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MEDICINSKE ZNANOSTI

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ZAGREB, 2014.

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CONTENTS / SADRŽAJ

EDITORIAL

Personalised Medicine

Dear readers, in front of you, there is volume 40 of the journal RAD of the Croatian Academy of Sciences and Arts – Medical Sciences. The present volume introduces the papers from the symposium entitled *The First Croatian Symposium on Personalised Oncology,* which was organised by the Department of Medical Sciences of the Croatian Academy, and held on October 25th, 2013. at the National Hall in Zagreb. We cordially thank our guest editors – Prof. Fedor Šantek, MD, PhD, and Prof. Nikola Đaković, MD, PhD – for their great effort in compiling the symposium papers.

I gladly state that in the course of the last year, this is the third symposium addressing medical issues and dealing with the topic of *personalised medicine*, which was organised by the Department of Medical Sciences and the Institute of Immunology and Tumour Genetics of the Croatian Academy of Sciences and Arts, but also coorganised by the Department of Clinical Oncology, School of Medicine, University of Zagreb.

Individuality is the only characteristic common to all people; diversity is the only thing common to all people: these sayings are commonly known and happen to be true. Hence it is obvious why the 21st century is the era of personalised medicine. The important role of personalised medicine becomes especially evident in the treatment intended for oncology patients. The meaning of this symposium and the contents of this volume should be comprehended in this sense. Personally, I like the definition of personalised medicine: *The right treatment for the right person at the right time.* Papers collected in this volume follow the idea of this definition. I therefore believe the audience will find great interest in reading them.

This volume further brings an article from the history of the study of pharmacy at the University of Zagreb, Croatia. The author is Prof. dr. Nikola Kujundžić, and we are very thankful to him for choosing our journal to publish this unique tribute to Vladimir Prelog, one of Croatian Nobel Laureates, in.

Dear readers, it is therefore with great pleasure that I present to you RAD 40 (2014), the tenth volume of our journal published in English, and I proudly announce that these 10 volumes of RAD have obtained about 100,000 hits on the web page hrcak.srce.hr.

Marko Pećina

Symposium

THE FIRST CROATIAN SYMPOSIUM ON PERSONALISED ONCOLOGY

Guest Editors

Fedor Šantek, Nikola Đaković

PREFACE

Current advances in the biology of cancer and emergence of new tools for genome analysis have opened clinical perspectives in oncology, generally termed as 'personalised medicine'.

Malignant diseases are no longer classified only by histology and tumour site, but are also separated into various molecular subtypes. Molecular characterisation of tumour cells enables the enhancement of classifications for many cancers and can sometimes guide treatment. The current scientific developments that stand behind molecular pathways lead to more personalised approach in the treatment of cancer.

Moreover, the precise sequence of the mutation can sometimes predict the outcome. On the other hand, there is a persistent gap between the growing number of identified genetic mutations or deregulated pathways and their actual implementation at the bedside as part of clinical routine. Overall, turning the dream of personalised oncology treatment into reality will require further collaborative endeavour.

The intention of the First Croatian Symposium – Personalised Oncology, which was held in October 2013, was to present the latest achievements in the fields of diagnosis, prevention and treatment of cancer. Regretfully, not all papers of all presentations are included in this issue.

Besides the Department of Medical Sciences of the Croatian Academy of Sciences and Arts and its Institute of Immunology and Tumour Genetics, co-organiser of the symposium was also the Department of Clinical Oncology, School of Medicine, University of Zagreb. The chairpersons of the symposium were Academy President Zvonko Kusić and Professors Lidija Beketić Orešković, Antonio Juretić, Fedor Šantek, Nikola Đaković.

On behalf of the
Organising Committee,
Fedor Šantek
Nikola Đaković

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PERSONALISED THERAPY OF BREAST CANCER – SIGNALLING PATHWAYS AND TARGETED THERAPY OF BREAST CANCER

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Summary

Personalised therapy of breast cancer is the optimal therapeutic approach to the patient, taking into consideration his personal characteristics, as well as clinical characteristics of the malignant deasease, involving pathohistological and molecular abnormalities of certain tumour. With the devolopment of molecular oncology methods, genetic profiling of each individual tumor is possible. Beside the major subtypes of breast carcinoma based on steroide receptors, Ki-67 proliferative index, and HER-2 receptors, numerous genetic subtypes of breast cancer have been found due to enormous genetic heterogeneity and instability of tumor cells. Some of genetic changes are considered as “driving” genes, resulting in dysregulations of crucial signalling transduction pathways involving in cell proliferation, angiogenesis, apoptosis, invasion or metastasis. Certain components of signalling transduction pathways can be targeted molecules of the so-called targeted biological therapy. Proper understanding of complexities of these dysregulated multiple intracellular signalling cascades in tumor cells is essential for the development of novel potential molecular therapeutic targets.

Keywords: breast cancer; signalling pathways; personalysed therapy.

The normal cells, as well as the tumor cells have capability to respond to the numerous external stimuli such as growth factors, hormons or cytokines. This complex processes comprise recognition on cellular mebrane by receptors, intracellular signalling transduction pathways, activation of numerous transcription factors, and expression of different genes. This is the way of cellular response to microenviroment, as well as regulation of cellular

proliferation and differentiation [1]. In this complex multifacorial nets of signalling transduction pathways from cellular membrane to nucleus, the crutial role have protein kynases, enzymes involved in methabolic pathways, protein phosphorilation, transport and activation, as well as in degradation of proteins. Changes in components of signalling transduction pathways can result in malignant cell transformation. In normal cells signalling transduction pathway is precisly regulated. In tumor cells, crucial molecules of this complex signaling transduction pathways can be changed by different mechanisms, envolving expression of some oncogenes, leading to abnormal signalling transduction pathways, inhibition of apoptosis, uncontrolled tumor cells proliferation, angiogenesis, tumor invasion and metastazing [2]. Activation of some oncogenes (e.g. ErbB2, PI3K, Akt, Myc,), or loss of function of some tumor supressor genes (TP53, or PTEN), resulting in changes in signalling pathways sach as PI3K/Akt/mTOR, or raf/MEK/ERK are implicated to be involved in carcinogenesis of breast cancer [3]. Certain components of signalling transduction pathways can be targeted molecules of so called targeted biological therapy. Understanding of complexities of these dysregulated multiple intracellular signalling cascades in the tumor cells is essential for the developing of novel potential molecular therapeutic targets [4].

Personalized therapy of malignant deseases, including breast cancer is, according to the definition, optimal therapeutic approach to the patient, taking into consideration his individual, personal characteristics (including genetic), as well as clinical characteristics of the malignant deasease, involving pathohystological and molecular abnormalities of certain tumor. With the devolopment of molecular oncology methods, especially with DNA microarray analyses, genetic profiling of each individual tumor is possible. Two biggest international projects of systematic genomic analyses of tumor samples are currently ongoing: The Cancer Genome Atlas – NIH project in USA, and International Cancer Genomic Consortium in the rest of the world (3). With these molecular genetic analyses, numerous genetic changes in breast cancer have been found, some of them are considered as „driving" genes, but the real role of some genetic changes in carcinogenesis remans to be determined [5].

According to the recommendations of St. Gallen conference (St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer, 2013) it is obligatory to determine major subtypes of breast cancer on the basis of immunohystochemical analysis of estrogene and progesterone

receptors, HER-2 receptors and Ki-67 proliferation index on the tumor tissue samples [6]. The therapeutic basis for luminal A and luminal B subtypes of steroide receptor positive breast cancer is hormonal therapy, imunotherapy shoulod be included in the treatment of HER 2 positive subtypes, while chemotherapy is still the basis for treatment triple negative („basal like") subtypes of breast cancer. However, molecular analyses of individual breast carcinoma tissues revealed that each of these subtypes can comprise futher new genetic subtypes of tumors which can deffer in prognosis and response to the therapy. According to numerous studes in the field of breast cancer, a great therapeutic problem in this desease is pronounced heterogeneity of tumor cells among apparently similar tumors, as well as diffent tumor clones in even one tumor. Tumor genom is very unstable, prone to numerous changes and mutations, even during therapy, which can lead to induction of different mecahisms of resistance and survival of mutated clones of tumor cells [3].

Hormonal therapy of steroid receptor positive breast cancer is one of the oldest and most succesful personalyzed therapeutic approach. With hormonal therapy steroide receptor expression on tumor cells is modulate or downregulate and/or hormone synthesis is blocked, resulting in decreased activation of estrogen signalling pathway. However, it has been shown that there is possibility of parallel activation of different signalling transduction pathways in breast cancer cells together with steroide receptor pathway (e.g. signalling pathways of EGFR, and PI3K/Akt/mTOR) which can be activated in resistance to hormonal therapy [7]. We block one signalling pathway with targeted therapy, but tumor cells activate different compensatory mechanisms and other numerous signalling pathways. This is the basis for therapeutic concept of necessity of parallel blocking of different signalling pathways – such as hormonal therapy together with mTOR inhibition in breast cancer patients (e.g. BOLERO-2 clinical trial of addition of mTOR inhibitor everolimus to aromatase inhibitor exemestan in the treatment of hormone receptor pozitive advanced breast cancer patients) [8,9]. Immunotherapy is the mainstain for the treatment of HER-2 pozitive breast cancer [10]. However, it has been shown that significant percent of HER-2 positive breast cancer does not respond to immunotherapy [11]. Different expression of TOPO2A enzyme in the group of HER 2 positive breast cancer could be one of the reasons of heterogeity. Tumors with mutation of HER-2, instead of HER-2 amplification could be another subgroup [12]. Second and third generations of anti-HER-2

therapies are in clinical studies, as well as so called „dual blocking" of ErbB2 pathway (e.g. anti HER2/neu receptor antibody together with tyrosine kinase inhibitors, as well as different combinations of chemotherapy, HER-2 blocking and blocking of some other signalling pathways by targeted agents [13]. A recent molecular anlyses of triple negative breast cancer (TNBC) revieled that different subgroups can be identified, defined by mesenchymal features, immune system-related genes, DNA damage response genes and activated androgen receptor signalling [14]. Potential novel therapeutic targets will be defined on the basis of this heterogeneity, like PARP inhibitors in deficiencies in the BRCA1 gene pathway [13].

In conclusion, we can say that reliable prognostic and predictive parametars for breast cancer, or biomarkers for identification of different tumor subpopulations are still widely needed [1,15]. A great progress on the way of personalisation in the therapy of breast cancer has been already achieved, but because of enormous genetic instability and heterogeneity, breast cancer is still largely unknown and the subject of numerous investigations.

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Sažetak

Personalizirano liječenje raka dojke – signalni putovi i ciljano liječenje raka dojke

Personalizirana terapija karcinoma dojke obuhvaća optimalni terapijski pristup, uzimajući u obzir pacijentove osobne karakteristike, kao i kliničke karakteristike pojedine maligne bolesti, uključujući patohistološke i molekularne abnormalnosti svakog pojedinog tumora. Razvojem metoda molekularne onkologije omogućeno je genetičko profiliranje svakog individualnog tumora. Pored osnovnih subtipova karcinoma dojke temeljem ekspresije steroidnih receptora, HER-2 receptora i čimbenika proliferacije Ki-67, a zbog velike hetrogenosti i nestabilnosti genoma tumorskih stanica, nalaze se i brojni drugi subtipovi. Neke od tih genetičkih promjena smatraju se tzv. pokretačkim genima odgovornim za poremećenu regulaciju ključnih signalnih puteva u stanici uključenih u staničnu proliferaciju, angiogenezu, apoptozu, invaziju ili metastaziranje. Određene komponente signalnih puteva su ciljne molekule tzv. ciljane biološke terapije. Razumijevanje kompleksnosti mehanizama ovih poremećenih prijenosa signala u tumorskim stanicama ključno je za razvoj novih potencijalnih meta ciljane biološke terapije karcinoma dojke.

***Ključne riječi*:** karcinom dojke; prijenos signala u stanici; personalizirana terapija.

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THE ROLE OF THE PATHOLOGIST IN THE INDIVIDUALISATION OF THERAPY IN PATIENTS WITH BREAST CANCER

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Summary

The diagnosis and treatment of breast cancer has rapidly evolved over the past 20 years. Starting in the 1980s, there have been important alterations in the diagnosis and treatment of breast cancer, having an important impact on the diagnostic procedure employed by pathologists. Numerous studies in recent years have identified many prognostic and predictive factors for breast cancer. Most of them have been determined pathohistologically, which resulted in a large responsibility for pathologists, therefore the pathologist has become a key person in a multidisciplinaty team of breast cancer and the person very responsible for the implementation of specific individual therapy.

Keywords: breast cancer; pathologist; personalised therapy.

Breast cancer is the most common cancer among women in both developing and developed regions in the world. Clinical cancer develops over a long period of time, requires multiple molecular alterations, and involves evolution of cellular populations with increasingly aggressive phenotypic characteristics [1]. Although the time required for the process of carcinogenesis is not well established for any human cancer, estimate suggest that this multistep process unfolds over many years and possibly several decades. Breast cancer represents a diverse collection of malignant diseases of the breast with highly variable clinical behaviors and disparate response to therapy [2].

Personalised oncology is evidence-based, individualised medicine that delivers the right care to the right cancer patient at the right time and results in measurable improvements in outcomes and a reduction on health care costs. The essence of personalised oncology lies in the use of biomarkers. The biomarkers can be from tissue, serum,urin or imaging and must be validated. Also, their have different importances: predictive, prognostic and early response biomarkers.

The diagnosis and treatment of breast cancer has rapidly evolved over the past 20 years. In the first part of the 20th century, treatment of breast cancer consisted of radical mastectomy, but adjuvant systemic treatment and adjuvant radiotherapy did not play a major role. Diagnosis of breast cancer was mostly made based on clinical presentation, later aided by mammography and often combined with frozen section pathology confirmation. Starting in the 1980s, there have been important alterations in the diagnosis and treatment of breast cancer, having an important impact on the diagnostic procedure employed by pathologists.

Histopathological features play an important role in guiding the treatment decisions. In addition, genetic research is starting to have an increasing impact on guiding therapy by providing prognostic and predictive factors [3].

To obtain optimal morphology in the histology sections, and to obtain optimal immunohistochemical staining results, the resection specimen should be cut into thin slices immediately after surgery.

For microscopic examination the pathologist should be obtained and processed for paraffin sections full diameter of the tumour and its surroundings, small part of the tumour to perform immunohistochemistry, if there are macroscopical or radiological abnormalities in the tissue surrounding the invasive tumour, these areas should be sampled. If the surrounding tissue is without abnormalities, it is necessary to take at least two sections from macroscopically normal breast tissue.

On slides stained with hematoxylin eosin (H.E.), pathologist must determine the prognostic and predictive factors for breast cancer. This includes the histological type of cancer [4], the degree of tumour differentiation [5], mitotic counts, lymphovascular invasion, estrogen and progesterone receptors [6], protein HER-2 [7] and proliferative index Ki-67 [8].

Receptors are determined by immunohistochemistry and the results are expressed as the percentage of positive cells and intensity of staining. Stai-

ning for estrogen and progesterone receptor is allways nuclear in localization and in most institutes all patients with a tumour in which more than 10% or more 1% of the tumour cells show positive staining regardless of the intensity of staining are candidates for adjuvant hormonal therapy. According to the consensus of the St Gallen 2014. cut-off of the progesterone receptors is 20%. This value best separating luminal type A from luminal type B breast cancer. Values below 20% indicate that the progesterone receptors are negative or low [9]. When negative staining for estrogen and/or progestrone receptor is seen, it is important to confirm that staining of the hormone receptor-negative case has been successful. This can usually be tested, since the majority of normal breast tissues contain some nuclei ducts and lobules that are positive for estrogen and progesterone receptor. If no normal breast epithelial cells are found to show positive staining, the hormone receptor assays should be repeated on another tumour block.

HER-2 gene amplification is observed in 15-30% of invasive breast cancers and leads to HER-2 receptor overexpression. HER-2 positive invasive breast cancers respond favourable to therapies that specifically target the HER-2 protein, therefore it is very important today to identify candidates for this type of targeted therapy. Several technologies are available for determining HER-2 status, but the two most commonly used are immunohistochemistry [IHC], which measures HER-2 protein expression and CISH [chromogen in situ hybridisation] which detects HER-2 gene amplification a method that is often used today in the pathology than FISH (fluorescence in situ hybridisation). The interpretation of the results is based on the intensity and percentage of stained cells. The most commonly used score system is 0, 1+ (negative results) , 2+ and 3+ (positive results). A 2+ is considered equivocal and should be followed by retesting by CISH. Women with IHC 3+ tumours are candidates for therapy with trastuzumab, but women with 2+ tumour should be retested and if the results show amplification of gene of those are candidates for trastuzumab. To ensure the highest possible accuracy, pathology centers must standardise methodologies and testing procedures.

Proliferative index is also very important and is determined by immunohistochemistry by monoclonal antibody Ki-67. Positive reaction is nuclear reaction and are counted positive nuclei in 1000 tumour cells on the high magnification and the results obtained is expressed as a percentage of positive nuclei. According to St Gallen consensus cut of value is 20% of positive

cells, which means that below this value is low and value above 20% is high proliferative indeks [9].

Based on the receptors, HER-2 status and proliferative index breast cancers are classified immunophenotypically into five subgroups: luminal type A, luminal type B HER-2 negative, luminal type B Her-2 positive, HER-2 positive (non-luminal type) and triple negative tumours. Based on the immunophenotype of the cancer patients receive appropriate therapy. The multigene testing remains inaccesible for the majority of women with early breast cancer, therefore is adopted clinico-pathological testing, now expressed in surrogate IHC-based classification.

Numerous studies in recent years have identified many prognostic and predictive factors for breast cancer. Most of them determined pathohistologically, which resulted in a large responsibility for pathologists. In addition, the pathologist has become a key person in a multidisciplinaty team of breast cancer and the person very responsible for the implementation of specific individual therapy.

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Sažetak

Uloga patologa u individualiziranoj terapiji bolesnica s karcinomom dojke

Unazad 20 godina dijagnostika i liječenje karcinoma dojke snažno je napredovalo. Počevši od 1980. godine kada su počele biti važne promjene u dijagnostici i liječenju karcinoma dojke, sve je to utjecalo na dijagnostički postupak i rad patologa. Brojna novija istraživanja utvrdila su većinu prognostičkih i prediktivnih čimbenika za karcinom dojke. Većina njih određuje se patohistološki a što je u konačnici rezultiralo velikom odgovornošću patologa koji je postao ključna osoba u multidisciplinarnom timu za karcinom dojke i vrlo odgovorna osoba za primjenu specifične individualizirane terapije.

Ključne riječi: karcinom dojke; patolog; personalizirana terapija.

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MOLECULAR DIAGNOSTICS OF HEREDITARY BREAST CANCER

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Summary

Mutations in BRCA1 and BRCA2 genes are associated with the family predisposition to breast and ovarian cancer. The main purpose of genetic testing is an early detection of the predisposition, because even early detection increases the effectiveness of treatment.

Keywords: breast cancer; hereditary breast cancer; BRCA1 and BRCA2 genes; mutations.

INTRODUCTION

Breast cancer is a leading cancer in female population worlwide. Epidemiological data indicate 5-10 % of all breast and /or ovarian cancer cases are hereditary, and germline mutations in BRCA genes account for the majority of hereditary breast nad ovarian cancers [1]. By today, those two genes Breast Cancer Gene 1 (BRCA1) and Breast Cancer Gene 2 (BRCA2) are only known breast cancer predisposing genes, whose mutations can be first sign of familial setting, before cancer occurs. Those genes are tumor suppressors, involved in DNA repair processes, and are the major breast and ovarian cancer susceptibility genes.

The penetrance of deleterious BRCA mutations has been variably estimated; a recent combined analysis of different reports [2] estimates the average cumulative risk (by the age of 70) in BRCA1 mutation cariers to be about 70 % for breast cancer and 40 % for ovarian cancer, whereas the corresponding risk for BRCA2 are 45 % and 11 %. So, person with inherited mutation in

BRCA1 and BRCA2 genes have 45-85% probability of developing breast cancer, and 11-62% probability of developing ovarian cancer by age 70, comparing to general population risk of 10%.

In familial setting, breast cancer occurs at young age and risk of bilateral breast and ovarian cancer is increased. Age of onset in subsequent generations of BRCA mutations carrying families is lower by 7,9 years [3]. Also, carriers of BRCA1 and BRCA2 mutations are at increased risk for other cancers: uterine, cervical, prostate, pancreatic, male breast, bile duct, stomac cancer and melanoma [4]. Breast cancer can occur in men as well, but the risk is 100 times lower than in women.

In general population the overall prevalence of BRCA1 and BRCA2 mutation carriers is estimated to be from 1 in 400 to1 in 800, the quite variable ratio among etnic groups and by geographic region.

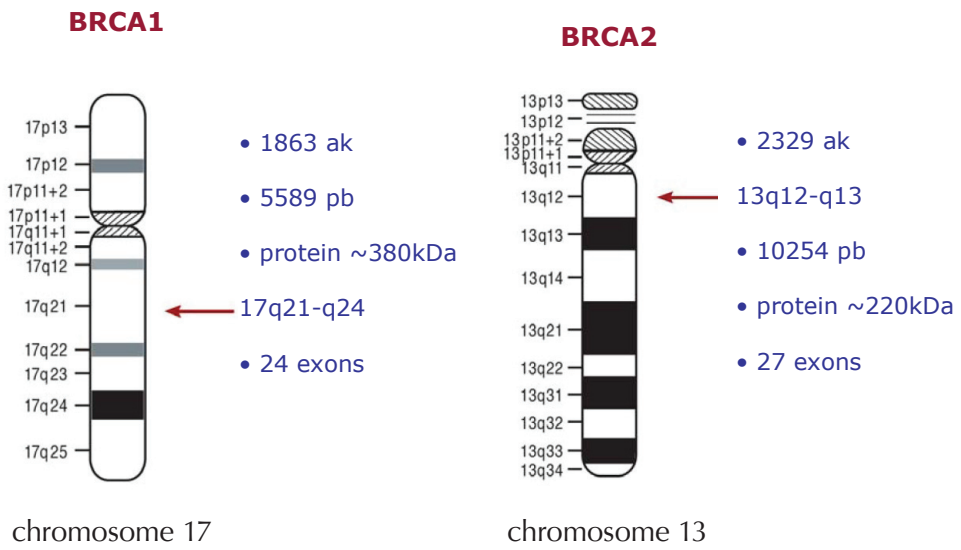

Figure 1. Shematic presentation of the location of BRCA 1 gene mapped on chromosome 17 and BRCA 2 gene on chromosome 13. There are listed also few data about size of the gene, exon number and protein size they code.

Most reported disease-associated alleles of BRCA1 and BRCA2 genes (Figure 1) have been attributed to frameshifts, nonsense or missense mutations, large rearrangements and splice alterations. They usually lead to truncated BRCA1 or BRCA2 protein or affect critical aminoacids for its structure or function. However, a large number of sequence variants, particularly missense variants, routinely encountered in clinical and research laboratories, cannot be readily distinguished as either disease-causing (deleterious) mutations or benign polymorphisms (clinically not significant) and thus are classified as variants of unknown clinical significance.

To date, more than 3600 BRCA1 and BRCA2 variants have been reported in locus-specific databases like Breast Cancer Infromation Core (BIC) - http://research.nhgri.nih.gov/bic/, than Universal Mutation Database (UMD) - http://www.umd.be/BRCA1/ and http://www.umd.be/BRCA2/, Leiden Open Variation Database - http://chromium.liacs.nl/LOVD2/cancer/home.php and kConFab - http://www.kconfab.org/Progress/Mutations.aspx.

Considering these numbers, genetic testing represents invaluable step forward in defynig these malignant disease. The testing is especially important in predicting the risk of developing ovarian cancer, as this kind of cancer is difficult to detect until late, when the chances of cure are less efficient.

Unfortunately, every year in Croatia 2500 women develop breast cancer and 450 develop ovarian cancer, and more than 900 die of breast cancer and almost 400 deaths of ovarian cancer. According to statistical estimates based on 4,3 million Croatian population, at least two hundred breast cancers have familial predisposition, and those estimates follow general worldwide statistics (data from Central Bureau of Statistics of Republic of Croatia and Croatian National Institute of Public Health, 2006). No data on BRCA variants in affected population of Croatia have been gathered so far.

EXPERIENCE WITH GENETIC TESTING IN CROATIA

In Croatia few years ago conditions were appropriate for providing genetic testing of inherited predisposition for breast and ovarian cancer . It was organized in Laboratory for Hereditary Cancer at Rudjer Boskovic Institute thanking to funds of Terry Fox donation and support by Croatian League against Cancer and Europa Donna Croatia.

The screening was performed by high-resolution melting approach, direct sequencing and semi-quantitative multiplex PCR method and provides

a comprehensive analysis on the type and distribution of BRCA1 and BRCA2 mutations and allelic variants [5].

The test entails very complex molecular-genetic methods that have been used in most developed countries for decades. The Laboratory is a member of EMQN (European Molecular Genetics Quality Network), international organization focused on standardizing protocols for molecular genetics diagnostics and on quality assurance of its members. Therefore, the methods used are at the European level of quality.

The main purpose of this testing was early detection of predisposition, because it lowers the cost of treatment as well as declines the need for long hospitalizations, chemotherapy, radiation and multiple surgeries. Even early detection increases the effectiveness of treatment. Targeted and preventive therapy, as well as the lifestyle changes can significantly decrease the mortality.

CANDIDATES FOR TESTING

Each candidate has to undergo genetic counseling before and after testing. The test is mainly for breast and ovarian cancer prone family members and is not informative for sporadic cases of breast and ovarian cancer. BRCA1 and BRCA2 mutations are inheritable with the same probability from either parent.

Potential candidates for BRCA1 and BRCA2 mutation testing are:

- persons with two or more relatives with breast cancer
- persons with early (before age of 50) breast cancer in the family
- persons with breast cancer in the family in more than one generation
- persons with bilateral breast cancer in the family
- persons with multiple ovarian cancer in the family
- persons with early (before age of 40) bilateral ovarian cancer
- persons with one or more relatives with BRCA1 and/or BRCA2 gene mutation in the family.

FIRST STUDY IN CROATIA

The results of BRCA1 and BRCA2 analysis in a group of 168 candidates from Croatia were selected for increased risk of breast and ovarian cancer on the basis of their personal or family history of the diseases. Candidates were

volunteers from several national associations and support groups for breast cancer patients, selected according to the set criteria.

In this study, 168 women from 147 families with positive family or personal history of breast and ovarian cancer were screened for mutations in BRCA1 and BRCA2 genes. All candidates gave their informed consent to perform DNA analysis on their blood samples before the samples were taken. The study was conducted according to the Declaration of Helsinki Principles.

The candidates were recruited from mostly all locations in Croatia (Kaštel Novi, Rijeka, Ploče, Šibenik, Osijek, Zagreb, Dubrovnik, Zadar, Čakovec, Karlobag, Gospić) and were classified into nine categories according to family or personal history of disease. Table 1. shows the specific characteristics of the candidates according to the inclusion criteria.

Table 1. Inclusion criteria for the candidates included in this study (from 6).

Group	Inclusion criteria	Cases (%)
A	affected individual with breast or ovarian cancer before 35 years of age	9 (5.3)
B	affected individual with breast cancer before the age of 35 with at least one case of breast or ovarian cancer in the family	4 (2.4)
C	affected individual with breast or ovarian cancer between the age of 35 and 55 with at least one case of breast cancer in the family	14 (8.3)
D	affected individual with breast or ovarian cancer between the age of 35 and 55 with at least one case of breast and at least one case of ovarian cancer in the family	7 (4.2)
E	unaffected individual with at least two cases of breast cancer in the family	65 (38.7)
F	unaffected individual with at least one case of breast cancer in the family before 50 years of age	39 (23.2)
G	unaffected individual with at least one case of breast and at least one case of ovarian cancer in the family	18 (10.7)
H	unaffected individual with at least one ovarian cancer in the family	8 (4.8)
I	unaffected individual with at least one case of breast cancer and male breast cancer in the family	4 (2.4)
Total		168 (100)

Candidates in group A are considered early onset breast cancer patients, as the usual cut off for early onset is 35 years of age.

From the 168 candidates which participated in this study, 32 (19%, age range 25-58, median 41) were affected with breast cancer, 2 (1.2%, ages 31 and 53) with ovarian cancer, and one with both breast and ovarian cancer (0.6%, ovarian cancer in 51 and breast cancer in 54). The remaining candidates were unaffected (133) but with positive family history (groups E-I).

Mutations in BRCA1 and BRCA2 genes detected

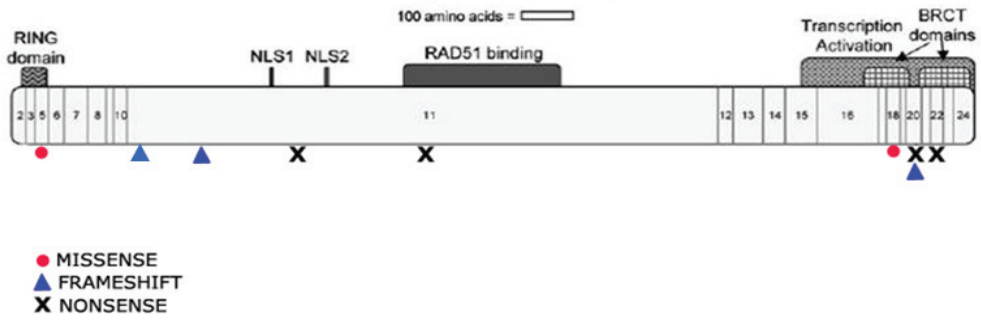

Figure 2. BRCA1 gene shematic presentation with marked type and position of detected mutations.

BRCA2

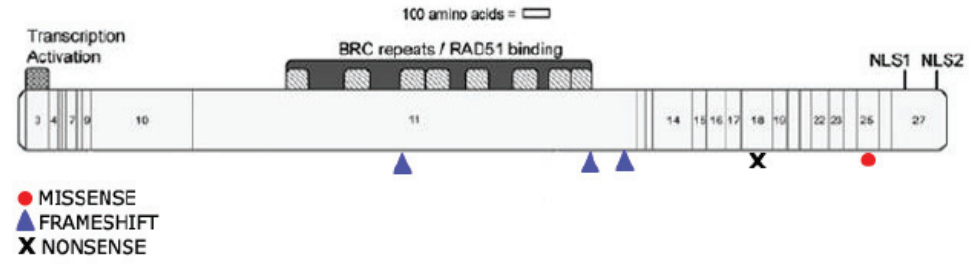

Figure 3. BRCA2 gene shematic presentation with marked type and position of detected mutations.

Al together (in 10 %) genetic testing identified 14 pathogenic mutations in17 candidates, 9 in BRCA1 and 5 in BRCA2 (Figures 1 and 2). Of those, 11 have been previously described and 3 were novel [6].

Nine pathogenic mutations in BRCA1 and five in BRCA2 were detected [6]. Croatia shares most of the mutations with Slovenia and Germany . No founder mutations were detected in Croatia, although one is a candidate. Further analyses on a larger number of samples is necessary.

The benefit of this testing is clear, since mutations were detected in young unaffected women who will be closely monitored.

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Sažetak

Molekularna dijagnostika nasljednog raka dojke

Mutacije u genima BRCA1 i BRCA2 povezuju se sa nasljeđenom sklonošću za nastanak raka dojke i jajnika. Svrha genetičkog testiranja je utvrditi dovoljno rano postoji li predispozicija, čime bi se dovoljno rano mogle poduzeti učinkovite mjere prevencije.

Ključne riječi: rak dojke; nasljedni rak dojke; geni BRCA1 i BRCA2; mutacije gena.

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PERSONALISED CANCER MEDICINE IN COLORECTAL CANCER - A SHORT OVERVIEW

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Summary

In this article, a short overview of the current clinical situation of colorectal cancer (CRC) personalised medicine is presented. CRC is a complex, heterogeneous disease that involves multiple signalling pathways and tumors that appear histologically identical, but may have different prognoses and different responses to treatment. Basically, the treatment for colorectal cancer varies by tumor location, stage at diagnosis, and patient's general condition. Recent newer polychemotherapy protocols, along with the use of inhibitors of the vascular endothelial growth factor (VEGF) and the epidermal growth factor receptor (EGFR) pathways, have enhanced the therapeutic responses and potentially also the patient's prognosis. These recent improvements in anticancer treatments and patient outcome in CRC were followed by a series of biomarker studies attempting to refine prognosis and predict patients who are likely to derive the most benefit from treatment. Consequently, validated predictive and prognostic biomarkers offer potential for personalised therapy for CRC patients. Microsatellite instability (MSI), as well as clinical pathological factors in stage II and III colorectal cancer, may now be considered to be a robust prognostic biomarker in the adjuvant setting. On the other hand, KRAS mutation status should be taken up as a part of routine clinical practice, as a predictive marker for response to EGFR-targeted therapies. The treatment of CRC is expected to become more and more routinely based on identified CRC subtypes and on validated prognostic and predictive biomarkers relatively soon (within several years), which should offer patients better therapeutic outcomes with less side effects.

Keywords: colorectal cancer; biomarkers; personalised medicine; chemotherapy; targeted therapy.

As regards its incidence and mortality, colorectal cancer (CRC) is a relatively common tumor (the third most common tumor) [1-3]. The colorectal cancer treatment strategy is still based on standard clinical and pathological parameters, where the assessment of the progression and spreading of the tumor and general conditions of the patient are given priority [4-7]. On the other hand, clinical observations indicate that this "equal" treatment strategy is not optimal because not all treated patients demonstrate analogous success. Its causes may lie both in patients' genetic differences and in other molecular heterogeneity between histologically "identical" tumors in different patients. In addition, as a result of further accumulation of genetic changes (clonal evolution of tumor) the genetic profile of metastases in a particular patient may be different to the original primary tumor finding, which in turn may affect the outcome of treatment if it is based only on molecular characteristics of the primary tumor. All this indicates that, whenever possible, treatment should be individualized according to patient's pharmacogenetic characteristics and the finding with respect to the predictive genetic alterations in autologous tumor cells. Such individualized approach (personalized cancer medicine) may result in more rational, higher-quality and more effective treatment with less adverse reactions. A genetic analysis and/or profile of both the patients and their tumors may provide information and/or parameters in connection with the pharmacodynamics, pharmacogenetics and sensitivity of tumor cells to potential oncological treatment modalities, which rationalizes the treatment strategy and the potential antitumor effect [6-12].

The pathogenesis of colorectal cancer is complex. The risk of occurrence and occurrence of colorectal cancer depends on the genetic characteristics of the individual (heritage and epigenetic changes), their diet, intestinal flora, and lifestyle. Colorectal cancer is hereditary in less than 5% of patients. For example, in patients with familial adenomatous polyposis, an adenomatous polyposis coli (APC) gene which act as a tumor suppresor gene is mutated and inherited. In case mismatch repair (MMR) genes are mutated and inherited, hereditary non-polyposis colorectal cancer (HNPCC) with microsatellite instability (the Lynch Syndrome) will occur, characterized by high genetic instability. Lynch syndrome follows an autosomal dominant inheritance pattern. People who have Lynch syndrome have a significantly increased risk of developoing colorectal cancer but also an increased risk of developing other types of cancers [6,7,13-18].

In most patients with colorectal cancer (approximately 95%), the impact of heritage is not so high, so we refer to it as sporadic colorectal cancer. Most of these tumors derive from malignant altered adenoma; in addition to genetic mutations, these tumors also demonstrate chromosome instability (with potential loss of tumor suppression genes). This is a so-called phenotype of chromosome instability (CIN) and microsatellite stability (MSS). Among the smaller patient population with sporadic tumors (approximately 15%), malignant alteration is caused by high microsatellite instability (MSI-high), as a result of epigenetic changes or remodulation, which most often result in DNA mismatch repair gene promoter region CpG island hypermethylation (CIMP, CpG Island Methylator Phenotype). These genes are silenced by hypermethylation of the promoter region. Patients suffering from this molecular type of tumor predominantly have a tumor finding in the right colon. As these two "groups" of sporadic tumors have different molecular profiles, that is, mutated genes, number of mutations and cellular molecular activation routes and mechanisms, we are, for the time being, able to identify at least two types of sporadic colorectal cancer. This difference is to a certain degree reflected in the prognosis and strategy of systemic adjuvant treatment. For example, stage II MSI-H patients have a better prognosis than CIN patients, and they seem to have no benefit from adjuvant treatment with 5-fluorouracil (5-FU) [4-7,13-18].

Systemic chemotherapy treatment of metastatic colorectal cancer is based on chemotherapeutic agents 5-FU, irinotecan, and oxaliplatin. Various studies have investigated whether molecular differences between patients can predict response to standard chemotherapy drugs to facilitate a more personalized approach to chemotherapy. Analysis regarding the genes or their products that may be targeted by such cytostatics or may be involved in the metabolism of these cytostatics or in repairing the damage to (tumor) DNA molecule caused by these agents are not in routine use. This might be due to the low number of studies, conflicting results between the studies, but also in not having standardized accepted laboratory procedures. The molecular target of 5-FU is the thymidylate synthase (TS) enzyme. TS is an important part of the folate–homocysteine cycle and purine and pyrimidine synthesis. Tumors with low expression of TS are less proliferative and may therefore be associated with a better prognosis. The metabolism of 5-FU is mediated by thymidine phosphorylase and dihydropyrimidine dehydrogenase (DPD).

Several variants in DPD have been associated with toxicity and DPD deficiency can result in severe and even fatal 5-FU toxicity [4-7,19,20].

Irinotecan is a topoisomerase-1 (Topo1) inhibitor and Topo1 is overexpressed in 43–51% of colorectal cancers. A large randomized FOCUS trial showed that patients with high levels of Topo-1 expression had improved OS with first-line combination chemotherapy compared with patients with low or moderate Topo1 levels. Irinotecan is detoxified by the enzyme "UDP glucuronosyltransferase 1 family, polypeptide A1" (UGT1A1). There is no current evidence of any benefit or harm of modifying irinotecan regimes based on an individual patient's UGT1A1 genotype [4-7,19,20].

The excision nuclease "Excision Repair Cross-Complementing 1" (ERCC1) is involved in the repair of platinum-induced DNA damage and early data suggest that there was an association between low ERCC1 expression and oxaliplatin effectiveness in patients with metastatic colorectal cancer. Enzyme "glutathione S-transferase" (GST) is involved in the oxaliplatin detoxification, and again, the relevance of specific polymorphisms seems clinicaly unclear [4-7,19,20].

On the other hand, predictive analysis is fortunately available for the monoclonal antibodies cetuximab and panitumumab. These two monoclonal antibodies may have an antitumor effect because they inhibit agonist binding to the epidermal growth factor receptor (anti-EGFR treatment) on colorectal cancer cells. Through this effect, these two monoclonal antibodies are able to inhibit the stimulation of tumor cells in case there are no activation mutations in downstream intracellular molecules of this activation pathway. The KRAS molecule is one of these downstream molecules in this molecular pathway. When KRAS gene is mutated, it stimulates this molecular pathway itself, irrespectively of the EGFR blockade. This is why the use of these monoclonal antibodies is conditional upon determining the KRAS and NRAS mutational status in autologous tumor cells. These two monoclonal antibodies can only be used in patients having non-mutated KRAS and NRAS genes. KRAS and NRAS testing is now part of routine clinical practice. The humanized monoclonal antibody bevacizumab inhibits the activity of the vascular endothelial growth factor (VEGF) where we have no predictive parameter or marker [4-7,19,20].

In conclusion, the existence of molecularly different cancer subtypes with different prognoses and potentially different treatment strategies may be said to be confirmed in colorectal cancer. A finding of MSI can be now consi-

dered to be a robust prognostic biomarker in the adjuvant setting, and KRAS and NRAS testing has been taken up as part of routine clinical practice as a predictive marker for response to EGFR-targeted therapies. Personalized medicine is making advances in colorectal cancer [6,7,20,21].

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Sažetak

Personalizirana medicina kolorektalnog raka: kratki pregled

U prikazanom radu dan je kratak pregled kliničke primjene personalizirane medicine kolorektalnog raka. Dijagnoza kolorektalnog raka bazira se na patohistološkim karakteristikama, ali rezultati molekularnih istraživanja ukazuju da se radi o skupini heterogenih tumora, koji se razlikuju u patogenezi, molekularnim aberacijama, prognozi i odgovoru na primijenjeno liječenje. Strategija liječenja raka kolorektuma još uvijek se prvenstveno temelji na smještaju tumora i procjeni uznapredovalosti i proširenosti tumora te općem stanju bolesnika. Razmjerno noviji polikemoterapijski protokoli kao i uporaba inhibitora vaskularnog endotelijalnog faktora rasta i receptora za epidermalni faktor rasta poboljšali su liječenje, a moguće i prognozu bolesnika. Ta novija poboljšanja paralelno prate i klinička ispitivanja s ciljem određivanja potencijalnih prognostičkih i prediktivnih biomarkera. Posljedično, validirani biomarkeri pružaju mogućnost personalizirane medicine za bolesnike s kolorektalnim rakom. Mikrosatelitska nestabilnost zajedno s kliničko-patološkim faktorima u stadiju bolesti II i III smatra se valjanim prognostičkim biomarkerom u strategiji adjuvantnog liječenja bolesnika stadija bolesti II i III. Nadalje, KRAS mutacijski status je parametar koji se mora odrediti ako se planira primjena inhibitora protiv receptora za epidermalni faktor rasta. Za očekivati je da će se unutar nekoliko slijedećih godina liječenje bolesnika s kolorektalnim rakom sve više i više temeljiti na nalazu molekularnih subtipova raka i prema validiranim prognostičkim i prediktivnim parametrima, jer bi takav pristup trebao osigurati bolju terapijsku učinkovitost i manje nuspojava.

Ključne riječi: kolorektalni rak; biomarkeri; personalizirana medicina; kemoterapija; ciljana terapija.

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COLORECTAL CANCER, NOVEL BIOMARKERS AND IMMUNOHISTOCHEMISTRY – AN OVERVIEW

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Summary

Colorectal cancer (CRC) is the most common cancer in Europe and the leading cause of death. A combination of therapy with targeted agents and the knowledge of many biomarkers is significantly increasing to better guide the selection of treatment. Biomarkers that are currently used as predictive and prognostic, as well as factors for therapy selection, are described in this overview. It refers to microsatellite instability (MSI), RAS-family of oncogenes, BRAF, TP53, Ki-67, Oncotype DX_, phosphatidylinositol 3-kinase (PI3K)/AKT, 18q LOH, and CpG island methylator phenotype.

Only a few biomarkers are currently used and in routine reported by pathologists. Future studies need to consider the combination of markers, standardising protocols and, if possible, simple and standardised assays for the detection of molecular markers.

Keywords: colorectal cancer; biomarkers; microsatellite instability; KRAS; BRAF.

INTRODUCTION

Colorectal cancer (CRC) is the most commonly diagnosed cancer in Europe and one of the leading causes of cancer death worldwide [1,2]. In 2008, 436,000 new cases of CRC were diagnosed in Europe and it was responsible for 212,000 (12.2%) deaths representing the second most common cause of cancer death after lung cancer (19.9%) [1]. In Croatia, combined, colon, rectum, rectosigmoid and anal cancers represented 15% in the male and 13% in the female cancer incidence in 2011 [3].

In the past years treatment and outcome of early and advanced disease has steadily improved. Currently, a broad variety of trials and retrospective analyses gave further insights into clinical questions like selection and duration of treatment, combinations with targeted agents and also knowledge of prognostic as well as predictive biomarkers is significantly increasing to better guide selection of treatment.

Therefore pathology report is becoming more complex and in the field of newfound and offered biomarkers it is becoming hard to identify and standardize those with truly predictive and prognostic value.

There are some factors definitively proven to be of prognostic importance based on evidence from multiple published trials and generally used in patient management. These are: the local extent of tumor assessed pathologically (the pT category ofthe TNM staging system of the American Joint Committee on Cancer and the Union Internationale Contre le Cancer [AJCC/UICC]); regional lymph node metastasis (the pN category of theTNM staging system); blood or lymphatic vessel invasion; residual tumor following surgery with curative intent (the R classification of the AJCC/UICC staging system), especially positive surgical margins [4].

Some factors biologically and clinically shown to have prognostic value for outcome and/or predictive value for therapy are also reported by pathologist, although it remains to be validated in comprehensive studies. It includes tumor grade, radial margin status and residual tumor in the resection specimen following neoadjuvant therapy (the ypTNM category of the TNM staging system) [4].

Factors shown to be promising in multiple studies are histologic type, histologic features associated with microsatellite instability (MSI) (ie, host lymphoid response to tumor and medullary or mucinous histologic type), high degree of MSI (MSI-H), loss of heterozygosity at 18q (DCC gene allelic loss), tumor border configuration (infiltrating vs pushing border), DNA content and all other molecular markers, perineural invasion, microvessel density, tumor cell–associated proteins or carbohydrates, peritumoral fibrosis, peritumoral inflammatory response, focal neuroendocrine differentiation, nuclear organizing regions and proliferation indices [4,5].

In recent years, colorectal cancer (CRC) has been divided into different subgroups with distinct precursor lesions, pathways of carcinogenesis, morphological, and molecular characteristics [6]. In spite of a tremendous amount of available literature on biomarkers only a few are nowadays used in da-

ily clinical practice, such as KRAS, BRAF, MSI and the Oncotype DX_ Colon Cancer Assay [7].

BIOMARKERS

Microsatellite instability

There are two forms of genomic instability that reflect different genetic pathways of tumorigenesis. One refers to a clonal change in the number of repeated DNA nucleotide units in microsatellites caused by deletions or insertions, and appears in tumors with deficient mismatch repair (MMR) [8].

The biochemical basis of this phenotype is explained by strand-specific mismatch repair defects and linked to germline mutations of the MMR gene hMSH2 and hMLH1. MSI phenotype is also found in Lynch Syndrome as mutations in PMS2 and hMSH6. If there is a clinical suspicion of Lynch Syndrome (Bethesda Guidelines), MSI testing with molecular screening and/or immunohistochemistry is recommended by the ESMO Consensus [9].

Different mechanism causes the sporadic type of MSI to develop in CRC and it is associated with hMLH1 promoter hypermethylation and lack of hMLH1 expression and subsequently loss of mismatch repair system function. This sporadic type of MSI could be investigated through testing for a BRAF V600E mutation that is strongly associated with a sporadic origin or by analysis of hMLH1 promoter hypermethylation [8,10].

It has been shown that MSI CRC is associated with a better prognosis than non-MSI CRC, but appears to be more pronounced for Lynch Syndrome [8,11,12]. MSI testing in molecular pathology laboratories is becoming increasingly available, but requires expertise and experience in testing and interpretation. Nowadays, immunohistochemistry (IHC) shows high sensitivity and specificity in detecting MSI and could therefore offer a relatively cheap, easy to perform and universally available test for MSI, instead of a more complex polymerase chain reaction (PCR)-based MSI test [13,14].

KRAS

The RAS-family of oncogenes consists of three members involved in tumor development, KRAS, HRAS and NRAS. Active KRAS mutations are found in 35–42% of CRCs and are thought to occur early in CRC carcinogenesis [15]. KRAS is part of the EGFR-signaling pathway downstream to EGFR, a

receptor tyrosine kinase which is activated through extracellular ligand binding. Activation of the pathway ultimately leads to the modulation of angiogenesis, cell migration, proliferation, cell adhesion, metastasis formation, and survival [16, 17]. Differences in KRAS mutations at codon 12 and 13 may result in different biological and functional consequences that could influence the prognosis of CRC. Initially, KRAS was found to be a strong prognostic factor in CRC, but this finding was later restricted to a codon 12 mutation, leading to a glycine to valine substitution (G12V).

American Society of Clinical Oncology recommends that all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations. If KRAS mutation in codon 12 or 13 is detected, then patients should not receive anti-EGFR antibody therapy as part of their treatment [18,19]. The attempt to predict response to EGFR treatment by assessing EGFR expression by immunohistochemistry in analogy to HER2/new in breast cancer turned out to be unsuccessful [6]. Mutation of the KRAS gene results in a constitutively active KRAS protein and mitogen activated protein kinase (MAPK) pathway signaling independent from EGFR [6].

In general, KRAS mutational analyses concentrate on mutations in codon 12 and 13 with commercially available kits such as the 'Therascreen KRAS Mutation Test kit', which make up for 96% of all observed mutations. Other activating mutations have been identified in these codons and, additionally, in codon 61 and 146 of the KRAS gene.

Approximately 1% of tumors with wild type at codons 12 and 13 will have mutations in codon 146 and an additional 7% of these will be mutated in codon 61 (6,8,18,19). These mutations may very well predict resistance to anti-EGFR treatment as may mutations of the Neuroblastoma RAS viral oncogene homolog (NRAS) gene. It remains to be seen, whether expanded mutational analyses of KRAS and NRAS adds substantial additional predictive value [6,8].

BRAF

The BRAF gene encodes a serine/threonine protein kinase belonging to the RAS-RAF-MEK-ERK kinase pathway regulated by KRAS protein activity and involved in CRC development. Nearly all oncogenic transformations of BRAF are the V600E mutations (8,20,21). The frequency of BRAF mutations

in CRC decreases with advancing UICC stage, approximately 8% of all CRC carry a BRAF mutation which is mutually exclusive to KRAS mutations [5,8,22].

After being primarily discussed as a potent predictive marker for resistance to anti-EGFR treatment, BRAF mutation has meanwhile been reported as a marker for poor prognosis in CRC in a number of retrospective analyses of large clinical trials.

The prognostic value of BRAF mutation is obviously influenced by the MSI status. In fact, patients with BRAF mutation and MSI had a favorable prognosis when compared with microsatellite stabile(MSS)/ BRAF wild-type patients [5,8,22,23].

Immunohistochemistry (IHC) can be used in detecting BRAF mutation; it shows high sensitivity and specificity for BRAF V600E mutation.

Other potential biomarkers

TP53 is a tumor suppressor gene on the short arm of chromosome 17, encoding a protein important in regulating cell division. It is aborting growth of potentially malignant cells. Mutations of the TP53 gene are detected in up to 85% of CRCs, usually occurring during the adenoma to adenocarcinoma transition (24,25,26). Lack of consensus on antibodies and scoring methods in immunohistochemical staining, lack of correlation between immunohistochemical overexpression and clinical data and discrepancies between immunohistochemistry and mutation analysis are responsible for conflicting results and are therefore important reasons for not justifying the use of TP53 in clinical practice [8,26,27].

Proliferation and ability to evade apoptosis is one of the most important attributes tumor cells must acquire for tumorigenesis. Ki-67 is used to determine proliferation in tumor cells but lack of uniformity in methodological approach and variations in the interpretation and reporting of pathologic findings are currently the most problematic issues associated with this factor. Further research should focus on combined analysis of proliferation and apoptosis, as a balance might exist between these two hallmarks of cancer [6,8,28-30].

Genomic signatures potentially have a high prognostic value and some are already in use in clinical practice, like Oncotype DX_. Other genomic signatures need to be validated before introducing them in clinical practice, preferably using tissues from randomized clinical trials [6,8,31-33].

Activation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway has been associated with the development of a human CRC, when stratified by KRAS status, a worse colon cancer-specific mortality associated with a PIK-3CA mutation was only found in KRAS wildtype tumors [8,34].

The prognostic value of 18q LOH also remains unclear and validation is necessary to draw further conclusions [35].

The existence of a new pathway for CRC pathogenesis which involves the transcriptional silencing of tumor suppressor genes by hypermethylation of CpG islands of the promoter region of various genes is increasingly studying. These tumors are classified as having the CpG island methylator phenotype (CIMP). CIMP could be used as a prognostic marker, but further research is necessary to confirm and validate these data [35,36,37].

CONCLUSION

Our knowledge of the process of tumorigenesis has been increasing in the past decades and it affects the development of new treatment modalities in human cancer. Only a few biomarkers are currently used and in routine reported by pathologist. Future studies need to consider the combination of markers, standardising protocols and if possible simple, cheap, automated and standardized assays for the detection of molecular markers. Most importantly, results need to be validated in larger studies, followed by prospective trials.

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Sažetak

Kolorektalni karcinom, novi biomarkeri i uloga imunohistokemije

Kolorektalni karcinom najčešći je maligni tumor u zemljama Europe te jedan od vodečih uzroka smrti od raka. Kombinirana terapija s lijekovima koji djeluju ciljano (tzv. pametni lijekovi) te sve više novih saznanja i novootkrivenih biomarkera koje tumor eksprimira značajno je povećala mogućnost selektivne terapije. Biomarkeri koji se trenutno sve više koriste kao prognostički i prediktivni, kao i oni koji su važni za izbor terapije opisani su u ovom pregledu. Među spomenute se najčešće ubraja mikrosatelitska nestabilnost zbog pogreške u popravku gena, RAS-obitelj onkogena, BRAF, TP53, Ki-67, Onkotip DX, fosfatidilinozitol-3 kinaza (PI3K)/AKT, 18q LOH i CpG metilacijski fenotip. Trenutno je u široj upotrebi svega nekoliko markera te se rutinski spominju u patološkom izvještaju.

Buduće studije bi centar istraživanja trebale usmjeriti prema kombinacijama različitih markera, uspostavi standardiziranih protokola te jednostavnih i dostupnih analiza za otkrivanje ekspresije molekularnih markera.

Ključne riječi: kolorektalni karcinom; biomarker; mikrosatellitska nestabilnost; KRAS; BRAF.

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PERSONALISED THERAPY FOR MELANOMA

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Summary

In the therapy of metastatic melanoma, prior to 2011 the only approved treatment option were dacarbazine and interleukin-2, with a small percentage of patients with good response; no study involving these agents had shown an improvement in overall survival. Researches in the last decades have contributed to a better understanding of melanoma. The discovery that BRAF is a driver oncogene in cancer and complementary improvements in our understanding of the immune system have resulted in new targeted and immune-therapies for metastatic melanoma. Targeted therapies can achieve impressive clinical results in large number of patients, but the resistance to the therapy is often present. Immune therapy can achieve long-term remission and cures, yet in a smaller proportion of patients, and we still have no biomarkers to predict which patients will respond. Nevertheless, melanoma has led the evolution of cancer treatment from relatively non-specific cytotoxic agents to highly selective therapies, which offer an improvement in the outcome for melanoma patients. Still, many open questions remain: how to avoid resistance to therapy; how to find biomakers to predict answer to therapy; and how to find the optimal treatment options for patients who relapse or do not respond.

Keywords: metastatic melanoma; immunotherapy; targeted therapy.

INTRODUCTION

Metastatic melanoma has a poor prognosis. The median survival for patients with stage IV melanoma ranging from 8 to 18 months after diagnosis,

depending on the substage. Few patients have a response to systemic therapies [1].

The only chemotherapeutic agent approved by the Food and Drug Administration for the treatment of metastatic melanoma for many years was dacarbazine. In phase 3 studies, dacarbazine was associated with a response rate of 7 to 12% and a median overall survival of 5.6 to 7.8 months after the initiation of treatment. Higher response rates can be achieved with combination chemotherapy, but these combinations have not resulted in improved rates of overall survival. Some other agents were used to treat metastatic melanoma- temozolomide, fotemustine, carboplatin, paclitaxel, and interleukin-2 and demonstrated limited efficacy, and no study involving these agents had shown an improvement in overall survival [1-5].

BRAF INHIBITOR

The era of targeted therapy in melanoma began with the identification of driver mutations in the serine threonine kinase *BRAF*. Approximately 40-60% of melanomas harbor activating (V600) mutations in the serine–threonine protein kinase B-RAF (BRAF). Melanomas carrying a BRAF V600E mutation constitutively activate the mitogen-activated protein kinase (MAPK) pathway, promoting cell proliferation and preventing apoptosis [6].

Vemurafenib was developed as a potent kinase inhibitor with specificity for the BRAF V600E mutation within cancer cells. It has marked antitumor effects against melanoma cell lines with the BRAF V600E mutation but not against cells with wild-type BRAF [7].

The oral BRAF inhibitor vemurafenib frequently produced tumor regressions in patients with BRAF V600–mutant metastatic melanoma. A phase 1 trial established the maximum tolerated dose to be 960 mg twice daily and showed frequent tumor responses [8].

A phase 2 trial involving patients who had received previous treatment for melanoma with the BRAF V600E mutation showed a confirmed response rate of 53%, with a median duration of response of 6.7 months [9].

A phase 3 randomized clinical trial comparing vemurafenib with dacarbazine in 675 patients with previously untreated, metastatic melanoma with the BRAF V600E mutation. Patients were randomly assigned to receive either vemurafenib (960 mg orally twice daily) or dacarbazine (1000 mg per square meter of body-surface area intravenously every 3 weeks). At 6 months, overall survival was 84% (95% confidence interval [CI], 78 to 89) in the ve-

murafenib group and 64% (95% CI, 56 to 73) in the dacarbazine group. In the interim analysis for overall survival and final analysis for progression-free survival, vemurafenib was associated with a relative reduction of 63% in the risk of death and of 74% in the risk of either death or disease progression, as compared with dacarbazine (P<0.001 for both comparisons). Response rates were 48% for vemurafenib and 5% for dacarbazine. Benefit was seen in all subgroups of patients who were included in the analysis, including patients with stage M1c disease or an elevated lactate dehydrogenase level, both of which are associated with particularly poor prognoses [10].

Similar to the first selective serine/threonine-protein kinase B-raf inhibitor vemurafenib, another selective inhibitor **dabrafenib** is highly efficacious in melanoma patients with BRAF V600E mutations, with response rates of approximately 50% and progression-free survival of 6 months. There is data to suggest that dabrafenib not only shows activity in V600E-mutated melanoma, but also in non-V600E BRAF mutated disease such as V600K. Dabrafenib, an inhibitor of mutated BRAF, has clinical activity with a manageable safety profile in studies of phase 1 and 2 in patients with BRAF(V600) mutated metastatic melanoma. In fase 3 randomised controlled trial dabrafenib (187 patients) or dacarbazine (63 patients). Median progression-free survival was 5,1 months for dabrafenib and 2,7 months for dacarbazine, with a hazard ratio (HR) of 0,30 (95% CI 0·18-0·51; p<0·0001) [11].

MEK INHIBITOR

As was already told, the most commonly observed BRAF mutation, V600E, and the next most common, V600K, account for 95% of the BRAF mutations found in all patients with cancer. Activated BRAF phosphorylates and activates MEK proteins (MEK1 and MEK2), which then activate downstream MAP kinases. The MAP kinase pathway is known to regulate proliferation and survival of tumor cells in many cancers.9 In preclinical models of human melanoma, selective BRAF and MEK inhibitors have inhibited growth and induced cell death in tumors bearing BRAF mutations [7].

Trametinib is an orally available, small-molecule, selective inhibitor of MEK1 and MEK2. In the phase 3 open-label trial, 322 patients who had metastatic melanoma with a V600E or V600K BRAF mutation were assigned to receive either trametinib, an oral selective MEK inhibitor, or chemotherapy in a 2:1 ratio. Patients received trametinib (2 mg orally) once daily or intravenous

dacarbazine (1000 mg per square meter of body-surface area) or paclitaxel (175 mg per square meter) every 3 weeks. Median progression-free survival was 4.8 months in the trametinib group and 1.5 months in the chemotherapy group (hazard ratio for disease progression or death in the trametinib group, 0.45; 95% confidence interval [CI], 0.33 to 0.63; P<0.001). At 6 months, the rate of overall survival was 81% in the trametinib group and 67% in the chemotherapy group despite crossover (hazard ratio for death, 0.54; 95% CI, 0.32 to 0.92; P=0.01). Rash, diarrhea, and peripheral edema were the most common toxic effects in the trametinib group and were managed with dose interruption and dose reduction; asymptomatic and reversible reduction in the cardiac ejection fraction and ocular toxic effects occurred infrequently. Secondary skin neoplasms were not observed. Trametinib, as compared with chemotherapy, improved rates of progression-free and overall survival among patients who had metastatic melanoma with a BRAF V600E or V600K mutation [12].

BRAF + MEK INHIBITOR

As with most targeted therapies that block a driver oncogene, cancer cells can develop acquired resistance. Resistance to BRAF inhibitors in melanoma is complex and mediated through multiple mechanisms with heterogeneous patterns of progression observed. The currently available data have indicated that the MAPK pathway is reactivated in resistant tumors. Some initial investigations suggest that reactivation of the MAPK pathway through the emergence of truncated hyperactive forms of BRAF, [13] secondary mutations in NRAS (the neuroblastoma RAS viral oncogene homologue) [14] or MEK (MAP kinase kinase), [15] up-regulation of COT (also known as TPL2 or MAP3K8), [16] or activation of alternative survival pathways induced by in-creased expression of receptor tyrosine kinases but not by secondary point mutations in BRAF32,35 are all mechanisms of resistance [17].

In vitro, MAPK signalling recovers rapidly following BRAF inhibition, in part through the relief of feedback inhibition in the pathway and an increased sensitivity to growth factors such as epidermal growth factor (EGF), neuregulin (NRG-1), hepatocyte growth factor (HGF) and fibroblasts growth factor (FGF). [17] In this context, reactivation of MAPK signalling following BRAF inhibition is important for therapeutic escape with increased levels of cell death and tumour regression being seen when BRAF and MEK are co-targeted [18-19].

Clinical trials have confirmed these preclinical observations with the BRAF/MEK inhibitor combination (**dabrafenib plus trametinib**) showing an increased PFS compared with BRAF inhibitor alone. In open-label study (Flaherty 2012.)162 patients were randomly assigned to receive combination therapy with dabrafenib (150 mg) plus trametinib (1 or 2 mg) or dabrafenib monotherapy. The combination therapy 150/2 (full-dose) group had significantly longer progression-free survival than did the monotherapy group (hazard ratio, 0.39; 95% CI, 0.25 to 0.62; P<0.001). The percentage of patients who were alive and progression-free at 1 year was also substantially higher (41% vs. 9%, P<0.001). The extent of tumor regression was also greater in the combination 150/2 group, with an objective response rate of 76%, as compared with 54% with monotherapy (P = 0.03). In addition, the median duration of response was substantially improved with combination therapy, as compared with dabrafenib monotherapy (10.5 months vs. 5.6 months) [20].

Another BRAF/MEK inhibitor combination **vemurafenib+cobimetinib** also appears promising, with newly released data from the BRIM-7 trial demonstrating an 87% confirmed response rate by RECIST and a median PFS of 13.7 months [21].

In treating *BRAF*-mutant melanoma a lot of progress has been made in developing oncogene directed therapies. With BRAf inhibitor response has been reported in about 25% and 50% of patients, and the median duration of response is about 6–7 months.10 Combination therapy with BRAF and MEK inhibitors results in an objective response rate of 76% and extends progression-free survival (PFS), but most patients develop resistance to these inhibitors. [20]. The major problem that limits the long-term responsiveness of the patients is resistance to these drugs. Current strategies to improve the durability of response are now focused on the development of personalized combination therapy strategies, the majority of which point upon the suppression of adaptive MAPK and PI3K/AKT signaling. At this time, the relationship between the genetic prolife of the tumor and patterns of adaptive signaling are not well understood. Better assays and biomarkers will be needed to explore the early treatment responses. The analysis of circulating tumor cells, circulating tumor DNA and proteomic methods are the strategies that are exploring [22].

CTLA-4 IMMUNOTHERAPY

Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) is an immune check-point molecule that down-regulates pathways of T-cell activation. **Ipilimumab**, is a fully human monoclonal antibody (IgG1) that blocks CTLA-4 on lymphocytes to promote antitumor immunity. [23] Ipilimumab has recently been associated with superior overall survival, with median overall survival of 10.1 months among previously treated patients and 11.2 months among previously untreated patients [24-25].

In the study leading to approval of ipilimumab, patients with unresectable stage III or IV melanoma, whose disease had progressed while they were receiving therapy for metastatic disease, were randomly assigned, in a 3:1:1 ratio, to receive ipilimumab plus gp100 (403 patients), ipilimumab alone (137), or gp100 alone (136). Ipilimumab, at a dose of 3 mg per kilogram of body weight, was administered with or without gp100 every 3 weeks for up to four treatments. The results showed median overall survival was 10.0 months among patients receiving ipilimumab plus gp100, as compared with 6.4 months among patients receiving gp100 alone (hazard ratio for death, 0.68; P<0.001). The median overall survival with ipilimumab alone was 10.1 months (hazard ratio for death in the comparison with gp100 alone, 0.66; P=0.003). No difference in overall survival was detected between the ipilimumab groups (hazard ratio with ipilimumab plus gp100, 1.04; P=0.76). Grade 3 or 4 immune-related adverse events occurred in 10 to 15% of patients treated with ipilimumab and in 3% treated with gp100 alone. There were 14 deaths related to the study drugs (2.1%), and 7 were associated with immune-related adverse events [24].

In the study with previously untreated metastatic melanoma, 502 patients were assigned to ipilimumab (10 mg per kilogram) plus dacarbazine (850 mg per square meter of body-surface area) or dacarbazine (850 mg per square meter) plus placebo, given at weeks 1, 4, 7, and 10, followed by dacarbazine alone every 3 weeks through week 22. Overall survival was significantly longer in the group receiving ipilimumab plus dacarbazine than in the group receiving dacarbazine plus placebo (11.2 months vs. 9.1 months, with higher survival rates in the ipilimumab-dacarbazine group at 1 year (47.3% vs. 36.3%), 2 years (28.5% vs. 17.9%), and 3 years (20.8% vs. 12.2%) (hazard ratio for death, 0.72; P<0.001). Grade 3 or 4 adverse events occurred in 56.3% of patients treated with ipilimumab plus dacarbazine, as compared

with 27.5% treated with dacarbazine and placebo (P<0.001). No drug-related deaths or gastrointestinal perforations occurred in the ipilimumab-dacarbazine group [25].

However, the majority of patients do not have a response to anti-CTLA4 antibody therapy and still need effective therapeutic options.

ANTI PD-1

In patients with ipilimumab-refractory melanoma the distinct mechanism of action of anti-programmed-death-receptor-1 (PD-1) antibodies, might have activity [26].

PD-1 is expressed on antigen-stimulated T cells and induces downstream signalling that inhibits T-cell proliferation, cytokine release, and cytotoxicity. Melanoma and many other tumors suppress cytotoxic T-cell activity by expressing PD-1 ligand (PD-L1) on the cell surface. Anti-PD-1 and PD-L1 antibodies can reverse this T-cell suppression and induce long-lasting antitumour responses in patients with advanced solid tumors, including advanced melanoma [26].

Pembrolizumab (MK-3475, previously known as lambrolizumab) is a highly selective, humanised monoclonal IgG4-kappa isotype antibody against PD-1 that has shown strong clinical activity with an acceptable safety profile. In phase I trial pembrolizumab at a dose of 2 mg/kg or 10 mg/kg every 3 weeks might be an efective treatment in patients who progresed to ipilimumab [27].

CONCLUSION

In melanoma we testify the evolution of cancer treatment from nonspecific cytotoxic agents to highly selective therapies. Recent advances in treatment of melanoma: the mitogen-activated protein kinase pathway inhibitors vemurafenib, dabrafenib, trametinib, anti-cytotoxic T-lymphocyte-associated-antigen-4 (CTLA-4) antibody ipilimumab, anti PD-1 pembrolizumab offer the patients improvements in outcomes. But still some challenges remain: how to avoid resistace to therapy, find the biomakers to predict answer to therapy and find the optimal treatment options for patients who relapse or do not respond.

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Sažetak

Personalizirano liječenje melanoma

U liječenju metastatskog melanoma prije 2011. godine, jedina dva odobrena lijeka bili su dakarbazin i interleukin 2., s malim postotkom bolesnika u kojih je zabilježen dobar odgovor. Niti jedna studija s tim lijekovima nije pokazala učinak na ukupno preživljenje. Istraživanja posljednjih desetljeća pridonijela su boljem razumijevanju melanoma. Otkriće BRAF mutacije kao važnog onkogena u melanoma i istovremeno razvijanje lijekova s djelovanjem na imunološki sustav, rezultirali su novim ciljanim i imunološkim terapijama za liječenje metastatskog melanoma. Ciljana terapija postiže impresivne kliničke rezultate u velikom broju bolesnika, ali se često razvija rezistencija na terapiju. Imunološka terapija postiže dugotrajnu remisiju, ali u manjem postotku pacijenata, a još uvijek nemamo biomarkere za predviđanje koji su to pacijenti koji će reagirati. Ipak, u terapiji melanom svjedočimo evoluciji u liječenju raka od relativno nespecifičnih citotoksičnih lijekova do vrlo selektivne terapije koja nudi poboljšanje u ishodu liječenja za pacijente s melanomom. Ipak, puno je još otvorenih pitanja: kako izbjeći pojavu rezistencije na liječenje, naći biomarkere kao prediktore dobrog odgovora na terapiju i utvrditi optimalan način liječenja za bolesnike nakon progresije i za one koji ne reagiraju na ove terapije.

Ključne riječi: metastatski melanom; imunoterapija; ciljana terapija.

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PROGNOSTIC BIOMARKERS IN MELANOMA

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Summary

Biomarkers are tumour- or host-related factors that correlate with tumour biological behaviour and patient prognosis. Modern analytical techniques have identified numerous possible biomarkers, but their relevance to melanoma progression, clinical outcome and the selection of optimal treatment strategies still needs to be established. In this review, we discuss common predictive biomarkers of melanoma.

***Keywords*:** biomarkers; melanoma; melanoma prognosis.

Malignant melanoma is one of the most aggressive malignancies in human and is responsible for almost 60% of lethal skin tumors. Its incidence has been increasing in white population in the past two decades. Melanoma metastasizes quickly and only 14% of patients with metastatic disease can expect to live for 5 years [1,2].

Melanoma is a very enigmatic and heterogeneous cancer. There is a complex interaction of environmental and endogenous, including genetic, risk factors in developing malignant melanoma [3]. Deregulation in oncogenes and tumour suppressors, as well as multiple molecular signals, are required for melanoma initiation and progression, leading to a range of interacting pathways. Attempts are ongoing to unravel this complex network, thus allowing the identification of novel genetic and molecular biomarkers, as well as potential therapeutic targets [4].

Current prognostic markers based on the conventional American Joint Committee on Cancer (AJCC) staging system (TNM) are Breslow tumour

thickness, presence of ulceration, mitotic count and extent of nodal involvement for primary cutaneous melanoma, as well as serum lactate dehydrogenase (LDH) and site of metastases for distant metastatic disease [1,5].

Biomarkers are tumour or host related factors that correlate with tumour biological behaviour and patient prognosis. In a very general sense, a biomarker describes any measurable diagnostic indicator that is used to assess the risk or presence of disease. Although longstanding, the quest to identify relevant and useful biomarkers for cutaneous melanoma, assessed by either serum or immunohistochemistry, has yielded few results. Biomarkers in melanoma may serve a variety of purposes, they may serve as a surrogate for identifying present disease burden, as with lactate dehydrogenase (LDH), identifying patients with more aggressive disease, and/or determining disease responsiveness to various therapies [4,5,6].

An explosion of molecular information over the years has unveiled an array of candidate biomarkers for enhanced prognosis and outcome prediction. More than 100 studies have published experiments using DNA microarrays to investigate

the gene expression profiles found in melanoma. Most expression studies designed to investigate the molecular mechanisms associated with melanoma progression used melanoma cell lines or metastatic tumour samples. Although many candidates have been reported, few have proven reliability or predictability at present to allow for routine use. Serum biomarkers are assessed by the peripheral blood, whereas immunohistochemical biomarkers may be evaluated on formalin-fixed paraffin-embedded tissue [7].

Modern personalised medicine intends to use individual molecular markers and patterns of markers to subdivide traditional tumour stages into subsets that behave differently from each other.

As early as in 1954, increased levels of **LDH** (*Lactate dehydrogenase*) were detected in serum of melanoma patients ever since, the value of LDH as a tumour marker for malignant melanoma has been discussed. LDH is of medical significance because it is found extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease [8].

LDH was reported to be an indicator for liver metastases, with a respective sensitivity and specificity of 95% and 83% in stage II patients, and 87% and 57% in stage III patients. Patients with abnormal LDH levels had a significantly decreased survival. Taken together, increasing evidence exists to

demonstrate that LDH is elevated in advanced disease, predominantly in cases with liver metastases. LDH might serve as a prognostic factor in late-stage malignant melanoma. This has been discussed in a study where LDH was evaluated in combination with other tumour markers such as S100B and MIA and identified, by multiple logistic regression analysis, as the only statistically significant marker for disease progression. LDH has been included in the AJCC staging system, and patients with distant metastases and elevated LDH are considered stage IV M1c [9,10].

The best-studied melanoma biomarker is currently **S100B**. First described in 1980 in cultured melanoma cells, S100B has quickly become a well-established and widely used immunohistochemical marker of pigmented skin lesions. S-100B protein is a 21-kd thermo-labile acidic dimeric protein consisting of two beta subunits, which was originally isolated from the CNS [11]. In 1995, a first study was published evaluating the clinical significance of serum S100B in melanoma. The study showed that observed death ratio was markedly increased with increasing concentrations of S100B ($P < 0.001$). In other studies, baseline serum S100B protein concentrations correlated with prognosis and stage, rising concentrations of serum S100B indicated progression of the disease and complete decline in serum S100B concentrations reflected remission [12,13].

Although determination of serum biomarkers such as LDH and S100B may have a prognostic value, it does not translate into an adequate therapeutic intervention and survival benefit due to limited efficacy of current treatment options in advanced melanomas.

MIA (*Melanoma-inhibiting activity*) was identified in the early 1990s as a soluble 11 kDa protein with growth-inhibiting activities secreted from malignant melanoma cells. The fact that it was strongly expressed in malignant melanocytic tumours, but not in benign human skin melanocytes or benign melanocytic nevi, indicated that MIA may represent a novel tumour marker for malignant melanoma [14,15].

TA90-IC (*Tumour-associated antigen 90 immune complex*) is a 90kD glycoprotein found in the serum and urine of 63% to 68% patients with melanoma. Since TA90 binds to endogenous anti TA90 monoclonal antibody immune complex (TA90-IC) may be detected in the serum of patients with melanomom by ELISA assay. Multivariate regression analysis revealed that TA90IC was an independent predictor of survival when elevation occurred between 2 weeks and 3 months, whereas MIA was an independent predictor

appearing at 4–6 months. In general, elevation of TA90IC preceded increase of MIA in patients who developed recurrence. Additional studies in populations not receiving vaccines will further clarify the clinical utility of these assays [16,17].

YKL-40 is a heparin- and chitin-binding lectin secreted by activated neutrophils and macrophages during the late stages of differentiation, but also by arthritic chondrocytes, differentiated vascular smooth muscle cells and fibroblast-like synovial cells. Elevated serum levels of YKL-40 are seen in a number of non- malignant diseases characterised by inflammation and remodelling of the extracellular matrix, and were shown to be an independent prognostic factor for poor survival in patients with cancer of the breast, colon, ovary, kidney and lung. Study analysis showed that serum YKL- 40 ($P = 0.004$) and serum LDH ($P = 0.004$) were independent prognostic factors for survival. A combination variable of elevated serum YKL-40 and LDH quadrupled the risk of early death ($P < 0.001$) compared with that of patients with normal levels of the markers. The use of serum YKL-40 has not received Food and Drug Administration approval for use as a biomarker for cancer [18,19].

Melanoma is a complex genetic disease, and multiple genetic alterations have been reported to play a role during disease progression. The mitogen-activated protein (MAP) kinase pathway is an important driver in melanoma and is made up of several potential targets providing therapeutic options. In this pathway, the activation of RAS proteins stimulates the RAF kinases ARAF, BRAF, and RAF1. This process causes phosphorylation of the MEK kinases, which phosphorylate the ERK kinases. Activated ERK regulates cyclin D1, which, in turn, regulates multiple cellular processes involved in cell division. Dysregulation of BRAF signaling has been shown to be one of these key drivers of the disease [20,21].

In 2002, Davies et al. first reported that **BRAF i**s mutated in approximately 8% of human tumors, most frequently in melanoma where the $BRAF^{V600}$ mutation is observed in approximately 50% of tumors. Mutations in $BRAF^{V600E}$ may cause the protein to become oncogenic. In preclinical studies, oncogenic BRAF signaling that is a result of this mutation may lead to increased and uncontrolled cell proliferation and resistance to apoptosis (programmed cell death) [22].

Drugs that treat cancers driven by *BRAF* have been developed. Two of these drugs, vemurafenib and dabrafenib are approved by FDA for treatment of late-stage melanoma. Vemurafenib (PLX4032) was the first drug to come

out of fragment-based drug discovery. BRAF V600E mutations are associated with increased sensitivity to BRAF inhibitors [23].

The novel BRAF V600E mutant-specific antibody, VE1 is currentlly used to detect the presence of the BRAF V600E mutation in patients with metastatic melanoma on paraffin-embedded, formalin-fixed melanoma biopsies. The antibody had a sensitivity of 97% and a specificity of 98% for detecting the presence of BRAF in immunohistochemical (IHC) analysis (*Figures 1, 2*). Clinical use of the V600E BRAF antibody should be a valuable supplement to conventional mutation testing and allow V600E mutant metastatic melanoma patients to be triaged rapidly into appropriate treatment pathways [24].

Since the discovery of $BRAF^{V600E}$ mutations in melanoma in 2002, scientists and clinicians have learned much about the role of mutated $BRAF^{V600E}$, but many questions remain unanswered and research is ongoing. The rapidly increasing incidence of melanoma, coupled with its highly aggressive metastatic nature and limited current treatment options, make this an active and exciting area of research.

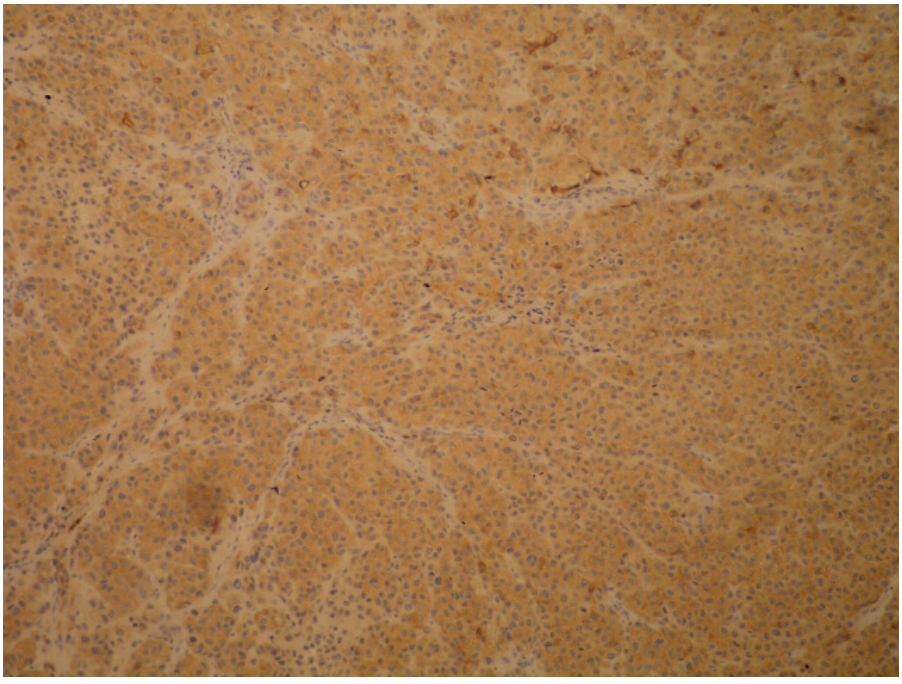

Figure 1. Metastatic melanoma positive with BRAF V600E (dot-like cytoplasmatic positivity X200).

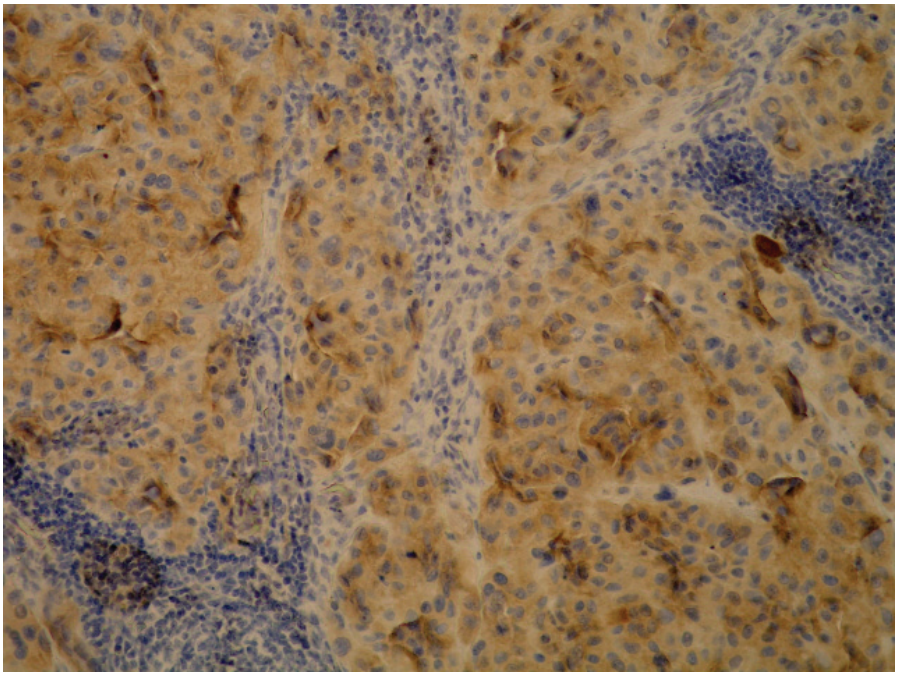

Figure 2. Metastatic melanoma in lymph node positive with BRAF V600E (dot-like cytoplasmic positivity X400).

Current molecular information indicates that melanoma should be viewed as a heterogeneous group of disorders with molecularly distinct defects in important cellular processes that include cell cycle regulation, cell signalling, cell adhesion, cell differentiation and cell death. The heterogeneity of these molecular signatures has two important implications: first, it accentuates the need for individualisation of melanoma diagnosis, prognosis and treatment; and second, it provides an array of potential biomarkers and novel putative drug targets to attain this individualisation. Careful dissection of melanoma into more homogeneous subgroups may be essential for identification of treatment benefits in specific subcategories of patient. At present, only LDH has been included in the AJCC staging system, no identified potential biomarker has undergone a large, rigorous, prospective trial with multivariate analysis that would allow it to be fully validated and developed for clinical practice. As such, there still remains an acute need for such markers in melanoma [25].

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Sažetak

Prognostički biomarkeri melanoma kože

Biomarkeri su faktori vezani za tumor ili domaćina koji koreliraju s biološkim ponašenjem tumora ili prognozom bolesnika. Moderne analitičke tehnike su dosada otkrile brojne moguće biomarkere, ali njihova važnost u razvoju i progresiji melanoma kože kao i kliničkom ishodu bolesti i odabiru najbolje terapije tek se treba utvrditi. U ovom preglednom članku navedeni su najčešće korišteni biomarkeri melanoma kože.

***Ključne riječi*:** biomarkeri; melanom kože; prognoza.

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MOLECULAR MARKERS FOR PERSONALISED APPROACH TO PATIENTS WITH MELANOMA

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Summary

The year 2011 was a breakthrough in melanoma treatment with two new targeted drugs, ipilimumab and vemurafenib, approved after showing overall survival benefit in patients with metastatic melanoma. Vemurafenib was approved only for treatment of patients with activating mutations in *BRAF* gene. Thus, the detection of activating *BRAF* mutations, which can be found in ≈50% melanomas, became a standard part of a routine protocol for the treatment of patients with metastatic melanoma. In this review, methods and protocols for the detection of *BRAF* mutations as an example of molecular marker for personalised approach to patients with melanoma are discussed, with an emphasis on the aspects that are still a matter of discussion and controversies. In addition to *BRAF* mutations, some other molecular markers for personalised approach to melanoma patients, such as predictive markers for ipilimumab therapy that are still not used routinely, are briefly discussed.

Keywords: melanoma; BRAF mutations; vemurafenib; ipilimumab; predictive markers; personalised medicine.

INTRODUCTION

Melanoma of the skin is the fifth most common cancer in the USA with the incidence rate of 21.3 per 100000 people per year [1]. The incidence of melanoma of the skin has been rising constantly with the average increase of 1.8% each year over 2002-2011 [1]. Unlike the incidence, the mortality was stable for the same period, the mortality rate being 2.7 per 100000 people per

year [1]. In the Republic of Croatia the incidence rate age-standardized to European Union population was 12.3 per 100000 in 2011 [2]. The discrepancy between incidence and mortality rate can be attributed to high survival rate for non-metastatic melanoma, the 5-year survival rate for localized melanoma being 98.1% [1]. However, the 5-year survival rate for melanoma patients with distant metastases is only 16.1% [1]. The poor outcome of patients with metastatic melanoma is mostly due to inefficient therapeutic options that were available until recently. The only two drugs approved and widely used for treating metastatic melanoma until 2011 were dacarbazine and interleukin-2, but neither of them showed improved overall survival benefit [3].

TARGETED THERAPY FOR MELANOMA

In 2011, two targeted therapy drugs, ipilimumab and vemurafenib, were approved for the treatment of metastatic melanoma. Unlike dacarbazine and interleukin-2, both ipilimumab and vemurafenib have shown overall survival benefit in patients with metastatic melanoma [4-6].

Ipilimumab, an immunotherapy drug, is a monoclonal antibody that targets immune checkpoint by binding specifically to cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 is an inhibitory receptor on T cells that binds CD80 and CD86 molecules on antigen-presenting cells during T-cell activation [7]. Blockade of CTLA-4 with specific antibodies like ipilimumab results with increased T cell activation and proliferation, and consequently with improved immune response against melanoma antigens [7]. Approval of ipilimumab for the treatment of unresectable or metastatic melanoma by the U.S. Food and Drug Administration (FDA) in 2011 was based on phase III clinical trial in which previously treated metastatic melanoma patients treated with ipilimumab and glycoprotein 100 (gp100) vaccine had improved survival compared to patients treated only with gp100 vaccine [4]. In another phase III randomized trial on previously untreated metastatic melanoma patients, median overall survival was significantly longer in patients treated with ipilimumab and dacarbazine compared to patients treated with only dacarbazine (11.2 months vs 9.1 months) [5]. In these clinical trials only 10-15% of melanoma patients responded to ipilimumab according to response evaluation criteria in solid tumors (RECIST) [4,5]. However, the response to ipilimumab treatment is different from typical response to cytotoxic cancer drugs. In some patients disease progression or development of new metastatic le-

sions was observed prior to ipilimumab induced disease control associated with improved survival [4,5,8]. Therefore different set of response criteria, immune-related response criteria (irRC), was developed as more appropriate to assess ipilimumab response compared to standard RECIST criteria [8]. The survival benefits in melanoma patients responding to ipilimumab seem to be long lasting. Pooled analysis of long-term survival data from different ipilimumab clinical trials has shown 22% three-year overall survival rate with survival curve reaching plateau around 3 years that extends through at least 10 years [9]. In clinical trials ipilimumab treatment was associated with more frequent and sometimes severe toxicities, notably specific immune-related adverse events [4,5]. Other targeted immunotherapy drugs have also been studied in melanoma patients. Most notable of them are drugs targeting programmed death 1 (PD-1) receptor, another inhibitory regulator of T cells, and it's ligand PD-L1 [10]. Examples of such drugs that have shown promising results in clinical trials on melanoma patients are nivolumab, lambrolizumab, and BMS-936559 [10-12].

Vemurafenib is a small-molecule inhibitor of V600 mutated BRAF kinase [13]. It was approved by FDA in 2011 for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation. In BRIM3 phase III trial on previously-untreated patients with BRAF (V600) mutation-positive metastatic melanoma both overall survival and progression free survival were significantly longer in patients treated with vemurafenib compared to patients treated with dacarbazine [6,14]. In that trial 57% of patients responded to vemurafenib treatment. In 2013 another small-molecule inhibitor of V600 mutated BRAF kinase, dabrafenib was approved by FDA for the treatment of treatment of BRAF V600E mutation-positive unresectable or metastatic melanoma. The approval was based on improved progression-free survival shown in a phase III trial comparing dabrafenib to dacarbazine [15]. In that trial 50% of patients responded to dabrafenib treatment [15]. In addition to other side effects, a significant proportion of patients treated with these BRAF inhibitors have developed cutaneous squamous-cell carcinomas (SCC) and keratoacanthomas [6,15]. That can be explained by the mechanism of paradoxical activation of RAS-RAF-MEK-ERK signaling pathway in cells with non-mutated BRAF by vemurafenib and dabrafenib [16]. A limitation of these BRAF targeted drugs is durability of response with median duration of response in different clinical trials being approximately 6 months and great majority of patients eventually progressing within 1 year [6,15]. Different

resistance mechanisms that can account for that have been described, some of them involving reactivation of mitogen activated protein kinase (MAPK) pathway [17,18]. Potential strategy of overcoming MAPK pathway reactivation resistance to BRAF inhibitors is MAPK pathway blockade downstream of BRAF. An example of such drug is a mitogen-activated protein kinase kinase (MEK) inhibitor, trametinib. Based on positive results of clinical trials trametinib was approved by FDA for the treatment of BRAF V600E or V600K mutation-positive unresectable or metastatic melanoma in 2013 as a single agent and in 2014 in combination with dabrafenib [19,20].

Drugs targeting other molecules and signaling pathways have also been studied in melanoma patients, like imatinib in melanoma patients with mutated *KIT*, drugs targeting vascular endothelial growth factor (VEGF), and drugs targeting PI3K-AKT-mTOR pathway [21-23].

BRAF FUNCTION AND *BRAF* MUTATIONS IN MELANOMA

Vemurafenib and dabrafenib are selectively targeting mutated and activated form of BRAF protein that is coded by *BRAF* gene [13,24]. BRAF is, together with ARAF and CRAF (RAF1), a member of RAF kinase family of serine/threonine protein kinases that are involved in RAS-RAF-MEK-ERK signaling pathway [25]. This signaling pathway is activated when an extracellular growth factor binds to a membrane-bound receptor with tyrosine kinase activity. Activation of different growth factor receptors leads to activation of RAS protein. RAS is a guanosine nucleotide-binding protein (G protein) that is critically involved in at least two different signaling pathways in addition to RAS-RAF-MEK-ERK pathway. In RAS-RAF-MEK-ERK pathway RAS activates RAF kinase, RAF phosphorylates and activates MEK and MEK phosphorylates and activates ERK mitogen activated protein kinases. ERK phosphorylates and activates different proteins: transcription factors that regulate gene transcription in the nucleus, and cytoplasmic proteins that regulate protein translation and other processes. In that way RAS-RAF-MEK-ERK pathway plays a central role in cellular proliferation, growth, differentiation and some other processes. This signaling pathway is often disrupted in cancer [25]. Due to activating mutations in genes coding for RAS and RAF and some other mechanisms, signaling trough this pathway is constitutive, unregulated and increased leading to uncontrolled cellular proliferation which is a hallmark of cancer [25,26].

Mutations in *BRAF* gene were found in different types of cancer, with high frequency in hairy cell leukemia (≈100%), melanomas (≈50%), papillary thyroid cancers (40-45%), colorectal cancers (8-15%), and ovarian cancers [26-30]. The most frequent *BRAF* mutation in different cancers is an amino acid substitution from a valine to a glutamic acid at position 600 in BRAF, known as V600E mutation. V600E mutation represents 80-90% *BRAF* mutations in melanomas [26,30,31]. Among remainder of BRAF mutations in melanomas the most frequent are other amino acid substitutions at the same position, V600K (found in up to 20% melanoma patients with *BRAF* mutations), V600D, and V600R [27,30,31]. BRAF mutations were more frequently found in younger patients and patients without chronic sun damage of the surrounding skin [27,32-35]. It was shown that presence of *BRAF* mutations is associated with worse prognosis in patients with metastatic melanoma not treated with BRAF-directed therapy [27].

TESTING FOR *BRAF* MUTATIONS

Testing for BRAF mutations is necessary before deciding about vemurafenib, dabrafenib, and trametinib therapy. All these drugs have shown activity and have been approved only for patients with melanoma that has V600 mutated BRAF. Furthermore, due to paradoxical activation of RAS-RAF-MEK-ERK signaling pathway in cells with non-mutated BRAF, vemurafenib and dabrafenib could promote cancer growth in patients with BRAF V600 non-mutated melanoma [16].

Different methods can be used to test for BRAF mutations: Sanger sequencing, pyrosequencing, mutation-specific real-time PCR, high resolution melting analysis, immunohistochemistry with VE1 antibody specific for V600E mutated *BRAF*, mismatch ligation assay, and others. These methods differ regarding sensitivity, specificity, cost, time and expertise required, and other parameters [36-38]. In the USA vemurafenib was approved by FDA for patients with BRAF mutations as detected by cobas® 4800 BRAF V600 Mutation Test (Roche Molecular Systems Inc.) as a companion diagnostic test, while dabrafenib and trametinib were approved for patients with BRAF mutations as detected by THxID™ BRAF Kit (bioMérieux, Inc.) as a companion diagnostic test. In the approval of these drugs in the European Union by the European Medicines Agency (EMEA) no companion diagnostic test was prescribed. The cobas test has shown very high specificity for *BRAF* V600

mutations (<1% false-positives), high sensitivity of >95% for *BRAF* V600E mutation, and capability to reliably detect *BRAF* V600E mutations present in as little as 5% of alleles present in a sample [39]. For comparison Sanger sequencing can reliably detect mutations if at least 20% of alleles present in a sample are mutant. The cobas method can detect also V600K and V600D mutations, but with lower sensitivity and limit for detection and it cannot distinguish between different mutations [39]. This is a potential disadvantage of that method because although vemurafenib was approved only for patients with V600E mutation, it was shown that melanoma patients with V600K mutation could also benefit from vemurafenib treatment (14,40).

BRAF mutations can be detected in fresh and frozen tissue samples but most often they are detected in paraffin-embedded tissue samples which are often only samples available. Parameters that could increase *BRAF* mutation detection failure rate are the age of samples, poor fixation of samples and high level of pigmentation. Another important parameter is percentage of cancer cells in the sample which should be high for *BRAF* mutation analysis to be reliable and representative. However, it was shown that cobas method has very low failure rate for *BRAF* mutation detection [39,41].

BRAF mutation testing is usually performed on patients with metastatic or unresectable melanoma who are candidates for therapy with vemurafenib, dabrafenib or trametinib. However, there are some valid arguments in favor of testing all patients with high-risk melanoma (American Joint Committee on Cancer stage IIb or higher) or even all patients upon diagnosis of melanoma [42]. When only patients who are candidates for therapy are tested, it is possible that time necessary to retrieve archived samples would delay initiation of therapy and thus decrease its potential benefit. It is also possible that only available archived samples from a patient would be old samples from initial biopsy what could increase *BRAF* mutation detection failure probability.

One finding that limits clinical value of *BRAF* mutation detection is intra-tumor and inter-tumor heterogeneity regarding the presence of *BRAF* mutations in a patient. It was shown that different regions within the same melanoma lesion can differ regarding the presence of *BRAF* mutations [43]. The results regarding the inter-tumor heterogeneity are conflicting. Several studies have shown relatively high discordance but other studies have shown high concordance between different melanoma lesions (primary and different metastases) in the same patients regarding the *BRAF* mutation sta-

tus [31,44-46]. Therefore, further studies are needed before making definitive conclusions regarding intra-patient *BRAF* mutation heterogeneity and its clinical consequences.

PREDICTIVE MARKERS FOR IPILIMUMAB THERAPY

Because of relatively low response-rate but durable and clinically significant response in patients responding to vemurafenib therapy, reliable predictive markers for vemurafenib would significantly improve management of patients with melanoma. However, in spite of different such potential markers being studied, none of them has shown the results that would justify its routine analysis. Markers that have shown promising results are increase in absolute lymphocyte count during treatment, presence of antibodies specific for NY-ESO1 antigen in serum, and baseline serum lactate dehydrogenase [47-49].

CONCLUSION

Analysis of *BRAF* mutations in melanoma patients prior to vemurafenib, dabrafenib, and trametinib therapy is one of a few examples of molecular predictive markers related to personalized cancer therapy that has become standard routine clinical practice. However, there are still controversies and opened questions regarding the optimal protocol for routine *BRAF* mutation testing in melanoma patients. Probably the most pertinent issue is intra-patient heterogeneity regarding the *BRAF* mutations. Also, there are different views on the best method for *BRAF* mutation detection and which patients to test. There would be a great clinical benefit from other types of molecular markers for personalized approach to patients with melanoma, like predictive markers for ipilimumab therapy or markers to predict resistance to vemurafenib therapy. No such marker has so far justified its routine analysis but several have shown promising results.

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Sažetak

Molekulski biljezi za personalizirani pristup bolesnicima s melanomom

2011. godina je bila prekretnica u liječenju bolesnika s melanomom budući da su te godine odobrena dva nova lijeka, vemurafenib i ipilimumab, koji su pokazali učinak na produženo preživljenje bolesnika s metastatskim melanomom. Vemurafenib je odobren za liječenje samo onih bolesnika koji imaju aktivirajuće mutacije u genu *BRAF*. Tako je detekcija aktivirajućih mutacija u genu *BRAF*, koje se mogu naći u ≈50% bolesnika s melanomom, postala standardni dio rutinskog protokola liječenja bolesnika s metastatskim melanomom. U ovom se radu raspravljaju metode i protokoli detekcije *BRAF* mutacija kao primjer molekulskog biljega za personalizirani pristup bolesnicima s melanomom, s naglaskom na one aspekte koji su još predmet rasprava i kontroverzi. Osim mutacija u genu *BRAF*, u radu se ukratko raspravljaju i neki drugi molekulski biljezi za personalizirani pristup liječenju bolesnika s melanomom, kao prediktivni biljezi za liječenje ipilimumabom, koji se još ne određuju rutinski.

Ključne riječi: melanom; BRAF mutacije; vemurafenib; ipilimumab; prediktivni biljezi; personalizirana medicina.

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From the history of the study of pharmacy in Zagreb

VLADIMIR PRELOG AND THE STUDY OF PHARMACY AT THE UNIVERSITY OF ZAGREB

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Summary

The study of pharmacy at the University of Zagreb was established relatively early (1882) as a consequence of a long and rich tradition of 700-year long Croatian pharmacy. Particularly large contributions to the development of both academic and industrial pharmacy were given by Gustav Janeček (1848–1929), Julije Domac (1853–1928), and Vladimir Prelog (1906–1998). The first two played an important role in the establishing of the study and its development during the first 40 years. They are the authors of the first original Croatian pharmacopoeia, *Croatian-Slavonian Pharmacopoeia* of 1901, which was considered by European scientists to be one of the best in the world, and which had political significance as well. Their lives and achievements are well analysed. Vladimir Prelog had a major influence on the development of organic and pharmaceutical chemistry at the Faculty of Pharmacy (FPh), later the Faculty of Pharmacy and Biochemistry (FPhB). However, his influence is only partly described in literature. During his seven-year tenure at the University of Zagreb (1935-1941) and his subsequent acceptance of number of graduate and postgraduate students in his Laboratory of Organic Chemistry at ETH, Prelog educated ten scientists whose careers have been associated with the study of pharmacy at the University of Zagreb. Their short biographies will be presented, with an accent on their common work with Prelog and their relations to FPh/FPhB. The events that preceded the proclamation of V. Prelog as honorary doctor of pharmaceutical sciences at the FPh and *Doctor honoris causa* at the University of Zagreb are described.

Keywords: Vladimir Prelog; pharmacy; study; Faculty of pharmacy and biochemistry; honorary doctor.

INTRODUCTION

The need for a scientific basis of pharmacy in Croatian lands was expressed and realized early, as a result of the long and rich tradition of Croatian pharmacy, going back for more than 700 years [1]. After several years of pressure, exerted by the Croatian-Slavonian Pharmaceutical Association and a few natural science professors from the Faculty of Philosophy, with the support of the Croatian ruler, *ban* Ivan Mažuranić, the study of pharmacy at the University of Zagreb was founded by the decision of Emperor Franz Joseph I on 4 October 1882 [2]. In the beginning, lectures were held at the natural history institutes of the Faculty of Philosophy. Separate university pharmacy departments were founded later. The Institute of Pharmacognosy, the first independent institute of its kind in the world, started operating in 1896 [3], the Institute of Pharmacy was founded in 1928, and the Institute of Pharmaceutical Technology in 1932. Finally, an independent Faculty of Pharmacy (FPh) was founded in 1942 and has been operating as the Faculty of Pharmacy and Biochemistry (FPhB) since 1963 [2]. Up until the middle 20th century, it was the only such faculty in Southeast Europe, attracting pharmacy students from Slovenia to Bulgaria. Despite three wars of 4 years each, and five different states and legal systems, the study of pharmacy at the University of Zagreb has been operating at the highest scientific level for 130 academic years without Yugoslav (now Croatian) Academy of Sciences and Arts (CASA) as well as 7 rectors of the University of Zagreb. A particularly large contribution to the development of not only academic but also Croatian industrial pharmacy was given by Gustav Janeček (1848 – 1929) [4], Julije Domac (1853 – 1928) [5], and Vladimir Prelog (1906 – 1998) [6]. The first two wrote the *Croatian-Slavonian Pharmacopoeia* in 1901. It was the first original Croatian pharmacopoeia, described by European scientists as one of the best in the world, and it had political significance too [7]. Vladimir Prelog had a major influence on the development of organic and pharmaceutical chemistry at FPh/FPhB.

Vladimir Prelog has been the subject of many books, scientific articles, reviews and other texts in Croatia. Articles about him became specially numerous after he won the Nobel Prize for Chemistry in 1975. Significant texts were published on the occasion of his death in 1998, the transfer of his urn to his homeland in 2001 and on the centenary of his birth 2006 [6,8-13]. This Croatian and Swiss Nobel Prize winner was the honorary citizen of several cities,

the honorary member of many associations and academies, the recipient of numerous honorary doctorates; symposiums were held about him; busts and plaques were put in his honor; streets, schools, societies, medals and awards were named after him; his face was printed on stamps etc. It would seem that Croatians have appropriately honored their great scientist, patriot and a great man in general. But this is not completely true. There are still unpublished and publicly unknown details and documents about his life and work. Moreover, the texts that have been published, especially those written for particular occasions – in other words, written in haste – contain incomplete and incorrect information.

ABOUT THE NOBEL PRIZE WINNER VLADIMIR PRELOG

The peculiar life of Prelog is hard to summarize in a few sentences, so we will cover only the key points of his biography. He was born in Sarajevo on 23 July 1906 and attended the comprehensive secondary school in Osijek (1918-1921), writing his first scientific work in 1921. He passed his matriculation exam at a comprehensive high school in Zagreb (1924). Then he studied in Prague, graduating chemistry in 1928 from the Department of Chemistry and Technology of the College of Technology, where he obtained his PhD in Chemistry in 1929. From 1929 to 1934, he worked as the head of the laboratory for fine chemicals of the chemical wholesale company of G. J. Dřize in Prague. In 1934, he came back to Zagreb, where he started lecturing Organic Chemistry at the Chemistry Department of the Technical Faculty as assistant professor until 1940, when he became associate professor. In autumn 1941 he started working in the Laboratory of Organic Chemistry at Eidgenossische Technische Hochschule (ETH) in Zürich, Switzerland, with the first Croatian Nobel Prize winner, Lavoslav Ružička. Working in this new environment, Prelog became assistant professor in 1947 and full professor in 1950. When Ružička went into retirement in 1957, Prelog became the head of the Institute of Organic Chemistry, holding that position until 1965. He published around 420 papers. He won the Nobel Prize for Chemistry in 1975 for his scientific achievements in the research of stereochemistry of large molecules. He obtained numerous honorary doctorates from many universities around the world (the University of Paris, the University of Cambridge, the University of Liverpool, the University of Zagreb etc.). He became a member, honorary member or associate of many academies and associations (the Croatian Academy of

Sciences and Arts, the American Academy of Arts and Sciences, the Academy of Pharmaceutical Sciences in Washington DC, the Academy of Sciences in Paris, the European Academy of Sciences and Arts, the Irish Royal Academy, the Danish Royal Academy of Sciences, the Pontifical Academy, the Chemical Society of London, the Pharmaceutical Association of Japan, the Japanese Chemical Society, the Austrian Chemical Society, the Royal Society of London, the Swiss Chemical Society, the Croatian Chemical Society, the Czechoslovak Chemical Society etc.). He won many prizes, medals and orders (the Croatian order *Hrvatska Danica s likom Ruđera Boškovića*, the *Werner* award and medal of the Swiss Chemical Society, the *William Marsh* order of merit of Rice University, Houston, Texas, the *Paul Karrer* medal in Switzerland, the *August-Wilhelm von Hoffman* medal of the German Chemical Society, the *Davy* medal of the Royal Society of London, the *Roger Adams* award of the Americal Chemical Society, the *Božo Težak* medal of the Croatian Chemical Society, the *Hamilton* award of the University of Nebraska, the *Evans* award of the University of Ohio etc.). He was the honorary citizen of Zagreb, Osijek and Sarajevo [6,14].

THE INFLUENCE OF VLADIMIR PRELOG ON CROATIAN CHEMISTRY AND PHARMACY

Prelog's influence on Croatian chemistry and pharmacy is huge. The *Zagreb School of Organic Chemistry* is the term used to describe the effect of V. Prelog during his seven-year tenure at the University of Zagreb (1935-1941) and his subsequent acceptance of an exceptional number of graduate and postgraduate students from Croatia in his Laboratory of Organic Chemistry at ETH. When they went back to Croatia, as a rule, they embarked upon distinguished scientific and academic careers, imparting their knowledge and Prelog's spirit to Croatians.

His contribution to the development of industrial pharmacy is also exceptionally large. In 1936, Prelog initiated the establishment of a research laboratory with a chemical and pharmacological department in "Kaštel", a pharmaceutical and chemical plant, thus linking university and industrial research. "Kaštel" went on to grow into PLIVA, a major pharmaceutical industry, while its research laboratory became an institute. Prelog enabled the doctoral and postdoctoral specializations of many PLIVA researchers in his laboratory at ETH.

This paper will highlight the influence of Vladimir Prelog on the development of organic and pharmaceutical chemistry at FPhB.

Figure 1. Vladimir Prelog in his laboratory at the ETH (Courtesy: Prof. M. Žinić).

PRELOG'S DOCTORAL STUDENTS AND COLLEAGUES AT THE FACULTY OF PHARMACY AND BIOCHEMISTRY

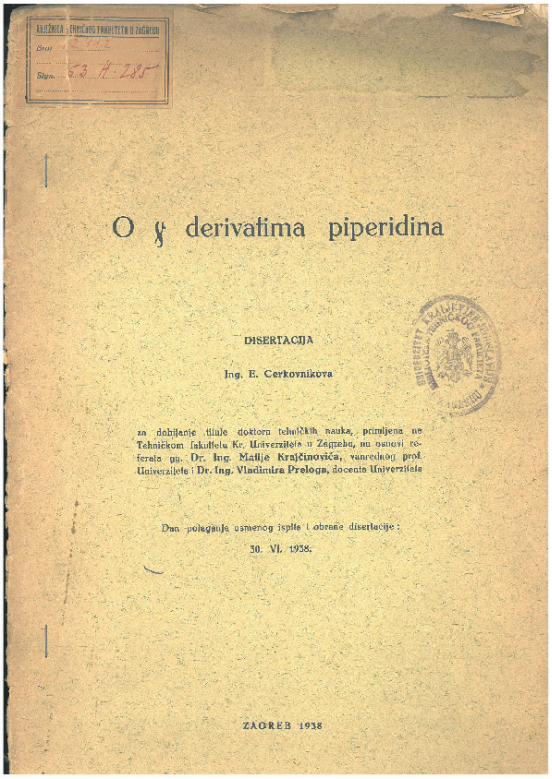
When books and other texts about Vladimir Prelog mention his numerous colleagues, they rarely mention that some of those colleagues spent a large part of their career at FPh/FPhB. It paints an incomplete picture of the scope of Prelog's influence on Croatian science and the biographies of some of his colleagues. We will present their short biographies here, pointing out the parts about their work with V. Prelog and their relations to FPh/FPhB.

Figure 2. V. Prelog with his former associates in the PLIVA Club, (1989). *Sitting from the left*: R. Seiwerth, D. Kolbah, V. Prelog, Mrs. Prelog and M. Prošteník; *Standing from the left*: M. Dumić, S. Mutak, B. Glunčić, K. Kovačević, B. Gašpert, M. Žinić, M. Kovačević, S. Borčić, V. Šunjić & S. Đokić. (*Bolded are names of Prelog's collaborators associated with FPhB).*

EUGEN CERKOVNIKOV (1904-1985)

He studied chemistry at the Chemical Department of the Faculty of Technology in Zagreb, graduating in 1929. From 1931 to 1932, he worked at the Faculté de Médécine in Paris, where he started doing scientific work on the synthesis of organic compounds with biologic effects. After returning to Zagreb, he volunteered at the Institute of Organic Chemistry of the Faculty of Technology in Zagreb, where he became Prelog's assistant in 1935. Prelog was his mentor for his PhD in technical sciences in the field of organic chemistry in 1938. From 1938 to 1947, he worked as research associate in the institute of the Kaštel plant (which would become PLIVA). In that period, he published 18 scientific papers together with Prelog. Cerkovnikov became assistant professor and was appointed as the first head of the Department of Organic Chemistry of the Faculty of Pharmacy in 1947. At the same faculty, he became associate professor in 1948 and full professor in 1956. Cerkovnikov was

among the first Croatian scientists examining the relationship between the structure of organic compounds and their pharmacological effects. In 1957, he moved to the newly established Faculty of Medicine in Rijeka, where he founded the Institute of Chemistry and Biochemistry and became its head. He was Dean of the Faculty of Medicine in Rijeka (1958/59). Cerkvenikov researched the synthesis of organic compounds, especially antimalarials, sulfonamides, spasmolytics and antihistamines, on the effects of ionizing radiation on living beings etc. He published some 200 scientific and 60 technical papers [10,14-16].



O derivatima piperidina

DISERTACIJA

Ing. E. Cerkovnikova

za dobijanje titule doktora tehničkih nauka, primljena na Tehničkom fakultetu Kr. Universitete u Zagrebu, na osnovi referata gg. Dr. Ing. Matije Krajinovića, vanrednog prof. Universitete i Dr. Ing. Vladimira Preloga, docenta Universitete

Dan polaganja usmenog ispita i obrane disertacije:

30. VI. 1938.

ZAGREB 1938

Figure 3. Cover page of Eugen Cerkovnikov´s dissertation [17].

DRAGUTIN KOLBAH (1912-1990)

He studied chemistry at the Department of Chemistry and Technology of the College of Technology in Prague, where he graduated in 1935. At that time, Prontosil (4,-sulfanilamido-2,4-diaminoazobenzene) began to dominate the pharmaceutical market as the first successful drug for bacterial infections. Prelog proposed to Kolbah to synthetize a number of azo dyes related to Prontosil within his doctoral thesis. One of those compounds, 4,-sulfanilamido-4-N-piperazyl-azo-benzene, known in literature as Kolbah's dye, showed excellent properties, so it was prepared for the market. In 1936, it was proven that Prontosil was reductively split in the organism and that the antibacterial effect was the property of one of its products, called sulfanilamide, which was the starting material in the synthesis of Kolbah's dye. Since sulfanilamide was not protected by a patent, it was quickly placed on the market by the Kaštel plant in 1937 as Streptazol, which was very successful. More than anyone else, it was the achievement of Prelog and his PhD graduate Kolbah. Kolbah earned his doctorate at the Faculty of Technology of the University of Zagreb as the first PhD graduate of Vladimir Prelog (1938). Kolbah worked as Prelog's private assistant until 1939. Then he did various jobs as a chemist until 1946, when he became the head of the sulfonamide facility in PLIVA. Kolbah worked on the isolation of alkaloids at ETH with Vladimir Prelog (1954) and on the preparation of compounds with antitumor effects at the Department of Chemistry, University of Michigan, Ann Arbor, Michigan, USA (1960-1962). He was appointed associate professor of organic chemistry at FPhB in 1962 and full professor in 1969, where he stayed until retirement. Kolbah was the head of the Institute of Organic Chemistry from 1962 to 1980. At the postgraduate studies in organic chemistry at the University of Zagreb, he gave lectures on selected topics in the chemistry of heterocyclic compounds. Kolbah researched the synthesis of biologically active compounds and the relationship between the structure of organic compounds and their biologic effects. He researched the synthesis of quinine, sulfonamides, benzodiazepines etc. Kolbah published around 50 scientific papers. Kolbah edited the well-known *Chemists' Textbook* (1951), which was republished in 1961 and 1986,and translated *Organic Chemistry*, the textbook by R. T. Morrison and R. N. Boyd, into Croatian. [8,14,15].

PAVAO ŠTERN (1913-1976)

P. Štern was born in 1913 in Varaždin, where he completed his secondary education in 1931. He studied medicine at the Faculty of Medicine in Zagreb, graduating in 1936. From 1937, Štern worked in the Kaštel plant (later PLIVA), where he established the pharmacological laboratory and closely cooperated with Vladimir Prelog. In 1945, he moved to the Institute of Pharmacology of the Faculty of Medicine. Examining antihistamines and the autonomous nervous system, Štern introduced the scientific concept of receptors. He gave lectures on pharmacology at the Faculty of FPh in Zagreb from 1945 to 1948. Štern continued his academic career as full professor at the newly established Faculty of Medicine in Sarajevo, where he founded the Institute of Pharmacology, which now bears his name. Štern had a wide range of scientific interests, from the antihistamines we mentioned, to muscle illnesses, leukemia and antileukemic drugs, mechanisms of inflammation, mechanisms of effects of psychopharmacological drugs etc. He published around 500 papers. Štern was Dean of the Faculty of Medicine in Sarajevo (1952/53), full member of the Academy of Sciences and Arts of Bosnia and Herzegovina, and corresponding member of CASA. [15,18].

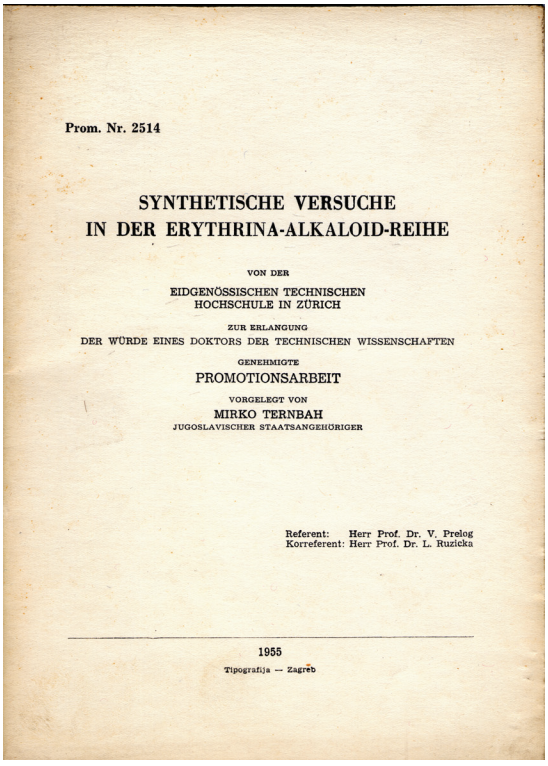
KREŠIMIR BALENOVIĆ (1914-2003)

K. Balenović graduated from the Department of Chemistry of the Faculty of Philosophy (1937) and obtained his PhD (1939) in Zagreb. He did his postdoctoral studies under the Nobel Prize winners Albert Szent-Györgyj at the University of Szeged (1942-1943) and Vladimir Prelog at ETH (1949/50), where he later worked as visiting professor (1968/1969). From 1939, Balenović worked as assistant at the Chemical Institute of the Faculty of Philosophy. In 1945, he was elected associate professor at same faculty, where he gave lectures on organic chemistry until 1946. He moved to the Faculty of Natural Sciences and Mathematics in Zagreb (FNSM) as associate professor in 1946 and full professor in 1952. He was Dean of FNSM in 1958/59. Balenović was one of the founders of chemistry at the Ruđer Bošković Institute. He was elected as associate member of YASA (CASA) in 1958 and full member in 1975. Balenović researched natural organic compounds, amino acids, polyketones, organic sulfoxides and selenoxides and published around 100 scientific pa-

pers. When Croatia gained independence, Balenović was among the first six vice-presidents of the country [14,15].

MIRKO TERNBAH (1920-?)

M. Ternbah studied pharmacy at the FPh, where he graduated in 1946. Then he was the principal of the Secondary School of Pharmacy in Zagreb for a year. Ternbah was elected assistant at the Department of Inorganic, Analytical and Physical Chemistry of the FPh in 1946. In 1948, and became assistant at the same faculty. He spent the period from 1952 to 1955 at the Laboratory for Organic Chemistry at ETH, where he made his doctoral dissertation under Vladimir Prelog and obtained his PhD in 1955. Ternbah worked at FPh until 1957, when he continued his scientific career abroad [15].



Prom. Nr. 2514

SYNTHETISCHE VERSUCHE
IN DER ERYTHRINA-ALKALOID-REIHE

VON DER
EIDGENÖSSISCHEN TECHNISCHEN
HOCHSCHULE IN ZÜRICH
ZUR ERLANGUNG
DER WÜRDE EINES DOKTORS DER TECHNISCHEN WISSENSCHAFTEN
GENEHMIGTE
PROMOTIONSARBEIT
VORGELEGT VON
MIRKO TERNBAH
JUGOSLAVISCHER STAATSANGEHÖRIGER

Referent: Herr Prof. Dr. V. Prelog
Korreferent: Herr Prof. Dr. L. Ruzicka

1955
Tipografija — Zagreb

Figure 4. Cover page of Mirko Ternbah´s dissertation whose second referee was Nobel Prize winner Lavoslav Ružička [19].

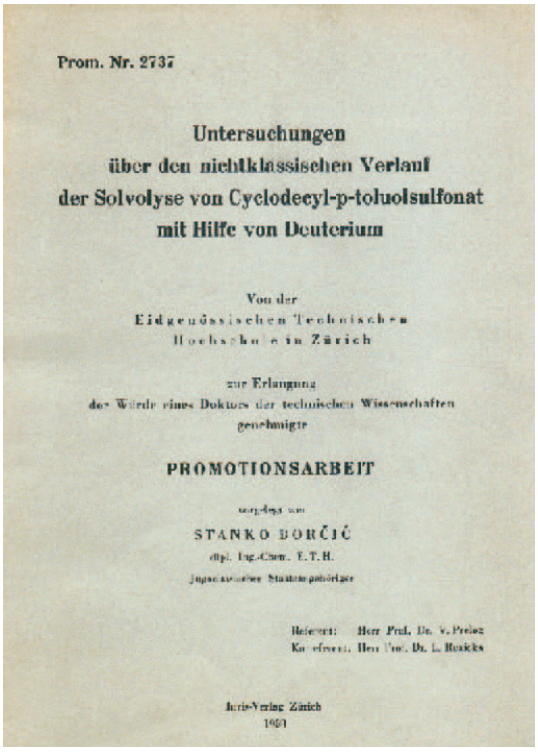
MIHOVIL PROŠTENIK (1916-1994)

M. Proštenik graduated from the Department for Chemistry and Technology of the Faculty of Technology in Zagreb (1939) and obtained his PhD from the same faculty (1944). He did his postdoctoral studies in the laboratories of L. Ružička and V. Prelog, at ETH (1948/49), and at the Department of Biochemistry, University of Illinois, Urbana, Illinois, USA. At the Faculty of Medicine of the University of Zagreb, Proštenik was appointed private docent in 1949, university docent in 1952, associate professor in 1953, and full professor in 1963. He gave lectures on organic chemistry at FPh from 1957 to 1962. He was elected associate member of YASA (CASA) in 1963 and full member in 1986. Proštenik mostly researched the chemistry and biochemistry of lipids and the heterocyclic compounds with nitrogen and published around 100 scientific papers and a dozen technical papers [14,15].

STANKO BORČIĆ (1931-1994)

S. Borčić studied chemistry at ETH (1949-1953), where he graduated (1953). He made his doctoral dissertation under two Croatian Nobel Prize winners, Lavoslav Ružička and Vladimir Prelog, and obtained his PhD in 1957. Upon the invitation of Ivan Supek, who was the director and founder of the Ruđer Bošković Institute, Borčić came to the Laboratory for Physical and Organic Chemistry in the institute, where he worked until 1967, when he was appointed associate professor at the FPhB. Borčić was appointed full professor in 1972. He spent a year at postdoctoral studies at the Department of Chemistry, California Institute of Technology, Pasadena, California, USA (1963/1964). Borčić was a visiting professor at several American universities: Department of Chemistry, University of Oregon, Corvallis, Oregon, USA (1970-1971); Department of Chemistry, University of Minnesota, Minneapolis, Minnesota, USA (1977); Department of Chemistry, Indiana University, Bloomington, Indiana, USA (1984); and Department of Chemistry, University of Ottawa, Ottawa, Canada (1981-1982). He gave lectures on Organic Chemistry at the FPhB, and lectures on Organic Reaction Mechanisms, Magnetic Resonance Methods and Organic Chemistry Methods at the postgraduate studies of the University of Zagreb. He was president (1984-1986) and vice-president (1986-1988) of the *Croatica chemica acta*. Borčić researched physical and organic chemistry, especially the organic reaction mechanisms and secondary isotope effects and NMR spectroscopy, publishing about 40

scientific papers on those topics. He was Vice Dean of the FPhB from 1972/73 to 1975/76 and Dean from 1985/86 to 1988/89. [14,15].


Prom. Nr. 2737

Untersuchungen
über den nichtklassischen Verlauf
der Solvolyse von Cyclodecyl-p-toluolsulfonat
mit Hilfe von Deuterium

Von der
Eidgenössischen Technischen
Hochschule in Zürich

zur Erlangung
der Würde eines Doktors der technischen Wissenschaften
genehmigte

PROMOTIONSARBEIT

vorgelegt von
STANKO BORČIĆ
dipl. Ing.-Chem. E.T.H.
Jugoslawischer Staatsangehöriger

Referent: Herr Prof. Dr. V. Prelog
Korreferent: Herr Prof. Dr. L. Ruzicka

Juris-Verlag Zürich
1960

Figure 5. Cover page of Stanko Borčić´s dissertation whose second referee was Nobel Prize winner Lavoslav Ružička [18].

MLADEN ŽINIĆ (1947-)

M. Žinić completed the Secondary School of Chemistry and Technology in Zagreb in 1966 and graduated from the Faculty of Technology in 1971. After graduating, he started working with D. Kolbah at the FPhB, first as an assistant volunteer and then as a resident assistant at the Department for Organic Chemistry of the FPhB. He obtained his MA after a postgraduate course in Organic Chemistry (1974) and obtained his PhD at FPhB (1978). After his postdoctoral studies under Vladimir Prelog at ETH, Žinić shortly returned to FPhB, but then went to the Ruđer Bošković Institute in 1985. He was

appointed full professor at the FNSM in 2004 and of the Faculty of Medicine at the University of Rijeka in 2009 as a titled full professor of chemistry. Žinić was elected associate member of CASA in 2004 and became Head of the Laboratory for Supramolecular and Nucleoside Chemistry in 1993. From 1989 to 1991, he closely cooperated with Jean-Marie Lehn, the French Nobel Prize winner and the founder of supramolecular chemistry. He published 107 scientific papers. Žinić performed the duty of the director of the Ruđer Bošković Institute from 2005 to 2009. Žinić was elected full member of CASA in 2014 [20].

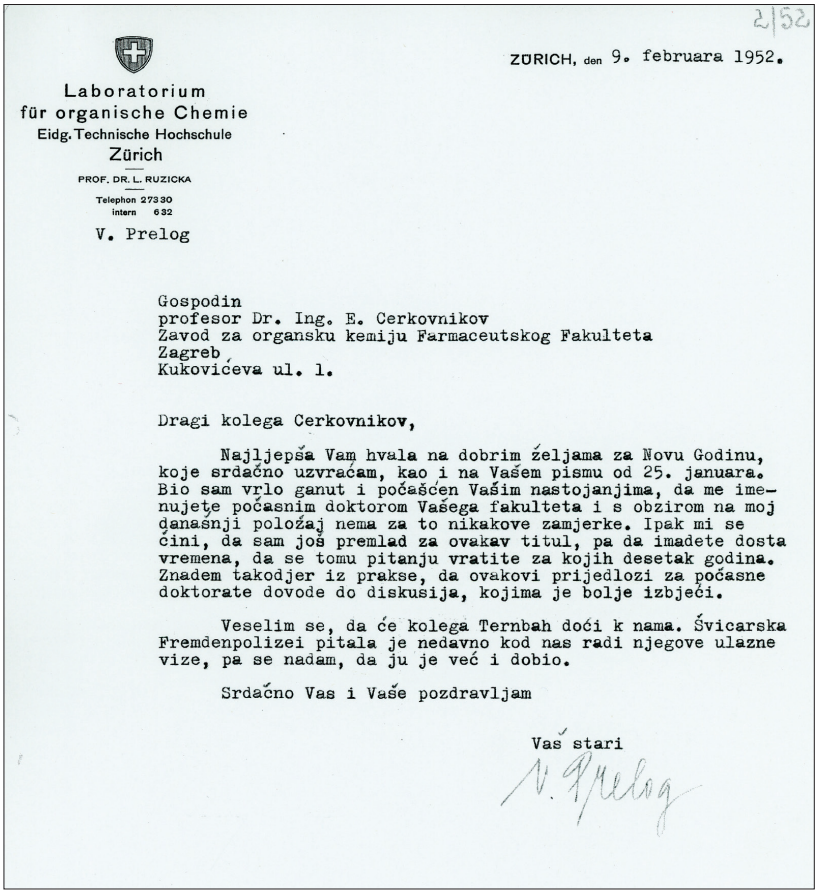
In addition, two more researchers were associated both with Vladimir Prelog and FPhB. **Miljenko Dumić** made his doctoral dissertation at FPhB and worked as postdoctoral fellow with Prelog at ETH (1983-1985). After thirty-five years working in pharmaceutical research and development in PLIVA industry he moved to the Department of Biotechnology of the Rijeka University where he was appointed as professor. Prof. Dumić was close with Prelog and wrote a number of texts about him. **Krunoslav Kovačević** received his M.S. degree from FPhB and during 1981-1982 he worked with Prelog at ETH on his PhD thesis which he finished at University of Zagreb. His career was associated with research and scientific management within PLIVA's Research Institute. Drs Dumić and Kovačević are authors of the most complete monography published about life and achievemet of Vladimir Prelog [6].

VLADIMIR PRELOG, HONORARY DOCTOR OF PHARMACEUTICAL SCIENCES AT THE FACULTY OF PHARMACY

In his letter of 25 January 1952, (Fig. 6) Dr Eugen Cerkovnikov, then a professor of organic chemistry and Head of the Institute for Organic Chemistry of FPh, informed Prelog about his efforts to make him an honorary doctor of FPh. In his reply of 09 February 1952, Prelog writes: "*...I was very moved and honored by your efforts to make me an honorary doctor of your faculty. Regarding my current position, your idea is not questionable. Yet I believe I am still too young for such a title, so you can take your time and reopen the issue in ten years or so. I know from practice that such proposals for honorary doctorates lead to discussions that are better avoided. I am happy that our colleague Ternbah is coming here. The Swiss Fremdenpolizei recently made inquiries here for his entry visa. I hope he has already obtained it...*" The letter reveals Prelog's proverbial modesty befitting a great man, and his care for the education of young Croatian scientists [21].

The proposal "*to give the diploma of the honorary doctor of pharmaceutical sciences to Dr. Eng. Vladimir Prelog, full professor at the Technical College in Zurich*", including an explanation, was submitted to the Council of FPh on 01 March 1952 by the professors Dragutin Barković and Eugen Cerkovnikov [21]. Already on 03 March 1952, there was an extraordinary session of the Council, presided by the dean, Prof. Fran Kušan. The session had only one agenda item: *Proposal of Prof Dr D. Barković and Prof Dr E. Cerkovnikov to give the diploma of the honorary doctor of pharmaceutical sciences to Dr Eng Vladimir Prelog, full professor at the Technical College in Zurich*. The faculty secretary Antun Kajfeš read the following explanation of the proposal:

Professor V. Prelog founded the school of organic chemists specialized in therapy, contributing to the development of pharmaceutical science in Yugoslavia and espe-



2/52

ZURICH, den 9. februara 1952.

Laboratorium
für organische Chemie
Eidg. Technische Hochschule
Zürich

PROF. DR. L. RUZICKA

Telephon 27330
intern 632

V. Prelog

Gospodin
profesor Dr. Ing. E. Cerkovnikov
Zavod za organsku kemiju Farmaceutskog Fakulteta
Zagreb
Kukovićeva ul. 1.

Dragi kolega Cerkovnikov,

Najljepša Vam hvala na dobrim željama za Novu Godinu, koje srdačno uzvraćam, kao i na Vašem pismu od 25. januara. Bio sam vrlo ganut i počašćen Vašim nastojanjima, da me imenujete počasnim doktorom Vašega fakulteta i s obzirom na moj današnji položaj nema za to nikakove zamjerke. Ipak mi se čini, da sam još premlad za ovakav titul, pa da imadete dosta vremena, da se tomu pitanju vratite za kojih desetak godina. Znadem takodjer iz prakse, da ovakovi prijedlozi za počasne doktorate dovode do diskusija, kojima je bolje izbjeći.

Veselim se, da će kolega Ternbah doći k nama. Švicarska Fremdenpolizei pitala je nedavno kod nas radi njegove ulazne vize, pa se nadam, da ju je već i dobio.

Srdačno Vas i Vaše pozdravljam

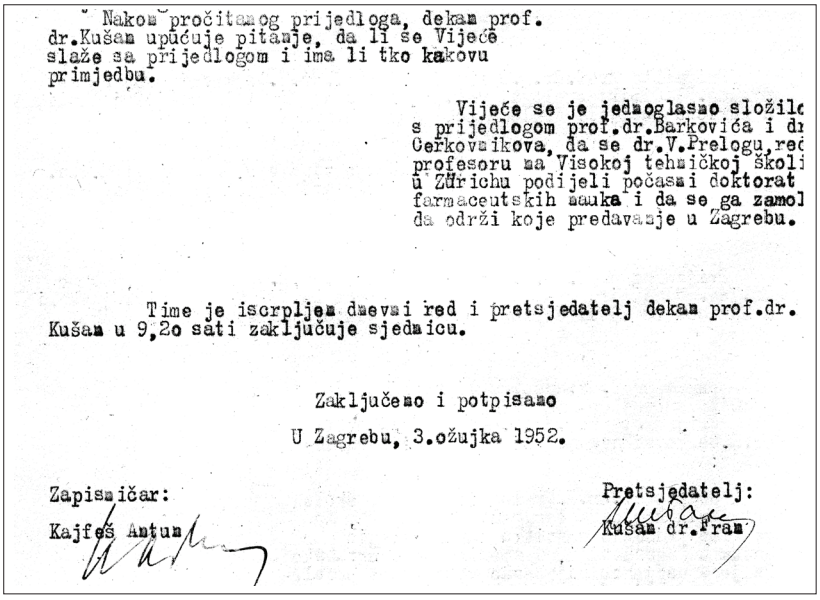
Vaš stari

V. Prelog

Figure 6. Prelog's letter of 09 February 1952.

cially in Croatia. He successfully resolved certain important problems in the field of pharmaceutical chemistry, such as the problem of malaria, and helped resolve the problem of the link between chemical constitution and antimalarial effect. He set the foundations of our production of synthetic drugs, and his co-workers and students rose to leading positions in the chemical and pharmaceutical industry in the field of synthetic chemistry and at the University (Prof Dr Balenović, Prof Dr Hahn, Prof Dr Režek, Doc Dr Prošteník, Dr Seiwerth etc.). Prof Dr Prelog has been worthily representing science abroad and is greatly admired in Europe and America. He has also had success in the area of natural compounds, contributing to the development of pharmaceutical science. While abroad, Prof Dr Prelog helps the work of our young scientists, thereby helping the development of science in Yugoslavia. The proposers are of the opinion that the awarding of a honorary doctor's diploma to Prof Dr Prelog would be visible proof that the University of Zagreb knows how to honor its deserving workers. If their proposal is accepted, Prof Dr Barković and Prof Dr Cerkovnikov also propose to ask Prof Dr Prelog to accept the honorary doctorate at our faculty and use the occasion to hold a few lectures in Zagreb.

The Council unanimously accepted the proposal (Fig. 7). Aside from the dean Fran Kušan, the following were present at the session: Hrvoje Iveković, Dragutin Barković, Božidar Vajić, Branka Akačić, Jaroslav Ječmen, Eugen Cerkovnikov, Marijana Fišer-Herman, Dragan Marković, Vera Vukčević-Ko-



Nakon pročitanog prijedloga, dekan prof. dr.Kušan upućuje pitanje, da li se Vijeće slaže sa prijedlogom i ima li tko kakovu primjedbu.

Vijeće se je jednoglasno složilo s prijedlogom prof.dr.Barkovića i dr Cerkovnikova, da se dr.V.Prelogu,red profesoru na Visokoj tehničkoj školi u Zürichu podijeli počasni doktorat farmaceutskih nauka i da se ga zamoli da održi koje predavanje u Zagrebu.

Time je iscrpljen dnevni red i pretsjedatelj dekan prof.dr. Kušan u 9,2o sati zaključuje sjednicu.

Zaključeno i potpisano

U Zagrebu, 3.ožujka 1952.

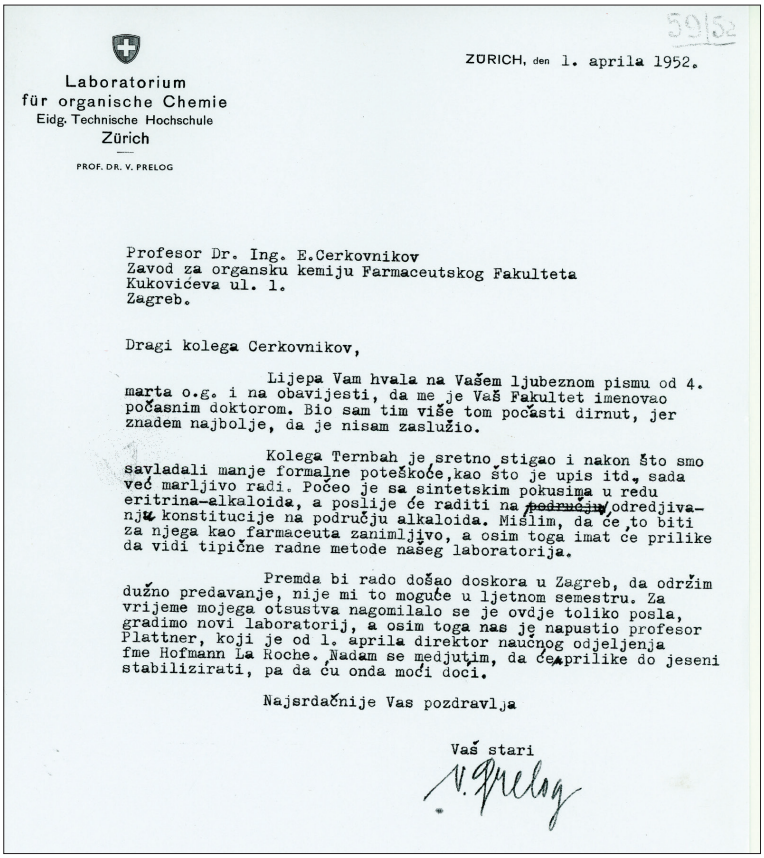
Zapisničar:
Kajfeš Antun

Pretsjedatelj:
Kušan dr.Fran

Figure 7. Part of the minutes from the FPh Council meeting of 03. March 1952.

vačević, Ivan Filipović, and Vladimir Seifert. They were the first in Croatia to recognize this great man of Croatian and world science, 23 years before he was awarded the Nobel Prize. They, too, are worthy of praise [19].

In his letter to Prof Cerkovnikov of 01 April 1952, (Fig. 8), Prelog expressed his gratitude at the news that FPh awarded him an honorary doctorate: *I was all the more moved by this news because I know best that I have not deserved it* [21].



Laboratorium
für organische Chemie
Eidg. Technische Hochschule
Zürich

PROF. DR. V. PRELOG

59/52

ZÜRICH, den 1. aprila 1952.

Profesor Dr. Ing. E.Cerkovnikov
Zavod za organsku kemiju Farmaceutskog Fakulteta
Kukoviceva ul. 1.
Zagreb.

Dragi kolega Cerkovnikov,

Lijepa Vam hvala na Vašem ljubeznom pismu od 4. marta o.g. i na obavijesti, da me je Vaš Fakultet imenovao počasnim doktorom. Bio sam tim više tom počasti dirnut, jer znadem najbolje, da je nisam zaslužio.

Kolega Ternbah je sretno stigao i nakon što smo savladali manje formalne poteškoće, kao što je upis itd., sada već marljivo radi. Počeo je sa sintetskim pokusima u redu eritrina-alkaloida, a poslije će raditi na ~~području~~ odredjivanju konstitucije na području alkaloida. Mislim, da će to biti za njega kao farmaceuta zanimljivo, a osim toga imat će prilike da vidi tipične radne metode našeg laboratorija.

Premda bi rado došao doskora u Zagreb, da održim dužno predavanje, nije mi to moguće u ljetnom semestru. Za vrijeme mojega otsustva nagomilalo se je ovdje toliko posla, gradimo novi laboratorij, a osim toga nas je napustio profesor Plattner, koji je od 1. aprila direktor naučnog odjeljenja fme Hofmann La Roche. Nadam se medjutim, da će prilike do jeseni stabilizirati, pa da ću onda moći doći.

Najsrdačnije Vas pozdravlja

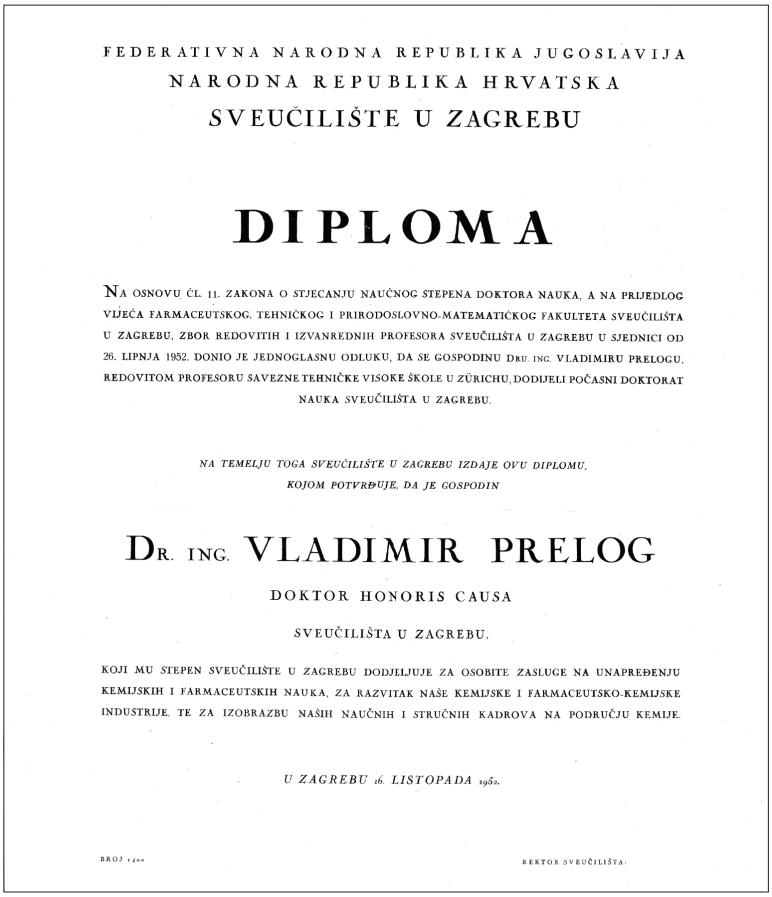
Vaš stari

V. Prelog

Figure 8. Prelog's letter of 01 April 1952

VLADIMIR PRELOG, HONORARY DOCTOR OF SCIENCE OF THE UNIVERSITY OF ZAGREB

On 29 March 1952, immediately after Prelog became honorary doctor of pharmaceutical science at FPh, professors Dragutin Barković and Eugen Cerkovnikov submitted a proposal to the faculty Council to get together with the Faculty of Technology, where Prelog taught for 6 years, and the Faculty of Natural Sciences and Mathematics to start a joint procedure at the University of Zagreb to award the honorary doctorate of the University to Vladimir Prelog. In the explanation, they pointed out that the Yugoslav Congress of Chemists will take place in Zagreb, coinciding with the 75th anniversary of practical teaching of chemistry at the University of Zagreb and the 25th anniversary of the establishment of the Croatian Chemical Society. They also noted that *this year* (1952) was the 70th anniversary of pharmaceutical studies at the University of Zagreb, and that it was planned to hold the *International Congress of Pharmacy Scientists*, so if the Council accepted their proposal, *all these celebrations and jubilees could be combined in a great common event of our chemical and pharmaceutical science and study at the University of Zagreb.* The session of the Choir of Full and Associate Professors of the University of Zagreb, called to award the honorary doctorate of science to Prof Vladimir Prelog, was held on 26 June 1952. The session, presided by Rector Prof Dr Franjo Bošnjaković, was attended by 107 out of 165 professors (only 83 were needed for a quorum). They included professors from the Faculty of Pharmacy: D. Barković, H. Iveković, F. Kušan, B. Vajić, B. Akačić E. Cerkovnikov and J. Ječmen. References were given by Prof D. Barković from FoPh, Prof V. Hann from the Faculty of Technology, and Prof K. Balenović from the Faculty of Natural Sciences and Mathematics, in that order, explaining the proposals in the name of their faculties. During the discussion, the proposals were supported by F. Bubanović, a professor from the Faculty of Medicine. In a secret ballot, the professors unanimously supported the proposal that Vladimir Prelog should receive the honorary doctorate of science (honoris causa) of the University of Zagreb. The awarding ceremony was held on 16 October 1952 [22]. It was also the occasion of the First Congress of Pure and Applied Chemistry, where Prelog held a lecture.



FEDERATIVNA NARODNA REPUBLIKA JUGOSLAVIJA
NARODNA REPUBLIKA HRVATSKA
SVEUČILIŠTE U ZAGREBU

DIPLOMA

NA OSNOVU ČL. 11. ZAKONA O STJECANJU NAUČNOG STEPENA DOKTORA NAUKA, A NA PRIJEDLOG VIJEĆA FARMACEUTSKOG, TEHNIČKOG I PRIRODOSLOVNO-MATEMATIČKOG FAKULTETA SVEUČILIŠTA U ZAGREBU, ZBOR REDOVITIH I IZVANREDNIH PROFESORA SVEUČILIŠTA U ZAGREBU U SJEDNICI OD 26. LIPNJA 1952. DONIO JE JEDNOGLASNU ODLUKU, DA SE GOSPODINU DRU. ING. VLADIMIRU PRELOGU, REDOVITOM PROFESORU SAVEZNE TEHNIČKE VISOKE ŠKOLE U ZÜRICHU, DODIJELI POČASNI DOKTORAT NAUKA SVEUČILIŠTA U ZAGREBU.

NA TEMELJU TOGA SVEUČILIŠTE U ZAGREBU IZDAJE OVU DIPLOMU, KOJOM POTVRĐUJE, DA JE GOSPODIN

DR. ING. VLADIMIR PRELOG

DOKTOR HONORIS CAUSA

SVEUČILIŠTA U ZAGREBU.

KOJI MU STEPEN SVEUČILIŠTE U ZAGREBU DODJELJUJE ZA OSOBITE ZASLUGE NA UNAPREĐENJU KEMIJSKIH I FARMACEUTSKIH NAUKA, ZA RAZVITAK NAŠE KEMIJSKE I FARMACEUTSKO-KEMIJSKE INDUSTRIJE, TE ZA IZOBRAZBU NAŠIH NAUČNIH I STRUČNIH KADROVA NA PODRUČJU KEMIJE.

U ZAGREBU 16. LISTOPADA 1952.

BROJ 1490

REKTOR SVEUČILIŠTA:

Figure 9. The diploma Doctor Honoris Causa of the Zagreb University, presented to Vladimir Prelog on October 16, 1952 [22].

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Sažetak

Vladimir Prelog i studij farmacije na Sveučilištu u Zagrebu

Studij farmacije na Sveučilištu u Zagrebu utemeljen je relativno rano (1882), kao posljedica više od 700 godina duge i bogate tradicije hrvatskog ljekarništva. Posebice velik doprinos razvoju akademske, kao i industrijske farmacije, dali su Gustav Janeček (1848– 1929), Julije Domac (1853–1928) i Vladimir Prelog (1906–1998). Prva dvojica odigrala su ključnu ulogu u formiranju studija i njegovu razvoju prvih 40 godina. Autori su prve originalne hrvatske farmakopeje, *Hrvatsko-slavonske farmakopeje* iz 1901, koju su europski znanstvenici smatrali jednom od najboljih na svijetu, a koja je imala i političko značenje. Njihovi životi i djela dobro su obrađeni. Vladimir Prelog imao je ogroman utjecaj na razvoj organske i farmaceutske kemije na Farmaceutskom (FPh), kasnije Farmaceutsko-biokemijskom fakultetu (FPhB). Međutim, njegov utjecaj je tek djelomično opisan u literaturi. Tijekom sedmogodišnjeg rada na Sveučilištu u Zagrebu (1935-1941), i kasnijeg prihvaćanja brojnih doktoranada i postdoktoranada u njegov Laboratorij za organsku kemiju pri ETH, Prelog je educirao deset znanstvenika čije su karijere povezane sa studijem farmacije na zagrebačkom sveučilištu. Ovdje ćemo ih predstaviti kratkim biografijama s naglaskom na njihov zajednički rad s Prelogom i vezu s FPh/FPhB. Opisani događaji su predhodili izboru V. Preloga za počasnog doktora farmaceutskih znanosti na FPh i *Doctor honoris causa* na Sveučilištu u Zagrebu.

Ključne riječi: Vladimir Prelog; farmacija; studij; Farmaceutsko-biokemijski fakultet, počasni doktor.

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