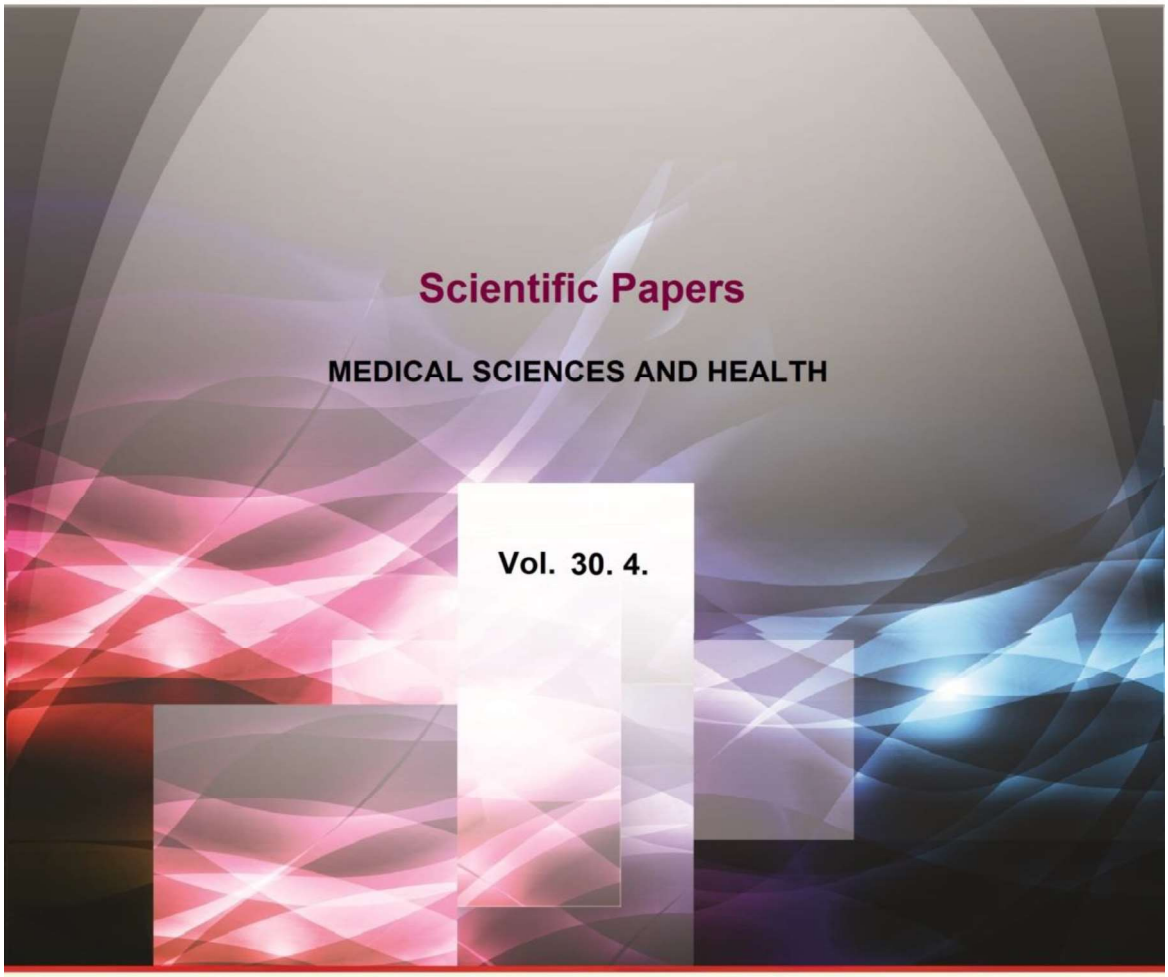
UDK 37

ISSN 2545 - 4439
ISSN 1857 - 923X

INTERNATIONAL JOURNAL

Institute of Knowledge Management

KNOWLEDGE



KIJ Vol. 30 No. 4 pp. 707 - 1084 Skopje 2019

Global Impact & Quality Factor 1.822 (2017) http://globalimpactfactor.com/knowledge-international-journal/

KNOWLEDGE

INTERNATIONAL JOURNAL

SCIENTIFIC PAPERS
VOL.30.4

Promoted in Vrnjacka Banja, Serbia
March, 2019

KNOWLEDGE – International Journal
Vol.30.4
March, 2019

INSTITUTE OF KNOWLEDGE MANAGEMENT
SKOPJE

KNOWLEDGE

International Journal Scientific papers Vol. 30.4

ADVISORY BOARD

Vlado Kambovski PhD, Robert Dimitrovski PhD, Siniša Zarić PhD, Maria Kavdanska PhD, Venelin Terziev PhD, Mirjana Borota – Popovska PhD, Cezar Birzea PhD, Ljubomir Kekenovski PhD, Veselin Videv PhD, Ivo Zupanovic, PhD, Savo Ashtalkoski PhD, Zivota Radosavljević PhD, Laste Spasovski PhD, Mersad Mujevic PhD, Nonka Mateva PhD, Rositsa Chobanova PhD, Predrag Trajković PhD, Dzulijana Tomovska PhD, Nedzad Korajlić PhD, Nebojsha Pavlović PhD, Nikolina Ognenska PhD, Baki Koleci PhD, Lisen Bashkurti PhD, Trajce Dojcinovski PhD, Jana Merdzanova PhD, Zoran Srzentić PhD, Nikolai Sashkov Cankov PhD, Marija Kostic PhD

Print: GRAFOPROM – Bitola

Editor: IKM – Skopje

Editor in chief
Robert Dimitrovski, PhD

KNOWLEDGE - International Journal Scientific Papers Vol. 30.4

ISSN 1857-923X (for e-version)
ISSN 2545 – 4439 (for printed version)

KNOWLEDGE – International Journal
Vol.30.4
March, 2019

INTERNATIONAL EDITORIAL BOARD

President: Academic, Prof. Vlado Kambovski PhD, Skopje (Macedonia)

Vice presidents:
Prof. Robert Dimitrovski PhD, Institute of Knowledge Management, Skopje (Macedonia)
Prof. Sinisa Zaric, PhD, Faculty of Economics, University of Belgrade, Belgrade (Serbia)
Prof. Venelin Terziev PhD, University of Rousse, Rousse (Bulgaria)
Prof. Mersad Mujevic PhD, Public Procurement Administration of Montenegro (Montenegro)
Prof. Tihomir Domazet PhD, President of the Croatian Institute for Finance and Accounting, Zagreb (Croatia)

Members:

- Prof. Aleksandar Korablev PhD, Dean, Faculty for economy and management, Saint Petrsburg State Forest Technical University, Saint Petrsburg (Russian Federation)
- Prof. Azra Adjajlic – Dedovic PhD, Faculty of criminology and security, Sarajevo (Bosnia & Herzegovina)
- Prof. Anita Trajkovska PhD, Rochester University (USA)
- Prof. Anka Trajkovska-Petkoska PhD, UKLO, Faculty of technology and technical sciences, Bitola (Macedonia)
- Prof. Alisabri Sabani PhD, Faculty of criminology and security, Sarajevo (Bosnia & Herzegovina)
- Prof. Ahmad Zakeri PhD, University of Wolverhampton, (United Kingdom)
- Prof. Ana Dzumalieva PhD, South-West University "Neofit Rilski", Blagoevgrad (Bulgaria)
- Prof. Aziz Pollozhani PhD, Rector, University Mother Teresa, Skopje (Macedonia)
- Prof. Branko Sotirov PhD, University of Rousse, Rousse (Bulgaria)
- Prof. Branko Boshkovic, PhD, College of Sports and Health, Belgrade (Serbia)
- Prof. Branimir Kampl PhD, Institute SANO, Zagreb (Croatia)
- Prof. Baki Koleci PhD, University Hadzi Zeka, Peya (Kosovo)
- Prof. Branislav Simonovic PhD, Faculty of Law, Kragujevac (Serbia)
  Prof. Bistra Angelovska, Faculty of Medicine, University "Goce Delcev", Shtip (Macedonia)
- Prof. Cezar Birzea, PhD, National School for Political and Administrative Studies, Bucharest (Romania)
- Prof. Cvetko Andreevski, Dean, Faculty of Tourism, UKLO, Bitola (Macedonia)
- Prof. Drago Cvijanovic, PhD, Faculty of Hotel Management and Tourism, University of Kragujevac, Vrnjacka Banja (Serbia)
- Prof. Dusan Ristic, PhD Emeritus, College of professional studies in Management and Business Communication, Novi Sad (Serbia)
- Prof. Dimitar Radev, PhD, Rector, University of Telecommunications and Post, Sofia (Bulgaria)
- Prof. Daniela Todorova PhD, Rector of "Todor Kableshkov" University of Transport, Sofia (Bulgaria)
- Prof. Dragan Kokovic PhD, University of Novi Sad, Novi Sad (Serbia)
- Prof. Dragan Marinkovic PhD, High health – sanitary school for professional studies, Belgrade (Serbia)
- Prof. Daniela Ivanova Popova PhD, Faculty of Public Health and Sport, SWU Neofit Rilski, Blagoevgrad (Bulgaria)
- Prof. Dzulijana Tomovska, PhD, Dean, Faculty of Biotechnical sciences, Bitola(Macedonia)
- Prof. Evgenia Penkova-Pantaleeva PhD, UNWE -Sofia (Bulgaria)

- Prof. Fadil Millaku, PhD, Rector, University "Hadzi Zeka", Peja (Kosovo)
- Prof. Fatos Ukaj, University "Hasan Prishtina", Prishtina (Kosovo)
- Prof. Georgi Georgiev PhD, National Military University "Vasil Levski", Veliko Trnovo (Bulgaria)
- Prof. Halit Shabani, PhD, University "Hadzi Zeka", Peja (Kosovo)
- Prof. Halima Sofradzija, PhD, University of Sarajevo, Saraevo (Bosnia and Herzegovina)
- Prof. Haris Halilovic, Faculty of criminology and security, University of Sarajevo, Saraevo (Bosnia and Herzegovina)
- Prof. Helmut Shramke PhD, former Head of the University of Vienna Reform Group (Austria)
- Prof. Hristina Georgieva Yancheva, PhD, Rector, Agricultural University, Plovdiv (Bulgaria)
- Prof. Hristo Beloev PhD, Bulgarian Academy of Science, Rector of the University of Rousse (Bulgaria)
- Prof. Hristina Milcheva, Medical college, Trakia University, Stara Zagora (Bulgaria)
- Prof. Izet Zeqiri, PhD, Academic, SEEU, Tetovo (Macedonia)
- Prof. Ivan Marchevski, PhD, Rector, D.A. Tsenov Academy of Economics, Svishtov (Bulgaria)
- Doc. Igor Stubelj, PhD, PhD, Faculty of Management, Primorska University, Koper (Slovenia)
- Prof. Ivo Zupanovic, PhD, Faculty of Business and Tourism, Budva (Montenegro)
- Prof. Ivan Petkov PhD, Rector, European Polytechnic University, Pernik (Bulgaria)
- Prof. Isa Spahiu PhD, AAB University, Prishtina (Kosovo)
- Prof. Ivana Jelik PhD, University of Podgorica, Faculty of Law, Podgorica (Montenegro)
- Prof. Islam Hasani PhD, Kingston University (Bahrein)
- Prof. Jova Ateljevic PhD, Faculty of Economy, University of Banja Luka, (Bosnia & Herzegovina)
- Prof. Jove Kekenovski PhD, Faculty of Tourism, UKLO , Bitola (Macedonia)
- Prof. Jonko Kunchev PhD, University „Cernorizec Hrabar" - Varna (Bulgaria)
- Prof. Jelena Stojanovic PhD, High medicine school for professional studies "Hipokrat", Bujanovac (Serbia)
- Prof Karl Schopf, PhD, Akademie fur wissenschaftliche forchung und studium, Wien (Austria)
- Prof. Katerina Belichovska, PhD, Faculty of Agricultural Sciences, UKIM, Skopje (Macedonia)
- Prof. Krasimir Petkov, PhD, National Sports Academy "Vassil Levski", Sofia (Bulgaria)
- Prof. Kamal Al-Nakib PhD, College of Business Administration Department, Kingdom University (Bahrain)
- Prof. Kiril Lisichkov, Faculty of Technology and Metallurgy, UKIM, Skopje (Macedonia)
- Prof. Lidija Tozi PhD, Faculty of Pharmacy, Ss. Cyril and Methodius University, Skopje (Macedonia)
- Prof. Laste Spasovski PhD, Vocational and educational centre, Skopje (Macedonia)
- Prof. Larisa Velic, PhD, Faculty of Law, University of Zenica, Zenica (Bosnia and Herzegovina)
- Prof. Lujza Grueva, PhD, Faculty of Medical Sciences, UKIM, Skopje (Macedonia)
- Prof. Lazar Stosic, PhD, Association for development of science, engineering and education, Vranje (Serbia)
- Prof. Lisen Bashkurti PhD, Global Vice President of Sun Moon University (Albania)
- Prof. Lence Mircevska PhD, High Medicine School, Bitola, (Macedonia)
- Prof. Ljubomir Kekenovski PhD, Faculty of Economics, UKIM, Skopje (Macedonia)
- Prof. Ljupce Kocovski PhD, Faculty of Biotechnical sciences, Bitola (Macedonia)

- Prof. Marusya Lyubcheva PhD, University "Prof. Asen Zlatarov", Member of the European Parliament, Burgas (Bulgaria)
- Prof. Maria Kavdanska PhD, Faculty of Pedagogy, South-West University Neofit Rilski, Blagoevgrad (Bulgaria)
- Prof. Maja Lubenova Cholakova PhD, Faculty of Public Health and Sport, SWU Neofit Rilski, Blagoevgrad (Bulgaria)
- Prof. Mirjana Borota-Popovska, PhD, Centre for Management and Human Resource Development, Institute for Sociological, Political and Juridical Research, Skopje (Macedonia)
- Prof. Mihail Garevski, PhD, Institute of Earthquake Engineering and Engineering Seismology, Skopje (Macedonia)
- Prof. Misho Hristovski PhD, Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Skopje (Macedonia)
- Prof. Mitko Kotovchevski, PhD, Faculty of Philosophy, UKIM, Skopje (Macedonia)
- Prof. Milan Radosavljevic PhD, Dean, Faculty of strategic and operational management, Union University, Belgrade (Serbia)
- Prof. Marija Topuzovska-Latkovikj, PhD, Centre for Management and Human Resource Development, Institute for Sociological, Political and Juridical Research, Skopje (Macedonia)
- Prof. Marija Knezevic PhD, Academic, Banja Luka, (Bosnia and Herzegovina)
- Prof. Margarita Bogdanova PhD, D.A.Tsenov Academy of Economics, Svishtov (Bulgaria)
- Prof. Mahmut Chelik PhD, Faculty of Philology, University "Goce Delchev", Shtip (Macedonia)
- Prof. Marija Mandaric PhD, Faculty of Hotel Management and Tourism, University of Kragujevac, Vrnjacka Banja (Serbia)
- Prof. Marina Simin PhD, College of professional studies in Management and Business Communication, Sremski Karlovci (Serbia)
- Prof. Miladin Kalinic, College of professional studies in Management and Business Communication, Sremski Karlovci (Serbia)
- Prof. Mitre Stojanovski PhD, Faculty of Biotechnical sciences, Bitola (Macedonia)
- Prof. Miodrag Smelcerovic PhD, High Technological and Artistic Vocational School, Leskovac (Serbia)
- Prof. Nadka Kostadinova, Faculty of Economics, Trakia University, Stara Zagora (Bulgaria)
- Prof. Natalija Kirejenko PhD, Faculty For economic and Business, Institute of Entrepreneurial Activity, Minsk (Belarus)
- Prof. Nenad Taneski PhD, Military Academy "Mihailo Apostolski", Skopje (Macedonia)
- Prof. Nevenka Tatkovic PhD, Juraj Dobrila University of Pula, Pula (Croatia)
- Prof. Nedzad Korajlic PhD, Dean, Faculty of criminal justice and security, University of Sarajevo (Bosnia and Herzegovina)
- Prof. Nikolay Georgiev PhD, "Todor Kableshkov" University of Transport, Sofia (Bulgaria)
- Prof. Nikolina Ognenska PhD, Faculty of Music, SEU - Blagoevgrad (Bulgaria)
- Prof. Nishad M. Navaz PhD, Kingdom University (India)
- Prof. Oliver Iliev PhD , Faculty of Communication and IT, FON University, Skopje (Macedonia)
- Prof. Oliver Dimitrijevic PhD, High medicine school for professional studies "Hipokrat", Bujanovac (Serbia)
- Prof. Paul Sergius Koku, PhD, Florida State University, Florida (USA)
- Prof. Primoz Dolenc, PhD, Faculty of Management, Primorska University, Koper (Slovenia)
- Prof. Predrag Trajkovic PhD, JMPNT, Vranje (Serbia)
- Prof. Petar Kolev PhD, "Todor Kableshkov" University of Transport, Sofia (Bulgaria)
- Prof. Pere Tumbas PhD, Faculty of Economics, University of Novi Sad, Subotica (Serbia)

- Prof. Rade Ratkovic PhD, Faculty of Business and Tourism, Budva (Montenegro)
- Prof. Rositsa Chobanova PhD, University of Telecommunications and Posts, Sofia (Bulgaria)
- Prof. Rumen Valcovski PhD, Imunolab Sofia (Bulgaria)
- Prof. Rumen Stefanov PhD, Dean, Faculty of public health, Medical University of Plovdiv (Bulgaria)
- Prof. Sasho Korunoski, Rector, UKLO, Bitola (Macedonia)
- Prof. Sashko Plachkov PhD, Faculty of Pedagogy, University Neofit Rilski, Blagoevgrad
- (Bulgaria)
- Prof. Snezhana Lazarevic, PhD, College of Sports and Health, Belgrade (Serbia)
- Prof. Stojan Ivanov Ivanov PhD, Faculty of Public Health and Sport, SWU Neofit Rilski, Blagoevgrad (Bulgaria)
  Prof. Snezana Stoilova, PhD, High Medicine School, Bitola, (Macedonia)
- Prof. Stojna Ristevska PhD, High Medicine School, Bitola, (Macedonia)
- Prof. Suzana Pavlovic PhD, High health – sanitary school for professional studies, Belgrade (Serbia)
- Prof. Sandra Zivanovic, PhD, Faculty of Hotel Management and Tourism, University of Kragujevac, Vrnjacka Banja (Serbia)
- Prof. Shyqeri Kabashi, College "Biznesi", Prishtina (Kosovo)
- Prof. Trayan Popkochev PhD, Faculty of Pedagogy, South-West University Neofit Rilski, Blagoevgrad (Bulgaria)
- Prof. Todor Krystevich, Vice Rector, D.A. Tsenov Academy of Economics, Svishtov (Bulgaria)
- Prof. Todorka Atanasova, Faculty of Economics, Trakia University, Stara Zagora (Bulgaria)
- Doc. Tatyana Sobolieva PhD, State Higher Education Establishment Vadiym Getman Kiyev National Economic University, Kiyev (Ukraine)
- Prof. Tzako Pantaleev PhD, NBUniversity , Sofia (Bulgaria)
- Prof. Violeta Dimova PhD, Faculty of Philology, University "Goce Delchev", Shtip (Macedonia)
- Prof. Volodymyr Denysyuk, PhD, Dobrov Center for Scientific and Technologogical Potential and History studies at the National Academy of Sciences of Ukraine (Ukraine)
- Prof. Valentina Staneva PhD, "Todor Kableshkov" University of Transport, Sofia (Bulgaria)
- Prof. Vasil Zecev PhD, College of tourism, Blagoevgrad (Bulgaria)
- Prof. Venus Del Rosario PhD, Arab Open University (Philippines)
- Prof. Yuri Doroshenko PhD, Dean, Faculty of Economics and Management, Belgorod (Russian Federation)
- Prof. Zlatko Pejkov, PhD, Faculty of Agricultural Sciences, UKIM, Skopje (Macedonia)
- Prof. Zivota Radosavljevik PhD, Dean, Faculty FORCUP, Union University, Belgrade (Serbia)
- Prof. Zorka Jugovic PhD, High health – sanitary school for professional studies, Belgrade (Serbia)

REVIEW PROCEDURE AND REVIEW BOARD

Each paper is reviewed by the editor and, if it is judged suitable for this publication, it is then sent to two referees for double blind peer review.

The editorial review board is consisted of 45 members, full professors in the fields 1) Natural and mathematical sciences, 2) Technical and technological sciences, 3) Medical sciences and Health, 4) Biotechnical sciences, 5) Social sciences, and 6) Humanities from all the Balkan countries and the region.

KNOWLEDGE – International Journal
Vol.30.4
March, 2019

Contents

KNOWLEDGE – International Journal
Vol.30.4
March, 2019

ANTICANCER MONOCLONAL ANTIBODIES AND THEIR RADIOIMMUNOCONJUGATES - GATEWAY TO THE MORE SUCCESSFUL THERAPY

Marija Sterjova Arev
University Goce Delcev - Stip , Faculty of Medical Sciences, Stip, Macedonia,
marija.sterjova@ugd.edu.mk
Emilija Janevik-Ivanovska
University Goce Delcev - Stip, Faculty of Medical Sciences,Stip, Macedonia,
emilija.janevik@ugd.edu.mk

Abstract: Target therapy is the result of many years research dedicated to identify the characteristics of cancer cells and confirmation the differences between cancer cells and normal cells. The treatment of cancer is focused on killing the fast dividing cells. The need to achieve specificity in the treatment of cancer has led to designing and synthesizing of high selective agents, like monoclonal antibodies. To choose the right monoclonal antibody, first is important to identify the antigen on the surface of tumor cells. Monoclonal antibody-based therapy is one of the most successful therapeutic approaches for solid tumors and malignancies. Antibodies have shown high complexity in the manner of action and their biological properties. Manipulation of genes of antibodies with microbiologic techniques has led to significant modification in murine proteins. In order to not be recognized by human immune system, murine antibodies can be transduced to human or humanized monoclonal antibodies. Various studies have shown the ability of the antibodies to target the specific tumors and localize in or around the tumor cells. Identification of specific antigen on the surface of the tumor cells provides the formulation of monoclonal antibodies from animals and humans, which selectively bind to antigen and allows less toxicity to healthy cells. Conjugation of antibodies with cytotoxic drugs, toxins and radioisotopes provides development of selective and specific reagents. This immunoconjugates have three parts: monoclonal antibody selective for specific antigen, molecule that has a capacity to kill the cancer cell, and linker that connect the antibody and the active molecule. Antibody-drug conjugates, have chemotherapy drugs attached to the antibody, target the surface of cancer cells and deliver the toxic substance to that specific area. This approach can eliminate some of the side effects of chemotherapy, which can damage healthy cells when used as a single agent. Novel processes of enzymatically conjugating small molecule toxins to antibodies allow formulation of high selectivity agents. Radioimmunotherapy is a treatment that uses monoclonal antibodies in combination with radiation. By attaching a monoclonal antibody to a radioactive molecule, this technique can deliver a dose of radiation therapy directly to tumor cells. The aim of this paper is to do a review of literature for most commonly used antibodies and radioimmunoconjugates in the treatment of different types of cancer showing in the same time our contribution with obtaining results.
Keywords: monoclonal antibodies, immunoconjugates, cancer.

1. INTRODUCTION

The use of monoclonal antibodies (MAbs) is a well-known type of targeted anticancer therapy. A typical strategy for the treatment of cancer is focused on targeting and killing fast dividing cells. This approach requires to achieve high target specificity and, as a result, has led to designing and synthesizing a number of highly selective monoclonal antibodies. Selective targeted therapy allows imparting less toxicity to healthy cells, thus causing insignificant side effects [1,2].

A monoclonal antibody is a laboratory-produced protein that is engineered to atach a specific cancer cells. The progress of genetic engieering over the years has provided many opportunities for design and production of four main categories of monoclonal antibodies (pure murine, chimeric, humanized and pure human). The suffix of the drug name is: -momab (murine), -ximab (chimeric), -zumab (humanized) and –mumab (human) [6].

1.1. Commonly used anticancer monoclonal antibodies

Several anti-cancer monoclonal antibodies are available for human usage in registered formulations and depending on the percentage of participation of human and animal fragments, the approved anticancer antibodies can be grouped into three categories [6]:

1.1.1. Naked murine monoclonal antibodies were first used antibodies, but they showed two major disadvantages. Derived from rodents they are recognized by the human immune system developing human anti-murine antibodies (HAMA). HAMA inactivate and eliminate the murine antibodies and generate allergic reactions and anaphylactic shock, result of antibody-HAMA complex. Murine Fc fragment provides a resticted binding to effector cells and

activation of inderect pathways CDC and ADCC. To overcome this problems and to minimize immunogenicity, the murine antibody was engineered and were obtained antibodies similar to human antibodies, providing better phramacological response [3].

1.1.2. Chimeric IgG1 antibodies, produced by cloning of recombinant DNA contain the genes of variable region of the murine antibody and the genes of the constant region of human antibodies. These antibodies have over 65% of the human parts and less immugenicity in comparison with a pure murine antibodies and include following representatives:

Rituximab is the first registered antibody, since November 1997. It's an anti-CD20 antibody and it works through direct induction of apoptosis and indirectly through ADCC and CDC. Is obtained by genetic engineering and composed from immunoglobulin constant region of human IgG1 and variable regions from the murine anti-CD20 antibody 2B8. Rituximab was approved for treatment of NHL (non-Hodgkin lymphoma) and CLL (chronic lymphocytic leukemia) [7]. Many studies have proven the clinical efficiency of rituximab arthritis in combination with methotrexate fot treatment of rheumatoid arthritis [8].

Cetuximab, younger chimeric antibody, produced ftom the anti-EGFR murine antibody 225 and the human IgG1 antibody. Cetuximab has almost the same affinity for the EGFR, as the primary murine antibody. It works against over-expressed EGFR via ADCC and is used in treatment of colorectal cancer [9], non-small cell lung cancer [10] and in combination with conventional radiotherapy is active for squamous-cell carcinoma of the head and neck [11].

1.1.3. Humanized IgG1 antibodies contains more than 90% of human protein sequences and are obtained by transplanting the CDR of the murine antibody in a human antibody. Because they have only 5-10% of mrine proteins have shown the lowest immunogenicity. The most used antibodies are following:

Trastuzumab is a first approved humanized antibody as a potent anti-HER2/neu antibody, originate from murine 4D5 antibody. By composition is a protein of 1328 amino acids and has a molecular weight of 148 kDa. The original murine antibody 4D5 inhibits the proliferation of cell lines that have overexpressed HER2 receptors. Like a potent inhibitor of the growth of human breast cancer cells 4D5 was chosen for further clinical development. To reduce the probability of generation of HAMA, a murine monoclonal antibody (4D5) was humanized. The new humanized 4D5 have improved clinical efficacy by reducing its immunogenicity and Fc region who support ADCC. This antibody has a higher affinity for the antigen (HER2/neu) binding than 4D5, approximately Kd = 0.1nM. Is registered for the treatment of breast cancer with overexpressed HER2/neu. Trastuzumab is binding to the IV subdomain of the receptor and manifests the effect through ADCC (Fc region contains carbohydrate residues that interact with other parts of the immune system – effector cells) [12]. Many clinical trials were shown the efficacy of trastuzumab in other types of cancer with overespressed HER2/neu, like prostate, ovarian and non-small cell lung cancer [13-15].

Alemtuzumab is anti-CD52 antibody and is used in CLL therapy (chronic lymphocytic leukemia) and T cell lymphoma. It works throught direct induction of apoptosis and indirectly through ADCC and CDC [16]. Except in the treatment of leukemia alemtuzumab is promising drug in therapy of multiple sclerosis [17] and like an immunosuppressant in bone marrow and kidney transplantation [18].

Bevacizumab is a humanized monoclonal antibody that was obtained from a murine anti-VEGF antibody A.4.6.1. and human IgG1. VEGF (vascular epidermal growth factor) is an important growth factor that regulate process of angiogenesis and in the case of metastases has overexpression of this receptor [19]. In 2004, is approved for treatment of metastatic colorectal cancer. Two years latet, in combination with FOLFOX4 (5-fluorouracil, leucovorin and oxaliplatin) is approved as a secondary therapy of metastatic colorectal cancer [20].

1.1.4. Human antibodies contain fully human amino acid sequence derived antibody region therapeutics where antigen specificity has been selected either in vivo by the use of genetically modified mice or by antibody engineering processes combined with screening.

Panitumumab is a first fully human monoclonal antibody used in therapy since 2006. Represent a IgG2 antibody and was generated by XenoMouse techology. [21]. *Scheider-Merck et al.* [22] described the mechanism of action of panitumumab in cells of myeloid lineage. Panitumumab can recruiting ADCC by neutrophils and monocytes, but can not recruiting NK cell-mediated ADCC. It is active in KRAS metastatic colorectal cancer. The studies was shown that this antibody significantly prolonged the life of a patients compared with conventional chemotherapy [22].

The secound fully human antibody **Ofatumumab** is a human anti-CD20 IgG1 antibody with the mechanism of action is through CDC and ADCC, but it doesn't activate direct apoptoss. In higher degree promotes CDC than ADCC and is used in various forms of leukemia (non-Hodkin's lymphoma, chronic lymphocytic leukemia, B-cell lymphoma) [23] and is also active in most autoimmune diseases (multiple sclerosis, rheumatoid arthritis) [24, 25].

Ipilimumab is a fully human IgG1 monoclonal antibody generated from unique transgenic mouse (HuMab), when the endogenous murine immunoglobulin genes have been repleaced with human loci. It is anti CLTA-4 (cytotoxic

T-lymphocyte-associated protein 4) antibody registered in 2011 for treatment of metastatic melanoma [26]. It works by binding CTLA-4, excliding inhibitory mechanism and allows destroying of tumor cells. The clinical trials have shown that this antibody alone or in combination with radiotherapy is active in prostate cancer [27] and in combination with paclitaxel and carboplastin is active in non-small cell and small cell lung cancer [28, 29].

Nivolumab is a youngest IgG4 monoclonal antobody registered in 2014 for therapy of metastatic melanoma. This antibody was generated by imunization of transgenic mice. It works by blocking the PD-1 (programmed death-1), which is an inhibitory receptor expressed on the surface if T cells [30]. The clinical trials have shown that nivolumab is active in non-small cell lung cancer and is registered for the therapy of this type of cancer [31].

Fully human and humanized antibodies carry a lower risk for inducing immune responses in humans than mouse or chimeric antibodies.

Monoclonal antibodies can be used as naked – unconjugated or conjugated with different types of drugs, toxins and radioisotopes, in order to improve the specificity and pharmacological response and to reduce the side effects [35].

1.2. Immunoconjugates

Conjugation of antibodies provides development of cancer-specific cytotoxic reagents. Each of these conjugates carrying their therapeutic capacity and intrinsic toxicity [32]. The drug conjugates have the potential advantage due to the higher drug release in tumor cells and reducing the drug resistance. The immunotoxins are very interesting for examinations because of their high specific activity [33], while the radio-labeled antibodies beside the therapeutic have the potential for molecular imaging [34]. Each immunoconjgate has an enhanced therapeutic specificity due to the conjugation with antibody which shows specificity to a particular antigen on the surface of tumor cells [32].

1.2.1. Radioimmunoconjugates

The high selectivity and specificity of monoclonal antibodies (mAbs) are usually exploited for the production of a variety of immunoconjugates where the antibody is linked either to cytotoxic drugs and toxins or to radioisotopes. Each of these conjugates carries its own therapeutic cargo and, therefore, exhibits an intrinsic toxicity [3]. Drug immunoconjugates have the potential advantage over the free drug substance of enhancing drug delivery to tumor cells with a concomitant reduction of drug resistance. Immunotoxins are very interesting tools for investigation because of their high specific biological activity [4], whereas radiolabeled antibodies have the additional advantage that can be used also for molecular imaging and therapy [5].

Significant radiopharmaceuticals based on immunoconjugates for diagnostic and therapeutical purpose used different radioisotopes (^{68}Ga, ^{177}Lu, ^{90}Y, $^{131/124/123}$I, ^{99m}Tc/^{188}Re). Several studies have shown that already existing radiolabeled formulations, using beta and alpha emitters (^{90}Y, ^{131}I, ^{177}Lu, ^{188}Re, ^{227}Th, ^{225}Ac) are potent therapy against many cancers. Many radioimmunoconjugates employing techniques of positron emission tomography (PET) and single photon emission tomography (SPECT) for imaging are used for diagnosis, follow the efficacy of the treatment and planning radiotherapy.

Until now, a series of clinical and preclinical trials of cancer imaging and therapy with radioimmunoconjugates has been carried out [36-38]. Many efforts have been devoted to obtain stable antibody conjugates with different radioisotopes and suitable chelators, while preserving the native antibody's immunoreactivity.

Full length mAbs and mAb fragments offer an excellent approach for creating high-affinity, labeling imaging preparation because of (1) the high specificity for targets, (2) an abundance of reactive amino acid residues (i.e., lysines and cysteines) for conjugation of chelators, (3) the plethora of therapeutic mAbs on the market and within clinical trials, (4) the clinical acceptance of radioimmunoscintigraphy, and (5) the versatility of imaging agent design offered by mAb engineering.

The chelator allows binding to the antibody on the one side and coordinative binding of radioisotpes on the other side. The most commonly used chelators are: DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), DTPA (diethylene triamine pentaacetic acid), TCMC (1,4,7,10-tetra-(2-carbamoyl methyl)-cyclododecane), HYNIC (succinimidyl-6-hydrazino-nicotinamide) and DTPA derivates. Radioimmutherapeutics used in the treatment of lymphoma are more advanced than any other agents. The only two approved RIT, tositumomab-^{131}I (approved 2003) and ibritumomab tiuksetin-^{111}In or ^{90}Y (approved 2002), are anti-CD20 and are indicated for the treatment of B-cell lymphoma. Tositumomab-^{131}I is a murine IgG2a monoclonal antibody labeled with iodine-131 and commercially is used for treatment of NHL and B-cell lymphoma. Ibritumomab tiuxetin kit contains murine IgG antibody and chelator tiuxetin (modified DTPA) which can be tagged with 111In (for imaging) and 90Y (for therapy) [34].

It was done a lot of efforts to formulate other stable immunoconjugates for therapy of different lymphomas and other types of cancer.

2. OUR CONTRIBUTION AND FUTURE REASEARCH

Over last 6 our group is involved in the several projects related to formulation of stable antibody conjugates in a form of freeze-dried (ready to use cold kit) for labeling with suitable radioisotope for therapy (^{90}Y, ^{177}Lu), including preclinical investigations as a step forward to the clinical studies [36-38].

To avoid problems related to the stability and transport of antibodies, technology of freeze-drying was introduced that can be applied as well for radiolabeled antibodies (Figure 1).

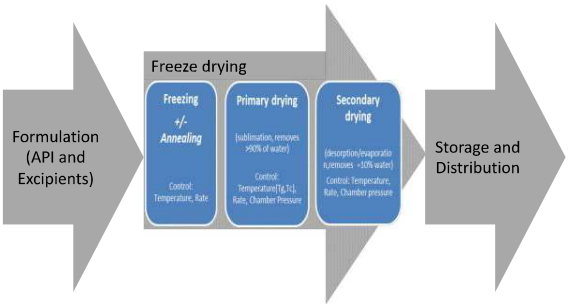


Figure 1. Freeze-drying technology for producing immunoconjugates

In our previous work we already indicated the possibility to introduce the available technology for "ready to use" preparation of cold kit freeze-dried formulation of conjugated antibody (rituximab, trastuzumab) and peptide-based radiopharmaceuticals for labeling with with ^{90}Y and ^{177}Lu [36-38].

We developed two freeze-dried trastuzumab conjugates using bifunctional chelators (BFC) - DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), DTPA DTPA (diethylene triamine pentaacetic acid), and DTPA derivative 1B4M-DTPA[IB4M-DTPA=2-(4-isothiocyanatobenzyl)-6-methyl-diethylene-triaminepentaacetic acid], as the most widely investigated for a successful labeling of antibodies with metallic radionuclides [14]. Schematic drawings of the chemical structures of these BFCs are illustrated in Figure 2.

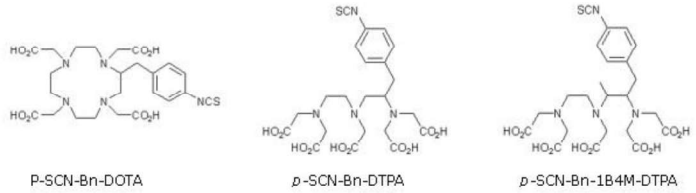


Figure 2. Chemical structure of the bifunctional chelators employed in this study

Radiolabeling was realized by combining the BFC-derivatized antibody with the appropriate radioisotope.

Several techniques have been used to characterize the stability of the antibody in the formulated immunoconjugates. A protein integrity and purity were accessed by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Vibrational (infrared and Raman) spectroscopy provided molecular structure information and was found convenient for verification of possible changes in the secondary structure. The number of chelating groups per one trastuzumab molecule was obtained by MALDI-TOF-MS. Quality control and stability were examined by ITLC using different mobile phases.

All our obtained results shows successful formulation of stable radioimmunoconjugates which makes this proposed freeze-dried kit as potential radiopharmaceutical *in vivo* investigations and possibility to introduce this e technology for preparation of freeze-dried formulation of conjugated antibody using different chelators for labeling with Zr-89, positron emitter (half-life of 78.4 h) compatible with the time needed to achieve optimal tumor-to-non tumor ratios (typically 2–4 days for intact monoclonal antibody).

3. CONCLUSION

The use of monoclonal antibodies in anti-cancer therapy is one of the big successes of the past decade. The success is based on long scientific researches in order to understand the complexity of the antibodies, target antigens, antibody-antigens functions and immune regulation of tumor growth. The affinity of the antibodies can be modified and adjusted to inhibit binding to normal tissues and to improve the penetration and retention in tumor tissue. The

selectivity toward cancer cells can be increased by modification of size, valence, structure and specificity of the antibodies. Improved conjugation technology allows the development of immunoconjugates and selective delivering of cytotoxic agents to tumor cells. These immunoconjugates are considered as next generation of anticancer antibodies. The progress of radiopharmacy provides development of radioimmunoconjugates which are more effective than immunotherapy with pure antibodies.

LITERATURE

[1] M. Mehren, G.A. Adams, L.M. Weiner, Monoclonal antibody therapy for cancer. Annu Rev Med, vol.54, pp.343-369, 2003
[2] J. Baselga, Clinical trials of herceptin (trastuzumab), Eur J Cancer, vol. 37 (1), pp.S18-S24, 2001.
[3] R.M. Sharkey, G.M. Goldenberg, Targeted therapy of cancer: new prospects for antibodies and immunoconjugates, CA Cancer J Clin, vol. 56 (4), pp. 226-243, 2006
[3] J. M. Reichert, V. E. Valge-Archer, Development trends for monoclonal antibody cancer therapeutics, Nature, vol. 6, pp. 349-356, 2007.
[4] I. Pastan, R. Hassan, D.J. FitzGerald, R.J. Kreitman, Immunotoxin therapy of cancer, Cancer, vol. 6(7), pp. 559-565, 2006
[5] D. E. Gerber, Targeted Therapies: a new generation of cancer treatments, American Family Physician, vol. 77, pp. 311-319, 2008.
[6] J. M. Reichert, C. J. Rosensweig, L. B. Faden, M. C. Dewirz, Monoclonal antibody successes in the clinic, Nature Biotechnology, vol. 23, pp. 1073-1078, 2005.
[7] J. Du, H. Wang, C. Zhong, B. Peng, M. Zhang, B. Li, S. Huo, Y. Guo, J. Ding, Structural basis for recognition of CD20 by therapeutic antibody rituximab, The Journal of Biological Chemistry, vol. 282, pp. 15073-15080, 2007.
[8] A. Ehlers, Rituximab: an autoimmune disease therapy, IG living, pp. 24-26 2013.
[9] D. Cunningham, Y. Humblet, S. Siena, D. Khayat, H. Bleiberg, A. Santoro, D. Bets, M. Mueser, A. Harstrick, C. Verslype, I. Chau, E. V. Cutsem, Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer, The New England Journal of Medicine, vol. 351, pp. 337-345, 2004.
[10] P. Steiner, C. Joynes, R. Bassi, S. Wang, J. R. Tonra, Y. R. Hadari, D.J. Hicklin, Tumor growth inhibition with cetuximab and chemotherapy in non-small cell lung cancer xenografts expressing wild-type and mutated epidermal growth factor receptor, Clinical Cancer Research, vol. 13, pp. 1540-1551, 2007.
[11] J. A. Bonner, P. M. Harari, J. Giralt, N. Azarnia, D. M. Shin, R. B. Cohen, C.U. Jones, R. Sur, D. Raben, J. Jassem, R. Ove, M. S. Kies, J. Baselga, H. Youssoufian, N. Amellal, E. K. Rowinsky, K. K. Ang, Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck, The New England Journal of Medicine, vol. 354, pp. 567-578, 2006.
[12] G. A. Hudis G.A, Trastuzumab-mechanism of action and use in clinical practice, The New England Journal of Medicine, vol. 357, pp. 39-51, 2007.
[13] P. N. Lara, K. G. Chee, J. Longmate, C. Ruel, F. J. Mayers, C. R. Gray, R. G. Edwards, P. H. Gumerlock, P. Twardowski, J. H. Doroshow, D. R. Gandara, Trastuzumab plus docetaxel in HER2/neu-positive prostate cancer, Cancer, vol. 100, pp. 2125-2131, 2004.
[14] M. A. Bookman, K. M. Darcy, D. Clarke-Pearson, R. A. Boothby, I. R. Horowitz, Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the gynecologic oncology group, Journal of Clinical Oncology, vol. 21, pp. 283-290, 2003.
[15] C. J. Langer, P. Stephenson, A. Thor, Vangel M, D. H. Johnson, Trastuzumab in the treatment of advanced non–small-cell lung cancer: Is there a role? Focus on eastern cooperative oncology group study 2598, Journal of Clinical Oncology, vol. 22, pp. 1180-1187, 2004.
[16] S. Demko, J. Summers, P. Keegan, R. Pazdur, FDA drug approval summary: alemtuzumab as single-agent treatment for B-cell chronic lymphocytic leukemia, The Ongologist, vol. 13, pp. 167-174, 2008.
[17] A. Minagar, J. S. Alexander, M. A. Sahraian, R. Zivadinov, Alemtuzumab and multiple sclerosis: therapeutic application, Expert Opinion on Biological Therapy, vol. 10, pp. 421-429, 2010.
[18] M. J. Hanaway, E. S. Woodle, S. Mulgaonkar, V. R. Peddi, D. B. Kaufman, M. R. First, R. Croy, J. Holman, Alemtuzumab induction in renal transplantation, The New England Journal of Medicine, vol 364, pp. 1909-1919, 2011.
[19] N. Ferrara, K. J. Hillan, W. Novotny, Bevacizumab (Avastin) a humanized anti-VEGF monoclonal antibody for cancer therapy, Biochemical and Biophysical Research Communications, vol. 333, pp. 328-335, 2005.

[20] M. H. Cohen, J. Gootenberg, P. Keegan, R. Pazdur, FDA drug approval summary: bevacizumab plus FOLFOX4 as second-line treatment of colorectal cancer, The Oncologist, vol. 12, pp. 356-361, 2007.
[21] R. M. Giusti, K. A, Shastri, M. H. Cohen, P. Keegan, R. Pazdur, FDA: drug approval summary: panitumumab (Vectibix), The Oncologist, vol. 12, pp. 577-583, 2007.
[22] T. Schneider-Merck, J. J. L.van Bueren, S. Berger, K. Rossen, P. H. C. van Barkel, S. Derer, T. Beyer, S. Lohse, W. K. Bleeker, M. Peipp, P. W. H. I. Parren, J. G. J. van de Winkel, T. Valerius, M. Dechant, Human IgG2 antibodies against epidermal growth factor receptor effectivevly trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG1, only by cells of myeloid lineage, The Journal of Immunology, vol. 184, pp. 512-520, 2010.
[23] S. J. Lemery, J. Zhang, M. D. Rothmann, J. Yang, J. Earp, H. Zhao, A. McDougal, A. Pilaro, R. Chiang, J. E. Gootenberg, P. Keegan, R. Pazdur, U.S. food and drug administration approval: ofatumumab for the treatment of patients with chronic lymphocytic leukemia refractory to fludarabine and alemtuzumab, Clinical Cancer Research, vol. 16, pp. 4331-4338, 2010.
[24] B. Barun, A. Bar-Or, Treatment of multiple sclerosis with anti-CD20 antibodies, Clinical Immunology, vol. 10, pp. 1-7, 2011.
[25] P. C. Taylor, E. Quattrocchi, S. Mallett, R. Kurrasch, J. Petersen, D. J. Chang, Ofatumumab, a fully human anti-CD20 monoclonal antibody, in biological-naive, rheumatoid arthritis patients with an inadequate response to methotrexate: a randomised, double-blind, placebo-controlled clinical trial, Annals of the Rheumatic Diseases, vol. 70, pp. 2119-2158, 2011.
[26] J. D. Wolchok, F. S. Hodi, J. S. Weber, J. P. Allison, W. J. Urba, C. Robert, S. J. O'Day, A. Hoos, R. Humphrey, D. M. Berman, N. Lonberg, A. J. Korman, Development of ipilimumab: a novel immunotherapeutic approach for the treatment of advanced melanoma, Annals of the New York Academy of Sciences, vol. 1291, pp. 1-13, 2013.
[27] S. F. Slovin, C. S. Higano, O. Hamid, S. Tejwani, A. Harzstark, J. J. Alumkal, H. I. Scher, K. Chin, P. Gagnier, M. B. McHenry, T. M. Beer, Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study, Annals of Oncology, vol. 24, pp. 1813-1821, 2013.
[28] M. Reck, I. Bondarenko, A. Luft, P. Serwatowski, F. Barlesi, R. Chacko, M. Sebastian, H. Lu, J. M. Cuillerot, T. J. Lynch, Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensivedisease-small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial, Annals of Oncology, vol. 24, pp. 75-83, 2013.
[29] T. J. Lynch, I. Bondarenko, A. Luft, P. Serwatowski, F. Barlesi, R. Chacko, M. Sebastian, J. Neal, H. Lu, J. M. Cuillerot, M. Reck, Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non–small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study, Journal of Clinical oncology, vol. 31, pp. 2047-2054, 2012.
[30] C. Wang, K. B. Thudium, M. Han, X. T. Wang, H. Huang, D. Feingersh, C. Garcia, Y. Wu, M. Kuhne, M. Srinivasan, S. Singh, S. Wong, N. Garner, H. Leblanc, R. T. Bunch, D. Blanset, M. J. Selby, A. J. Korman, In Vitro Characterization of the Anti-PD-1 Antibody Nivolumab, BMS-936558, and In Vivo Toxicology in Non-Human Primates, Cancer Immunology Research, vol. 2, pp. 846-856, 2014.
[31] J. Brahmer, K. L. Reckamp, P. Baas, L. Crino, W. E. E. Eberhardt, E. Poddubskaya, S. Antonia, A. Pluzanski, E. E. Vokes, E. Holgado, D. Waterhouse, N. Ready, J. Gainor, O. A. Frontera, L. Havel, M. Steins, M. C. Garassino, J. G. Aerts, M. Domine, L. V. Ares, M. Reck, C. Baudelet, C. T. Harbison, B. Lestini, D. R. Spigel, Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer, The New England Journal of Medicine, vol. 10, pp. 1-14, 2015.
[32] B. A. Teicher, R. V. J. Chari, Antibody conjugate therapeutics: challenges and potential, Clinical Cancer Research, vol. 17, pp. 6389-6397, 2011.
[33] I. Pastan, R. Hassan, D. J. FitzGerald, R. J. Kreitman, Immunotoxin therapy of cancer, Cancer, vol. 6, pp. 559-565, 2006.
[34] C. A. Boswell, M. W. Brechbiel, Development of radioommunotherapeutic and diagnostic antibodies: an inside-out view, Nuclear Medicine and Biology, vol. 247, pp. 757-778, 2007.
[35] C.A. Boswell, M.W. Brechbiel, Development of radioommunotherapeutic and diagnostic antibodies: an inside-out view, Nucl Med Biol, vol. 34(7), pp.757-778, 2007
[36] D. Gjorgieva Ackova, K. Smilkov, E. Janevik-Ivanovska, Formulation and characterization of "Ready to Use" 1B4M-DTPA-rituximab for Lu-177 labeling, World J Med Sci. vol.11(4), pp.535-540, 2014

[37] D. Gjorgieva Ackova, K. Smilkov, E. Janevik-Ivanovska, T. Stafilov, Z. Arsova-Sarafinovska, P. Makreski, Evaluation of non-radioactive lutetium- and yttrium-labeled immunoconjugates of rituximab – a vibrational spectroscopy study, Maced J Chem Eng. Vol.34(2), pp.351-362, 2015
[38] M. Sterjova, P. Džodić, P. Makreski, A. Duatti, M.Risteski, E. Janevik-Ivanovska, Vibrational spectroscopy as a tool for examination to the secondary structure of metal-labeled trastuzumab immunoconjugates, Journal of Radioanalytical and Nuclear Chemistry, vol.319 (on-line doi.org/10.1007/s10967-019-06450-8), 2019