and intoxication in chronic myeloid leukemia [34]. Increased mortality rate is reported due to the known ability of the parasite to induce suppression of the immune system and to

increase susceptibility to secondary infections [38]. Malaria is not classified as carcinogenic by WHO-IARC and no connection between these two diseases - malaria and leukemia, has been proven so far [37,39].

The impact of the infectious and inflammatory process on bone marrow function has been revealed to a considerable extent, but it is still not known whether the changes affect body's ability to control pathogenic agents. Despite the reversibility of bone marrow populations' changes in response to inflammation, it remains unclear whether the disorders have a longer-term impact on hematopoiesis, including in chronic, prolonged infections [4]. The microenvironment restoration processes are still not defined so it is not elucidated whether there are possible differences from normal hematopoiesis that may provide conditions for malignant transformation as well as infection-induced aberrant immune responses that promote the development of a malignant process.

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Роботизирана стереотактична радиохирургия с кибернож в България – начални резултати

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Robotic stereotactic radiosurgery with cyberknife in Bulgaria – initial results

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РЕЗЮМЕ:

Лъчелечението е един от основните методи за лечение на онкологични заболявания. Около 60% от всички раково диагностицирани пациенти ще постъпят в лъчетерапевтичен център за дефинитивно или палиативно лечение. Създаването на нови поколения линейни ускорители като КиберНож позволява лечение с роботизирана стереотактична радиохирургия. Това дава възможност за приложение на голяма доза в малък обем, с висок дозов градиент при запазване на здравите тъкани. Устройството комбинира линеен ускорител, разположен на роботизирана ръка с интегрирана система за проследяване на мишената. Методиката позволява да се локализира и следи формацията по време на цялото облъчване и при необходимост ново центриране на пациента.

Ключови думи: радиационна онкология, стереотактична радиохирургия, КиберНож

ABSTRACT

Radiotherapy is one of the main and vital methods of treatment for oncological diseases. Around 60% of all cancer diagnosed patients will be hospitalized at radiation oncology clinic for definitive or palliative treatment. The development of new generation of linear accelerators such as CyberKnife allows to perform a robotic stereotactic radiosurgery. This gives an opportunity to deliver a high dose in low volume with a high dose gradient while maintaining healthy tissues. The device combines a linear accelerator mounted on a robotic arm and an integrated image guidance system. The method allows to locate and to track the formation during the whole treatment and if it is necessary to re-center the patient.

Key words: radiation oncology, stereotactic radiosurgery, CyberKnife

INTRODUCTION

Modern treatment of oncological diseases requires a complex approach involving doctors of various specialties – radiation oncologist, medical oncologist, surgeon, pathologist, and a specialist of imaging diagnostics. Radiation therapy (RT) occupies a decisive place, especially after the introduction of a new class of linear accelerator for stereotactic radiosurgery (SRS) - CyberKnife. The method allows irradiation of patients with both primary and secondary malignancies of the head and neck, lung, prostate, liver and spinal metastases. Glioblastoma is the most common brain tumor and one of the most aggressive, the World Health Organization classifies it as an astrocytoma grade IV. According to the National Cancer Institute, newly diagnosed patients with brain and other neurological tumors for the year 2014 are about 23,380 - 15% of them are glioblastomas [1,2]. Brain metastases are 4-5 times more common than primary intracranial carcinomas, occurring in 20-40% of patients with oncological diseases [3]. The most common initial disease in these patients is lung (40%) and breast (20-25%).

MATERIAL AND METHODS

For a period of year and a half (April 2017 – September 2018) 69 patients were treated with CyberKnife system in Clinic of Radiotherapy, UMHAT "Sv. Georgi"- Plovdiv. The age rate is between 41 and 82 years old patients, average 61.5 years. The male/female distribution is 44/25. The reason for this huge difference between men and women is because the biggest group of the patients is with brain metastases (28), with primary lung carcinoma (over 90%) which is more common for males. On the other hand there is a small group with prostate carcinoma.

For every patient with head and neck carcinoma there are individual thermoplastic masks which fix the head during CT and allow to take the same position during treatment. The purpose is to restrict the movements and rotation of the head.

The pre-prepared and approved by a physician and physician plan is put on phantom before the radiation treatment starts. This checks whether the set dose is the same as the dose that the patient will receive. During each procedure, the patient's movements are monitored by a tracking system and X-rays are taken every 20 seconds, and when the patient moves more than 1mm, the table's position is automatically adjusted. If the displacement is more than 5mm, the radiation stops and the patient is returned to the starting position.

Used isodose line is between 60-90% depending on the localization and the coverage is over 95%. Fraction time is between 40 – 65mins.

Patients are divided into two groups with several subgroups **(table 1, 2):**

Intracranial formations:

- **Primary benignant or malignant**

The whole group includes 23 patients, 9 of them with benignant and 14 with malignant formations. Including criteria: only 1 lesion with diameter up to 5cm, ECOG status 0-2, life expectancy over 6 months. Number of the fractions is between 1 – 5, day dose between 5Gy – 15Gy.

- **Brain metastases**

The group includes 28 patients – 23 of them with primary lung carcinoma, 1 breast carcinoma, 1 kidney carcinoma, 1 colon transversum carcinoma and 2 with unknown primary. Including criteria: maximum 4 metastases with diameter up to 4cm, ECOG status 0-2, life expectancy over 6 months. Number of fractions is between 1 – 5, day dose between 5-22Gy. Some of the patient had been treated with whole brain RT before that.

Table 1. ***Intracranial formations – primary and secondary***

Type	Patients	Formations	Size	Fractions	Day Dose
Primary	23	Only 1	Up to 5cm	1-5	5-15Gy
Secondary	28	Up to 4	Up to 4cm	1-5	5-22Gy

Extracranial carcinomas:

• Head and neck cancer

The whole group is with 10 patients. Most of them had been operated with progression after that. Including criteria: maximum diameter 4-5cm, EGOG 0-2, life expectancy over 6 months. Number of fraction between 2-5, day dose between 5-10Gy.

• Prostate cancer

Only 4 patients. Two weeks before SRS must be replaced 4 fiducial markers in the prostate. The purpose of this gold markers is to detect and to track the prostate during treatment. Every patient must urinate and after that drink 250ml water 20min before SRS. Including criteria: T1/2N0M0 , EGOG 0-2, life expectancy over 6 months. Number of the fractions is 5, day dose 7,25Gy.

• Spine metastasis

Only 4 patients. Spine tracking system can detect and track the vertebrae. Including criteria: single spine metastasis, EGOG 0-2, life expectancy over 6 months. Number of fractions 3, day dose 9Gy in the metastasis and 7Gy in the vertebrae.

RESULTS AND DISCUSSION

Precision-oriented treatment planning loses its meaning if the positions of the target and organs at risk (OAR) are deviated during actual treatment because of setup uncertainty or motion. Patient immobilization is thus crucial, especially for tumors in the brain and the head and neck. For relatively few and small formations, ablating each lesion precisely with exceptionally high level of radiation dose using the so-called SRS technique may be indicated [4]. The International Commission on Radiation Units ICRU defines OAR as those normal tissues which lie adjacent to tumours and may therefore be included within treated volumes, with a risk that the radiation may impair their normal functioning. Preparation of a treatment plan involves outlining. [5] The possible radiation complications for CNS can be divided in 3 groups:

Acute: alopecia, radiation dermatitis, fatigue, transient worsening of symptoms due to edema, nausea, and vomiting (particularly with brainstem [area postrema] and posterior fossa [PF] radiation), and otitis externa.

Table 2. ***Extracranial formations***

Location	Patients	Fractions	Day Dose	Fiducials
Head and Neck	10	2-5	5-10Gy	0
Prostate	4	5	7,25Gy	4
Spine	4	3 in metastasis	9Gy	0
		3 in vertebrae	7Gy	

Mucositis, esophagitis, and myelosuppression are associated with cranio-spinal irradiation. Subside within 4–6 weeks after radiation. Doserelated.

Subacute (6 weeks to 6 months after RT): somnolence, fatigue, neurologic deterioration, perhaps caused by changes in capillary permeability and transient demyelination.

Late (6 months to many years after RT): radiation necrosis, diffuse leukoencephalopathy (especially with chemo, but not necessarily correlated with clinical symptoms), hearing loss, retinopathy, cataract, visual changes, endocrine abnormalities (if hypothalamic-pituitary axis is irradiated), vasculopathy, Moyamoya syndrome, decreased new learning. [6]

The most complex follow-up (blood tests and MRI) was conducted to a patients with brain metastases. Life expectancy in this group is low and that's why there are not enough clinical studies.[7,8] More than 1/3 of all patient that are treated with CyberKnife are with brain metastases. Before SRS on each patient was made a CT and MRI on head and blood samples were taken to assess the activity of the primary disease. Studies include blood tests, coagulogram, growth factors (EGFR), apoptosis (p53), angiogenesis (VEGR, angiopoetin2, HIF-1Alpha). Integrating biological information to improve the radiotherapy strategy and therapeutic response can be defined as a personalized RT. To do this, reliable predictive biological markers are needed which can be used to determine optimal dose, choice of fractionation schemes, or combined modalities. Biomarkers can also be classified according to modality of assessment, and this has implication for how particular biomarkers might be developed.[9,10,11] These results will be analyzed and presented in another article. The control visits of patients with secondary malignancies are on the first, third, sixth and twelfth months after SRS. Every visit includes physical examination, MRI and haematological tests, to assess metastatic activity. Preliminary data from imaging methods show a very good therapeutic response with minimal or missing side effects. In all patients, there has been a reduction or lack of progression in the extent of irradiated metastasis, as well as an improvement in the overall condition and regression of the symptoms. This shows excellent local control of the disease as well as minimal damage to healthy tissue around the formations. Only five of the patients with brain metastases showed new formations in the CNS (the treated ones are with smaller size). Two of them had only 1 new lesssion that was treated with CyberKnife, and the other 3 patients was treated with whole brain RT because they had more than 1 new metastasis.

CONCLUSION

Stereotactic radiosurgery with CyberKnife is a widespread radiotherapy method in Europe and America with proven clinical outcomes. The ability to administer a high dose in a small volume with a high dose gradient protects the healthy tissues and the side effects are minimized.

DISCLOSURE

The authors have declared no conflicts of interest.

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