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Impact of air pollution in Poland to the incidence of the lung cancer in adults (2010-2014)

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Abstract: Cancer is one of the diseases with highest rates of morbidity in the world and one of the leading causes of death. One of the most dangerous is lung cancer, which in men is first in morbidity and mortality, in women in third place in morbidity (in Poland on second) and in the first in mortality. Among the many factors that contribute to the disease is air pollution.
We have analyzed the databases of the Central Statistical Office, the Chief Inspectorate for Environmental Protection, to determine the air condition in Poland in 2010-2015. We compared these parameters with air quality standards proposed by the WHO and the EU, and also in the light of Polish law. Changes in concentrations of SO2, NO2, PM2.5, PM10 over the past six years have compared the number of cases of lung cancer in the same period, obtained from the National Cancer Registry. Unfortunately, the number of lung cancer cases is constantly increasing, with a slight improvement in some of the air quality parameters.
Due to the observed trend, we have been focusing on the issue of screening for lung cancer and their cost-effectiveness. According to the authors, the increase in the number of lung cancer cases is worth considering a screening program in the risk group. This will not, of course, replace education and campaigns aimed at reducing risk factors.
Keywords: Lung cancer, PM 2,5, PM10, screening

1. Cancer

Cancer is a term for a group of diseases in which diseased cells begin to divide without limitation from the immune control of our body. Cancer can develop almost anywhere, normally cells die due to age and previous damages in order to give space for new cells, which are currently needed in the body. When the disease develops, this regimen stops working. Damaged cells multiply more and more frequently and they resemble their prototype less. This excess of cells can form solid structures – tumors, but for example blood cancers usually do not form solid structures. Malignant tumors along with their growth obtain the ability to infiltrate neighboring tissues as well as create metastases via the blood or lymphatic system in distant tissues [1].

1.1. Cancer morbidity and mortality

Cancers have one of the highest morbidity rates – about 12 million new cases worldwide in 2012, which means that for every 100,000 people, 182 of them will develop cancer. Unfortunately, this is also one of the main causes of death, approximately 8 million people have died due to cancer in the same period. By showing this in a more graphical way, every 100,000 people 102 of them died during the study year because of the cancer. As for the mortality rates, subsequent to cancer were the following causes: ischemic heart disease, stroke, pneumonia. It is interesting that the highest incidence of cancer occurs in highly developed countries (North America, Western Europe, Japan, South Korea, Australia, New Zealand), but as many as 60% of all cancers occur in Africa, South and Central America, Asia and results in 70% of fatalities [2].

1.2. Risk factors

Cancer is a multifactorial disease, we can draw here attention to some of the types of cancer (e.g. colorectal, breast cancer) [3, 4], in some viral factors are more important (cervical cancer, Merkel cell carcinoma) [5÷7]. A wide range of risk factors are the most common in our environment, one can say that for those we can influence the most in our

daily lives. First of all, the environmental pollution should be mentioned – further discussed in more detail on the issue of air pollution in the context of lung cancer. Stress, lifestyle, working conditions (exposure to pollution), diet and alcohol consumption are factors that affect our health and the potential development of cancer. Active and passive smoking is a significant risk factor and threat to our health, which is important not only in the case of lung cancer but also other cancers, not to mention even other lung and cardiovascular diseases [2, 8].

2. Lung cancer

2.1. World distribution of lung cancer

According to the World Health Organization report from 2014 (data for 2012), lung cancer is the malignant tumor with the highest morbidity among all cancer types. Gender inequality in cancer distribution is worth noting – among men, lung cancer is in the top place in the world – frequency 34.2/100,000 worldwide, resulting in 16.7% of all cancers. In women the situation is only slightly better, lung cancer occupies third place (8.7% of all cancers) behind breast cancer (25.2%) and colorectal cancer (9.2%).

Unfortunately, lung cancer is also responsible for the highest mortality of cancers in the world – it accounts for 19.4% of cancer deaths, which gives us almost 1.6 million people in 2012. It is characterized by a very poor prognosis – statistically only in 5.8% patients diagnosed with lung cancer 5-year survival rate is predicted [2].

2.2. Distribution of lung cancer in Poland

According to the data available in the National Cancer Registry of Poland we can see that in Poland (data for 2013) the most common malignancy cancer in men was lung cancer 48.1/100.000, resulting in 18.7% of all cancers in Poland. In women, same for the worldwide statistics, lung cancer was in third place – 8.8% of cases (31/100 000). Unfortunately, in the case of cancer mortality in Poland, lung cancer is at the top place mortality rates of both men and women. The 5-year survival rate was 13.1%, which is better than world-wide statistics. Sadly, however, we must say that in the case of both morbidity and mortality Poland is above the European Union average [9].

2.3. Gender differences in lung cancer

Based on the available data [9, 10], we see a growing trend for lung cancer in women over the years, while in men it is on a similar level, in some parts of the world, decreasing. Women are more likely to be diagnosed with adenocarcinoma, which is strongly associated with smoking, but the more commonly diagnosed men in squamous cell carcinoma has an even stronger correlation. We can see an upward trend in the incidence of adenocarcinoma, which may also suggest other risk factors [10, 11]. Certainly, cultural changes and the increasing popularity of smoking among women are not without reason.

3. Tobacco smoking epidemic

Tobacco smoking has a significant negative impact on our body, as it contributes to numerous cardiological, pulmonological and oncological diseases [8, 12÷16]. The World Health Organization estimates that around 6 million people die each year from tobacco-related illnesses. We are pleased to welcome the fact that the number of cigarettes smoked in developed countries is decreasing. It is difficult to say clearly whether this is due to awareness of the harmfulness of this addiction or legal regulations (high taxes, restrictions on smoking places, anti-smoking campaigns) [2]. Unfortunately, in developing countries, the consumption of tobacco products is constantly increasing.

4. Air pollution

Air purity is recognized as one of the important health parameters and welfare criteria. The World Health Organization has been dealing with air quality since 1987, when the first guidelines for standards and norms for pollutants were issued. Since then many researches and studies have been done on the harmfulness of those substances (most commonly SO_2, NO_2, Benzene, Ozone, PM2.5, PM10), which are largely the result of human kind activities, especially the development of technology and industry. Since the publication of the first guidelines, it has become important for public opinion to take care of the quality of the surrounding environment. This issue is constantly gaining momentum.

4.1. Sulfur dioxide (SO_2)

Sulfur dioxide is a chemical compound which is being emitted into the atmosphere from both natural and industrial sources. It is not a neutral compound – exposure to its increased concentration in the atmosphere has a negative impact. We can distinguish short-term exposure to elevated concentrations of over 500 μg / m3 within 10 minutes and long-term exposure of over 20 μg / m3 per day [17]. A result of increased exposure may be irritation of the airways, intensification of chronic respiratory diseases, chronic cardiovascular diseases. A measurable index to this correlation lies in the increased frequency of patients reporting intensification of the symptoms to Hospital Emergency Departments during periods of elevated levels of SO_2 in the atmosphere [18÷19].

Tab. 1 Average daily concentrations of SO_2 in Poland in 2010-2014, SO_2 emissions in 2010,2014, Lung cancer incidence ICD-10 C33 + C34 in 2010-2014

	2010	2011	2012	2013	2014
Concentration of SO_2 (μg/m3)	6,1	4,6	6,6	2,4	2,1
Emission of SO_2 (in thousands of tons)	970	-	-	-	800
Morbidity of lung cancer cases (ICD-10 C33 + C34) (absolute numbers)	20871	20837	21870	21556	22032

Source: Prepared by the author on the basis of data of the Chief Inspectorate for Environmental Protection, Central Statistical Office– Ochrona Środowiska 2016 oraz Didkowska Joanna, Wojciechowska Urszula. Zachorowania i zgony na nowotwory złośliwe w Polsce. Krajowy Rejestr Nowotworów, Centrum Onkologii – Instytut im. Marii Skłodowskiej-Curie. Available on the website http://onkologia.org.pl/k/ epidemiologia/ dostęp z dnia 13/06/2017

Based on Table 1, we can see that the standards proposed by the World Health Organization are respected in Poland and the SO_2 emission from industrial sources in the country is decreasing. Despite optimistic data, the incidence of lung cancer still continues to increase.

4.2. Nitrogen dioxide (NO_2)

Nitrogen oxides mainly represented by nitrogen dioxide are pollutants mainly attributed to road transport. Most of them are formed by NO reaction with ozone. This is another chemical with proven harmful effects, whether in animal studies or humans. Here we also have guidelines prepared by the World Health Organization – the annual norm of 40 μg / m^3 and hourly 200 μg / m^3 [17]. Any crossing of standards may have a negative effect, it is particularly worth emphasizing in the context of the difference between the WHO guidelines and the Polish and European legislation. The effects of exposure to elevated levels appear in every age group and may occur even in fetus as a low birth weight, low weight in relation to the gestation week or premature birth [20]. This also increases the

risk of children patients in the Emergency Departments due to intensification of symptoms of respiratory diseases [21]. In adult life through long-term exposure to car exhaust fumes our cardiovascular diseases risk may be increased [22], and we can not forget the increased risk of cancer including lung and breast cancer [23, 24].

Tab. 2 Average annual concentrations of NO_2 in Poland in 2010-2014, NO_2 emissions in 2010, 2014, Incidence of lung cancer ICD-10 C33 + C34 in 2010-2014

	2010	2011	2012	2013	2014
Concentration of NO_2 (μg/m3)	19,7	19,7	18,54	18,3	18,09
Emission of NO_2 (in thousands of tons)	874	-	-	-	723
Morbidity of lung cancer cases (ICD-10 C33 + C34) (absolute numbers)	20871	20837	21870	21556	22032

Source: Prepared by the author on the basis of data of the Chief Inspectorate for Environmental Protection, Central Statistical Office – Ochrona Środowiska 2016 oraz Didkowska Joanna, Wojciechowska Urszula. Zachorowania i zgony na nowotwory złośliwe w Polsce. Krajowy Rejestr Nowotworów, Centrum Onkologii – Instytut im. Marii Skłodowskiej-Curie. Available on the website http://onkologia.org.pl/k/ epidemiologia/ dostęp z dnia 13/06/2017

Based on the data in Table 2 we see a decrease in both NO_2 concentration and its emission, analogically to SO_2. This fact can be explained that the greater risk is the exposure to the harmful factor itself and the its duration – the greater risk lies in the longer duration and not the intensity [25]

4.3. Benzene

The main source of benzene is the processing of crude oil, road transport. It is used in industrial production and we can also find it in tobacco smoke. Under the Polish and European legislation, its annual air quantity is 5 $\mu g/m^3$ – the 2005 WHO guidelines do not comment on this. What worries us that it can be also found in food and water. It has toxic and narcotic effect on the human body [26], which can result in increased mortality, including acute and chronic myeloid leukemia and lung cancer [27]. Negative impact is not limited to cancer proliferation, but also to the damages within reproductive, nervous, immunological, cardiovascular and respiratory systems [28].

Tab. 3 Average annual concentrations of benzene in Poland in 2010-2014, Benzene emissions in 2010,2014, Incidence of lung cancer ICD-10 C33 + C34 in 2010-2014

	2000	2010	2011	2012	2013	2014
Concentration of benzene (μg/m3)	2,51	2,25	2,06	2,29	1,88	1,78
Emission of benzene (in thousands of tons)	57	-	-	-	-	-
Morbidity of lung cancer cases (ICD-10 C33 + C34) (absolute numbers)	-	20871	20837	21870	21556	22032

Source: Prepared by the author on the basis of data of the Chief Inspectorate for Environmental Protection, Central Statistical Office – Ochrona Środowiska 2016 oraz Didkowska Joanna, Wojciechowska Urszula. Zachorowania i zgony na nowotwory złośliwe w Polsce. Krajowy Rejestr Nowotworów, Centrum Onkologii – Instytut im. Marii Skłodowskiej-Curie. Available on the website http://onkologia.org.pl/k/ epidemiologia/ dostęp z dnia 13/06/2017

The average concentration in Poland in the case of benzene falls within the limits set by the law. (Tab. 3) But what can cause anxiety is the increased emissions of this harmful substance, especially given its wide negative impact on the human body.

4.4. Particulate Matter (PM)

Particulate Matter is a mixture of organic and inorganic substances, including toxic substances such as dioxins, furans, or heavy metals suspended in gas. We distinguish two types: PM 10 – that is particles up to 10 microns in diameter – they can reach the airways and lungs. PM 2,5 are particles with a diameter of up to 2.5 micrometers, due to its small size, they can reach not only to the lungs, but also the bloodstream. Major sources of the PM include burning low-quality coal and waste in old unregulated boilers, chemical, energy and mining industries [26]. Many consequences of exposure to dust can be attributed to circulatory symptoms: arrhythmias, especially ischemic stroke [29÷32] respiratory system: sore throat, cough, intensification of COPD, lung cancer [21, 32, 33]. According to the guidelines proposed by the World Health Organization, the average annual concentration for PM 2,5 should not exceed 10 $\mu g/m^3$ and for PM 10-20 $\mu g/m^3$.

Tab. 4 Average annual concentrations of PM2,5 i PM10 in Poland in 2010-2014, PM emissions in 2010,2014, Incidence of lung cancer ICD-10 C33 + C34 in 2010-2014

	2010	2011	2012	2013	2014
Concentration of PM2,5 (μg/m3)	34,2	31,7	27,9	26,5	26,3
Concentration of PM10 (μg/m3)	37,6	36,7	34	32,1	34,5
Emission of PM (in thousands of tons)	462	-	-	-	383
Morbidity of lung cancer cases (ICD-10 C33 + C34) (absolute numbers)	20871	20837	21870	21556	22032

Source: Prepared by the author on the basis of data of the Chief Inspectorate for Environmental Protection, Central Statistical Office– Ochrona Środowiska 2016 oraz Didkowska Joanna, Wojciechowska Urszula. Zachorowania i zgony na nowotwory złośliwe w Polsce. Krajowy Rejestr Nowotworów, Centrum Onkologii – Instytut im. Marii Skłodowskiej-Curie. Available on the website http://onkologia.org.pl/k/ epidemiologia/ dostęp z dnia 13/06/2017

As can be seen from the information presented in Table 4, the standards for PM 2,5 have been exceeded in many cases over the last few years – and the more liberal EU and Polish norms have been exceedingly disturbed as well. In the case of PM 10 similar standards were not met, but the scale of their exceedance was lower than in PM 2.5. Optimistic seems to be a downward trend for both concentrations and emissions.

5. Air Quality

Due to the nature and authority of the organization, when writing about air quality, in many places we refer to the standards of the World Health Organization issued in 2005 [17]. Other proposed standards that could be taken into consideration are the guidelines adopted by the European Commission under Directive 2008/50/EC of 2008 and the Regulation of the Minister of the Environment of Poland 2012 (Official Journal of 2012, item 1032). Unfortunately, these values are more liberal than those proposed by WHO. Presumably, the explanation of this fact may be the economic nature of the European Union and the desire to take care of the Polish economy. Sadly, caring for the surrounding environment is associated with costs and limitations. With the ability to trace and compare the various standards of Tab. 4, we should be more attentive to the numerous media coverage of air purity and compliance. By reading the limit values of the norms in the EU and Poland guidelines it is worth mentioning that the WHO determines each exceedance as harmful to human health [17].

Tab. 4 Comparison of air quality for PM2,5;PM10;NO_2;SO_2 between WHO, UE, Poland

	WHO	UE	Poland
Annual concentration PM2,5 (μg/m3)	10	25	25
Annual concentration PM10 (μg/m3)	20	40	40
Daily concentration PM10 (μg/m3)	50	50	50
Number of exceedance days allowed for PM10	0	35	35
Daily concentration SO_2 (μg/m3)	20	125	125
Number of exceedance days allowed for SO_2	0	3	3
Annual concentration NO_2 (μg/m3)	40	40	40
Hourly concentration NO_2 (μg/m3)	200	200	200
Number of hours exceeded for NO_2	0	18	18

Source: Prepared by the author on the basis of data of the WHO Air Quality Guidelines 2005, Dyrektywa nr 2008/50/EC Komisji Europejskiej, rozporządzenie ministra środowiska Polski z 2012 roku (Dz. U. z 2012 r., poz. 1032)

6. Screening of cancer

Early detection and treatment of cancer can reduce the mortality and other complications caused by the disease. This allows to prolong the life expectancy in good health. However, to speak about the legitimacy of conducting such research we have to answer a few questions:

- does the disease included in the research affect the health of individuals or public health and is of the significant importance;
- does its prevalence increases of probability of detection of appropriate (justifying the study) number of cases;
- does the disease has a fairly long pre-clinical (latent) phase in order to detect the disease in the screening test, which precedes the onset of symptoms;
- are there concrete opportunities to implement preventive or curative measures, implementation of which can be cause early prevention, cure the disease or alleviate the course of the disease;
- are there technical capabilities available to carry out such studies in the form of an organizational structure of appropriately sensitive and specific diagnostic tests and financial means for carrying out the study itself and further care for people with positive screening results [34]?

Responding to these questions in the context of lung cancer, we can say that this disease is so important to the health of individuals due to the long treatment as well as public health – the high cost of advanced disease treatment. As we can see from the statistics, the spread of this disease is constantly increasing. There is a preclinical phase of the disease in which there are no symptoms, and the first symptoms that may disturb us are very unspecific f.ex. cough, hoarseness. There are specific early treatment options – whether in the case of non-small cell lung carcinoma surgery or small-cell carcinoma chemotherapy or immunotherapy. The last question is hard to give an unequivocal answer, because we have research that could be used in this matter- a low-dosage CT or a two-screen X-ray picture. In Poland there is a health care system in which the patient will find care – especially

within the oncological package proposed by the government. But are there adequate financial resources? – in the light of calculations, should be found, due to the profitability [35÷38].

7. Conclusion

Summarizing previous findings and discussions, air quality in Poland requires further attention. Emissions of pollutants tend to be diminishing, but the concentration parameters themselves do not meet the purity standards, which should be cause for concern. We can not judge to what extent the value of measurements is due to pollution produced in Poland and in which the flow of air masses from neighboring areas. Similarly, we can not clearly determine whether the increase in lung cancer incidence is due to exposure to harmful substances in Poland or during migration to foreign countries where concentration is higher. Its necessary to keep an eye on the air quality in the context of different standards, and examine the exact difference between the different standards. In this study, we have highlighted the growing problem of lung cancer, because of the multifactoral etiology of cancer, we can not blame one factor, whether it is air pollution or tobacco smoking (especially over 30 packs a year). Air pollution can not only increase the risk of disease but also worsen prognosis of existing diseases [39]. In view of the above, it would seem reasonable to consider the appropriateness of screening for lung cancer – which would allow early treatment. Because of the costs of treating lung cancer, nothing will replace the simplest method of raising awareness about risk factors and discouraging smoking [40]. Our study was aimed to identifying the current air quality in Poland based on selected pollutants and assessing their potential impact on lung cancer. In this case we want to point out the necessity of the screening program. We hope that this study will allow you to familiarize yourself with the important issue of the wider audience.

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Importance of the peer education in cancer prophylaxis and prevention among high-school students in Poland

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Abstract: Cancer is one of the deadliest groups among non-communicable diseases, with fatalities over 8 million people annually. More than half million Poles live with diagnosed cancer. Despite intensive prophylaxis efforts, cancer morbidity is still increasing. Therefore, authors would like to present the importance and legitimacy of peer education in cancer prophylaxis among high-school students, conducted for 116 youth participants by medical students according to the European Code Against Cancer guidelines. The results show significant increase in performance of knowledge, as well as provision of the opportunity for youth to open up about cancer concerns and health matters in peer-to-peer environment.
Keywords: peer education, cancer prevention, prophylaxis in Poland

1. Introduction

Cancers are a group of diseases included to non-communicable diseases (NCDs) – civilizational illnesses that result from genetic, physiological, environmental, and behavioral factors. Cancer is the second most fatal NCD after cardiovascular diseases, with more than 8 million fatalities each year [1].

According to the Constitution of the World Health Organization (WHO) "Health is a state of complete physical, mental and social well-being and not the absence of disease or infirmity" [2]. In the world of public health, a vigorous discussion is currently held on the new definition of health. Definition, which will also take into account the socioeconomic approach to this most important human good. Within this approach, Health is seen as a Product, generated by the sum of individual, state and healthcare efforts taking care of prevention, early detection and treatment of illnesses in their earliest stages. In the case of cancers, according to the International Agency for Research on Cancer (IARC), more than 30 to 50% of cancers are prevented, which is mainly related to the elimination of: smoking, alcohol abuse, obesity and physical inactivity, infections, environmental pollution, radiation, occupational carcinogens [3].

Health and cancer prevention are undoubtedly linked to the Sustainable Development Goals 2015-2030 – 17 United Nations priorities which will ensure humanity a balanced existence and harmony of life [4, 5]. In particular correlates to Goal 3 – Ensuring healthy living and promoting wellbeing for everyone of all ages, and Goal 1 – Ending poverty in all forms, in all countries. It has been found that over 60% of cancer deaths occur in low- and middle-income countries [6].

Prevention of lifestyle diseases is also one of the most important priorities of the WHO, which aims to reduce the incidence and premature mortality due to NCDs by 25% by 2025 within the Global Action Plan for Prevention and Control of Non-Communicable Diseases [7].

2. Purpose of the study

The purpose of this study is to present the legitimacy of promotion of health and cancer prevention within peer education, based on the results of the questionnaire regarding European Code Against Cancer recommendations as well as the subjective questions, feedback and suggestions of participants of the study.

3. Cancer morbidity in Poland

According to the Polish National Cancer Registry, over two times increase of the incidence of malignant tumors has been reported in Poland over the past three decades [8]. Within four years from 2010 to 2014, there was an increase of over 20,000 new cases of malignant cancer diagnoses (up to almost 160,000 diagnoses in 2014) [9]. More than half a million Poles live with cancer diagnosed in the last 10 years.

The most common malignancies diagnosed in men are lung cancer (18.5%), prostate cancer (15.6%) and colorectal cancer (6.8%), while in women most common are breast cancer (21.7%), lung cancer (9.2%) and endometrial cancer (7.4%) [8].

Over the past 60 years, cancer mortality in Poland has also increased almost 2.5 times. In both sexes, the highest rate of mortality is lung cancer (30% in men 17% in women), second in prostate cancer in men (8.4%) and breast cancer in women (13.9%), and colorectal cancer as the third most common cause of cancer death in both sexes – 7.5-7.9% [9].

Comparing the European statistics, the incidence of cancer in Poland is lower than the EU average, which unfortunately does not correlate with the significantly higher malignancy mortality compared to the EU average (20% higher for males, 10% higher for females).

4. Prophylaxis of morbidity and promotion of health in Poland

Measures for the prevention of cancer in Poland have been enacted under the National Program for the Prevention of Cancer of the Ministry of Health. Earlier editions of the Program resulted in, e.g. increase in the availability of radiotherapy throughout Poland, an increase of 31% of patients operated in stage I lung cancer, increase of 5 years survival rate in patients with colorectal cancer by 26% [10÷11]. The program for 2016-2024 consists of five priorities, including (1) health promotion and primary prevention, (2) secondary prevention, diagnosis and detection of cancer, (3) support for treatment, (4) oncological education and (5) support for the cancer registration system. The project described in the study is organized as part of volunteering without the support of governmental and non-governmental organizations by medical students from the Medical University of Lublin, supports the goal of promoting health and primary pre-vention of cancer among Polish society.

5. Methodology

Within the framework of the undertaken actions, cancer prevention classes were conducted for high school students. Classes in the form of peer education were conducted by students of the Medical University of Lublin in English with simultaneous translation to Polish. Educators have been trained in the methodology of teaching and the subject of the project, including monitoring the effects of educators education (post-training survey) before the initiation of the project. During the seminary for pupils, the recommendations included in the European Code Against Cancer and the most common misconceptions and myths about cancer functioning in Polish society were presented. The seminar was preceded by a questionnaire covering the most important preventive actions. The survey was repeated after conducting didactic activities in order to monitor the achievements of the didactic objectives. The evaluation of the project included both objective percentages as well as questions and feedback from project participants.

5.1. Peer education

Peer education is a form of health promotion, the main purpose of which is to provide knowledge about health prevention by volunteers – members of the community pre-viously trained in the methodology of conducting didactic classes [12, 13]. Guidelines and recommen-dations are presented to participants by educators of a similar social profile and experience, enriched with locally important aspects of functioning in a given society. This aspect consequently provides an accessible form of behavioral change in their lives.

The effectiveness of peer education and its positive aspect is demonstrated by several behavioral theories. In Kelly's popular opinion leader theory, educators play the role of opinion

leaders – people whose opinions shape the worldview of those who respect them [14]. The theory of social learning is based on imitation on the basis of classical conditioning in the psychological aspect, while the differential association theory is based on the sociological aspect of teaching certain behaviors [15÷16].

5.2. European Code Against Cancer

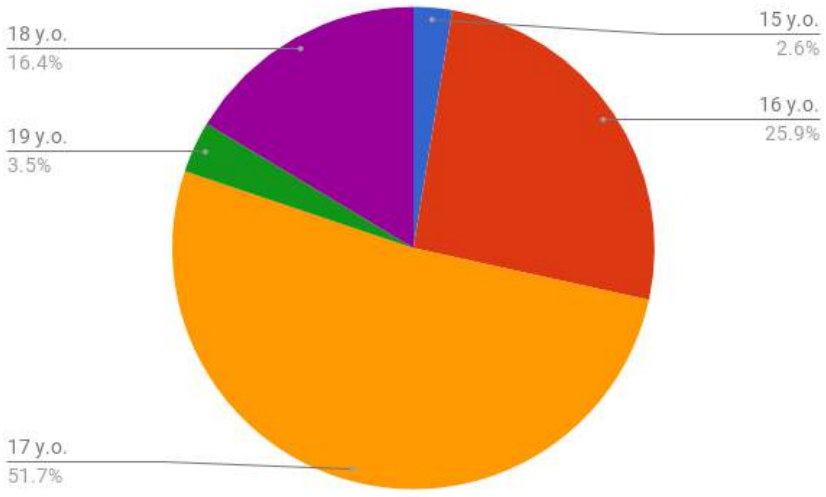
The European Code Against Cancer is an initiative of the European Commission established in 1987 [17]. The Fourth Edition of the Code currently adopted in years 2012-13, resulted from the project of independent European Union Expert Groups in cooperation with IARC. The aim of the project is to deliver accessible recommen-dations for citizens of the European Union who can easily prevent the development of cancer in their families and their immediate surroundings. It is also to serve as an in-formation platform containing scientifically proven evidence of a viable lifestyle in accordance with the Code [18÷19].

The Code contains 12 recommendations, which include:

- refrain from smoking tobacco;
- create a tobacco-free environment;
- maintain a healthy weight;
- include physical activity in daily schedule;
- follow the recommendations of healthy eating;
- limit the consumption of alcohol;
- avoid excessive sun exposure;
- protect yourself against carcinogens at work;
- reduce exposure to radon;
- for women – promotion of breastfeeding and reducing hormone replacement therapy;
- vaccine against HBV and HPV;
- participate in early colon cancer, breast cancer, cervical cancer screening programs [20].

5.3. Study group

The study included 116 students from 4 high schools. Classes were held in groups of up to 20 and 30 people. 64% of the respondents were women, 36% men. The average age of respondents is 17 years (the youngest respondent 15, the oldest 19). 56% came from small and medium-sized towns, 48% from large towns (over 250,000 inhabitants).



Picture 1. Age distribution in study group participants. Source: prepared by the authors.

5.4. The most important aspects of the Code Against Cancer discussed in the survey

5.4.1. Tobacco smoking

Smoking is one of the most important factors for lifestyle diseases. Every 6 seconds, one person suffers from smoking-related disease. In addition, over 90% of lung cancer cases are caused by this addiction. At the age of 18-24 most people start smoking habitually. Each cigarette is a loss of 11 minutes of one's own life, and is able to lead to: lung, tongue, lips cancer, chronic obstructive pulmonary disease, myocardial infarction, hypertension, aortic aneurysm, urinary cancer and esophageal cancer [21, 22].

Poland has banned smoking in public places since 2010, which has resulted in a significant reduction in exposure to tobacco smoke – according to the Sanitary Inspec-torate, more than double decrease of the exposure in food and beverages premises [23].

5.4.2. Breast cancer

Breast cancer is responsible for about 22% of the cases of women cancer in Poland [8]. The risk of developing the disease is increasing by the age of 50, the occurrence of the cancer is also increasing in younger patients. The most important risk factor is the genetic predisposition associated with BRCA1 and BRCA2 mutations. Hormonal factors and age-related increases can not be ruled out [24]. Even in the absence of genetic mutation, primary prophylaxis should be performed – breast self-examination, breast ultrasound and mammography [25].

5.4.3. Colorectal cancer and healthy diet

Colorectal cancer is responsible in Poland for 12% of cancers in men and 10% of cancers in women. For almost 30 years there has been an almost fourfold increase in morbidity in the Polish population [8÷9]. The most important risk factors include: age (increase in morbidity after 50), genetic factors, diet (excessive red meat consumption, lack of fiber rich foods, vegetables and fruits), physical inactivity and smoking [26÷27]. In Poland several stereotypes about the disease exists, e.g. that this is a malignant disease only, only invasive screening methods are available and it is only an incurable cancer [28].

Modern medicine offers variety of non-invasive screening programs such as latent blood in stool screening and screening recommended after 50 years or earlier if there are cases of colorectal carcinoma in first-degree relatives, as well as invasive screening such as classic and virtual colonoscopy [29].

5.4.4. Physical activity

Regular physical activity, a minimum of 30 minutes of intensive or 60 minutes of moderate intensity training per day affects oncoprotectivity of every organism [30]. Not only because of the reduced risk of obesity, which is the second most common cause of non-communicable diseases, but also for individual types of cancer, there is a lower risk of occurrence in people who regularly exercise than people leading sedentary lifestyle (from 25% to even 50 lower risk of colorectal cancer, 25% lower risk of breast cancer in postmenopausal women, 20% lower risk of endometrial cancer) [30÷31]. According to the 2012 Central Statistical Office, about 50% of Poles declare participation in sports and leisure activities, 48.8% of men and 43.3% of women – a significant increase of 8% compared to 2008 [32]. Few respondents, however, in their motivational aspect quote health purposes (9.9%) and maintaining proper physical fitness (17.8%).The majority of participants in this study mention pleasure and entertainment as the most important aspect which physical activity provides (66%). According to the TNS study in 2015, Poland is still below the average for physical activity for EU countries. Cycling (53%), jogging (33%), swimming (29%) are the most popular sports among Poles [33].

5.4.5. Carcinogens in chemical substances

Carcinogenic substances are primarily found in tobacco smoke, automobile exhausts, industrial areas as well as in household chemicals such as e.g. paints, pesticides. The most important recommendation of the Code for these substances is their conscious and careful use in accordance with attached instructions, preferably outdoors or in well ventilated areas. Another important aspect is the responsible disposal of chemicals to prevent soil and drinking water contamination [17].

5.4.6. Hepatitis type B

The hepatitis B virus (HBV) infected already more than 2 billion people worldwide [34]. About half a million people are infected in Poland, with more than 1,500 annual new infections being reported [35]. HBV is a virus 100 times more infectious than HIV (only one drop of infected blood can lead to infection), counts also as one of the most important carcinogens in the population – causing more than 80% of cases of hepato-cellular carcinoma [36]. In case of HBV infection, it is not possible to eradicate it afterwards. The treatment aims at suppressing the replication of the virus, spreading of the further disease and consequently reducing the risk of liver lesions, cirrhosis and hepatocellular carcinoma. HBV infection is caused by exposure to blood contaminated by the virus, also on sharp tools – such as needles, knives, but also toothbrushes; through sexual contacts and vertically during childbirth [37, 38].

Prevention of hepatitis B infection in Poland includes basic infant vaccination – 3 doses in the schedule – 24h postpartum (including tuberculosis vaccination) then after 1 month and 6 months. Revaccination is recommended for people close to HBV infected patients, healthcare workers exposed to potential virus infected material, people traveling to regions of increased HBV infections (Central and Southern Africa, South-East Asia) to people sharing syringes and HIV-positive patients. Additional vaccination is specifically indicated in patients with immunosuppressive treatment, immunodeficient patients and diabetic patients, depending on serum antibody levels [39÷40].

In primary prophylaxis attention is drawn to the need for vaccination and the avoidance of exposure to contaminated materials.

5.4.7. Human papillomavirus

Human papillomavirus (HPV) is the most common sexually transmitted pathogen. Transmissions occur in more than 50% of men and women [41]. Out of more than 100 types, the most dangerous are HPV 16 and 18 – types of high oncogenic risk responsible for cervical cancer [42].

In Poland more than 40% of early diagnosed cases of cervical cancer equal with progressive stage and indications for only palliative treatment [9]. While the EU countries (Sweden, Great Britain) managed to increase the rate of cytology performed by over 60-70%, in Poland despite common preventive campaigns, only about 30% of Polish women perform cytological screening [8].

Prophylaxis is primarily a vaccination against human papillomavirus, included in the National Vaccination Program as one of the additional vaccines suggested to conduct before sexual initiation. Vaccination is carried out free of charge in numerous Polish cities for children aged 14-15 [40].

5.5. Questionnaire

The single choice test questionnaire included questions on oncoprotection: physical activity, healthy eating, responsible use of harmful substances, and prophylaxis for colorectal cancer, breast cancer, cervical cancer, hepatocellular carcinoma, and lung cancer. In addition, data on gender, age and place of residence of respondents were obtained.

5.6. Results

5.6.1. Pre-questionnaire

Correctness of response in the survey before the study was 18% (13% lowest response, 33% highest). The most common correct answers in the pre-survey were to physical activity (62% correct answers) and a healthy diet questions (44% correct answers). In remaining questions, most of the participants failed to get the correct answers, which remained at 4-8% of the correct answers. In questions about documents and regulations related to cancer prevention recommendations, only 5% of respondents knew about the existence of a European Code Against Cancer.

5.6.2. Post-questionnaire

Post-survey results presented as follows – average score of correct answers 83.6% (highest score 100%, lowest score 73%). The highest score was obtained by 35 people (30% of respondents). The most common errors were questions about the prevention of HBV, HPV infections. 100% of the respondents after the seminar were able to identify documents containing recommendations for cancer prevention, in particular the European Cancer Code.

Table 1. Results of the study

	Pre-questionnaire	Post-questionnaire	Increase
Average correctness	18%	83,6%	65,6%
Highest score	33%	100%	67%
Lowest score	13%	73%	60%
ECAC knowledge	5%	100%	95%

Source: Prepared by the authors

As shown in the table 1, the general increase in knowledge as a result of conducted peer education was 65.6%.

5.6.3. Questions and feedback

Apart from objective estimation of the results of conducted activities, authors found also very important to analyze the questions and the feedback of the project participants. The most commonly asked questions included possibility of "cancer infection" in the presence of HBV or HPV, as well as genetic predisposition for breast cancer, colorectal cancer, lung cancer. The presence of volunteers was significantly important. Volunteers combined qualities of both student community members with whom participants more easily identify themselves as well as representatives of future health care professionals, with more comprehensive medical responses than high school textbooks. Medical students provided participants with opportunity to ask even very personal questions about their own and family health. Volunteers answered questions in accordance with the guidelines of the Code Against Cancer and their knowledge, but always stressed out that it is important to contact your GP or specialist for any oncological distress.

In the feedback received from the participants, 100% of the respondents considered the project as an accessible form of obtaining knowledge, as well as 93% indicated their willingness to participate in other editions of the project on other medical education courses offered by educators. 83% of the respondents did not have any problem understanding the content of the English version with the simultaneous translation into Polish. Any doubts of the participants were dispersed during the last questions and answers session and with didactic materials prepared by the educators in Polish language (27% of participants with few problems with the understanding of the presentation, after the completion of the project, the questions session and the materials considered measures taken sufficient to fully understand the topic).

5.7. Discussion – challenges of peer education

Peer education is a form of learning with many advantages – primarily peer health promotion from volunteers – peers with whom a given age group can easily identify, providing knowledge in an accessible way, enriching it with locally important aspects of functioning even unknown to teachers or physicians. These factors will facilitate and enable accessible the introduction of specific changes in participants' behavior.

The most commonly mentioned challenges include:

- few peer education reports;
- lack of standardized global guidelines and performance statistics;

- need of monitoring peer volunteers knowledge with the help of experts or expe-rienced people in the particular field.

Peer assisted learning (PAL) – a form of peer-to-peer tutoring, a concept already known since antiquity and "Archons" – peers from Aristotle's School, accompanies medical education since its early beginning [43]. Attempts of the didactic regulation of this form of education began in 1973 at the University of Missouri-Kansas City, at the UMCK-SI Center, main purpose of which is to train senior students in imparting knowledge to junior students [44÷45]. Similar centers were also established in the United Kingdom, and in 2003 new medical education standards in UK were introduced, according to which medical university graduates need to understand the principles of medical education in the education cycle, learn a variety of teaching and learning techniques, and assume responsibility for supporting their peers in education [46]. Peer assisted learning has been recognized in the United States and the UK as one of the most valuable learning and development methods – both for tutees and for tutors [47÷49].

In the case of peer education, international structuring is a difficult task. Due to the cultural and traditional aspect of health prevention activities, the methodology of ongoing projects to achieve the best possible learning outcomes can vary widely between countries or regions. This makes it impossible to compare and universalize this form of education. Nevertheless, the authors of this project wish to pay particular attention to the need to carry out numerous studies comparing teaching methods in terms of short and long term learning outcomes – increasing knowledge and introducing behavioral change. The authors express their hope that their research will have an impact on increasing the interest in the method and its refinement through the monitoring of learning outcomes.

5.8. Conclusions

In the case of the presented project, peer education was based on the principles of PAL, introduced in the aspect of health promotion and education on medical topics to students not originally associated with medical sciences. The knowledge was provided to tutors in accordance with the latest data available in the Polish nomenclature and statistics and in accordance with the European Code Against Cancer.

The results presented by both the objective part – increasing the knowledge about presented medical issues by more than 60%, as well as the interest of the participants in the knowledge of the projects carried out testify to the success of the method, the need for social initiatives in the field of medical issues and the need to continue actions for the prevention of cancer and health promotion.

5.9. Summary

Cancer is one of the most important challenge in the eradication of non-com-municable diseases, largely dependent on many environmental and behavioral factors. Peer assisted learning is a teaching method already known since ancient times, accom-panying medicine since its beginnings, and in the form of peer education – prevention and health promotion – used since the 80-90s of the previous century. The presented paper showcases the success of the method in the fight against false beliefs and social conditions which are conducive to the development of cancer and inadequate prevention.

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Knowledge of cervical cancer prevention among teenagers

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Abstract: Introduction: Cervical cancer is one of he most common cancers in women. It is estimated that more than 500.000 new cases of intraepithelial changes are detected in young women each year. Cervical cancer can develop painlessly and have no symptoms for many years, therefore, extremely important is to be aware of possible preventive actions in this area.
Aim of the study: Assesment of knowledge of teenagers about prevention of cervical cancer.
Material and Method: The study was conducted in Lublin in 2012 among 121 girls aged 18-19 years using author's questionnaire on selected aspects of cervical cancer prevention. Statistical tests were performer on the basis of computer software STATISTICA 10.0 (StatSoft, Poland)
Result: According to respondents, most frequently cited risk factors for cervical cancer are papilloma virus infections (79.34%), untreated inflammation of the vulva (71.07%) and frequent change of sexual partners (55.37%). Most of the tenagers (84.3%) knew, that the primary diagnostic test of cervical cancer is a cervical cytology (Pap) smear and preventive methods are: HPV vaccination, regular visits to the gynecologist or the treatment of cervical erosions. However, 88% of respondents had only elementary knowledge about cerviacal cancer. Much information about prevention of cerviacal cancer high school girls received from the media (66.94%) and internet (47.11%). In the option of teen activities relating to cervical cancer prophylaxis should be extended for talks (51.24%) and meetings with sick women (26.45%).
Conclusions: Respondents have basic knowledge about the prevention of cervical cancer, however there is the lack of knowledge of the nature of the disease. The main sources of knowledge about cervical cancer are mass media. Teenagers expect to extend existing general, screening preventive methods on educational programs (talks, meetings with women affected by this cancer).
Keywords: girls, knowledge, prevention of cervical cancer

1. Introduction

Cervical cancer is second most common (after breast cancer) malignant neoplasms in women. It is so called „silent killer", because it can progress for a long period of time without any symptoms. It is estimated that in our country the incidence of cervical cancer amounts over 4,000 new cases per year and about 2,000 of them die [1]. This cancer most offen develops in women aged 45-55, while the greatest exposure to risk factors, in particular infection with human papilloma virus (HPV), takes place between 16 and 25 years of life [2÷3].

In most cases cervical cancer is squamous cel cancer derived from multilayer squamous cervical disc (about 95%). The second most common is adenocarcinoma of the cervix, which comes from glandular epithelium of the cervical canal (3-5%). Other cancers and other types of cervical tumors are very rare (0.1%) [4÷5].

Cervical cancer is not hereditary disease or genetic condition. Modern literature provides many proven and probable risk factors for cervical cancer.

The documented risk factors include:

- age (the risk of developing cancer and high-grade change decreases with age);
- infection of HPV type 16 and 18 lasting more than 12 months (or other highly oncogenic types of HPV);
- early sexual initiation (<18 years of age);
- a large number of births (multiparous infected with HPV giving rise to 7 or more times have 4-fold higher risk than women giving birth 1-2 times);
- a large number of sexual partners (more than 4 per year);
- tobacco smoking;
- low socioeconomic status, which consists of poor living conditions,

inadequate personal hygiene and malnutrition;
- cervical intraepithelial neoplasia (CIN) [6÷9].

In turn, the likely risk factors for cervical cancer qualifies:
- long-term use of hormonal contraceptives.

Use of oral contraceptives for over 10 years increased 4-fold risk
- coexisting HIV infection;
- recurrent vaginal infections caused by *Chlamydia trachomatis, Cytomegalovirus*, or chronic vaginal infection of *Chlamydia trachomatis;*
- a diet deficient in vitamins A, C, E, β-carotene, folic acid (antioxidants) [10÷12].

Polish Gynecological Society recommends diagnosis and staging of cervical cancer based on the result of the physical examination, per vaginum and per rectum examination as well as Pap smear or colposcopy The growing importance begins to play molecular diagnostics of DNA HPV HR. The results of recent studies have confirmed that this method has a higher sensitivity and specificity in detecting similar CIN 2 as compared to 3 times with a Pap test performed [13]. To verify the diagnosis it is useful to turn to download targeted for histopathological examination.

Complementary methods to determine the clinical stage of the disease is cystoscopy, proctoscopy, transvaginal ultrasonography and microscopic examination of material from suspicious places in the bladder and rectum [14].

Despite the enormous progress of diagnosis and treatment, as well as the development of public awareness of the need for regular screening testing, mortality from cervical cancer is not decreasing. A major impact on decreasing the cancer incidence have individual decisions of women concerning the widely understood lifestyle. Knowledge of risk factors and methods of early detection of cervical abnormalities can save many women from cancer.

The correct strategy is to visualize the modern populations need to take multi-faceted measures to effective planning and implementation of prevention programs.

A special group to which educational activities should be directed constitute teena-gers. The role of education in their homes, in schools and health centers is to familiarize teens with the consequences of their lifestyle. At this age, young people discover their sexuality, visit gynecologist for the first time, often have a period of rebellion and start risky behaviors. This is also the period of life in which it could come to HPV infection. An adequate knowledge of risk factors and prevention including one of the simplest ways of prevention such as the possibility of immunization against HPV in teens should be a top priority of education in this particular population. Yet it is also the age at which we learn quickly and efficiently.

Cervical cancer is one of the malignancies of known etiology and risk factors, so it is possible to prevent and detect the disease at an early stage of its development.

According to the World Health Organization (WHO) definition there are 3 types of cervical cancer prevention:
1) primary prevention, or prophylaxis of disease;
2) secondary prevention, which is the early detection of prancerous lesions and early forms of cancer;
3) tertiary prevention which consists of reducing mortality from cervical cancer through effective diagnosis and treatment of the disease [15].

Primary prevention that aims to reduce the incidence of cervical cancer depends largely on the involvement of nurses in the education of children and their families [16].

2. The aim of the study

The main objective of this study was to investigate the knowledge of a teenagers on the epidemiology, diagnosis and methods of prevention of cervical cancer. The specific objective was to determine the role of the school nurse in the implementation of pre-ventive measures in this respect, in the opinion of the respondents.

3. Material and methods

The study was conducted in the period from March to April 2012 at the XXIII High School in Lublin during educational classes. The study group consisted of 121 girls attending class III.

To achieve the objectives of the study we exploited a diagnostic survey method using a questionnaire.

The research tool was the original questionnaire prepared for this study. The questionnaire consisted of 19 closed questions of one or multiple-choice on the issue of cervical cancer, ie. epidemiological data, the factors conducive to the development of the disease, methods of diagnosis, sources of knowledge and the educational expectations of the respondents to the school nurses in terms of prevention of cervical cancer.

In order to verify a research tool, we conducted a pilot study, which involved 20 girls. Analysis of the results of the pilot project showed the correctness of our research tool, which enabled to start conducting proper research. The study was approved by the school principal, school counselor, teachers and adult respondents. Before the observation started teenager respondents were informed of the purpose and total anonymity of the tests. Participation in the research was voluntary.

4. Statistical analysis

The results of the survey were statistically analyzed using the computer program Statistica 10.0 (StatSoft, Poland). Measurable variables were calculated by mean value and standard deviation values and unmeasurable variables were shown by the frequencies and percentage. To compare the qualitative characteristics of homogeneity chi-square test was used. To investigate the existence of the relationship between the studied traits Chi-Square Test for Independence was used. The level of significance was set at 0.05.

5. Results

The study included 84 high school girls at the age of 18 (69%) and 37 19-year-old (31%) (Figure 1). Most of the respondents were students from the city (57.85%; n=70).

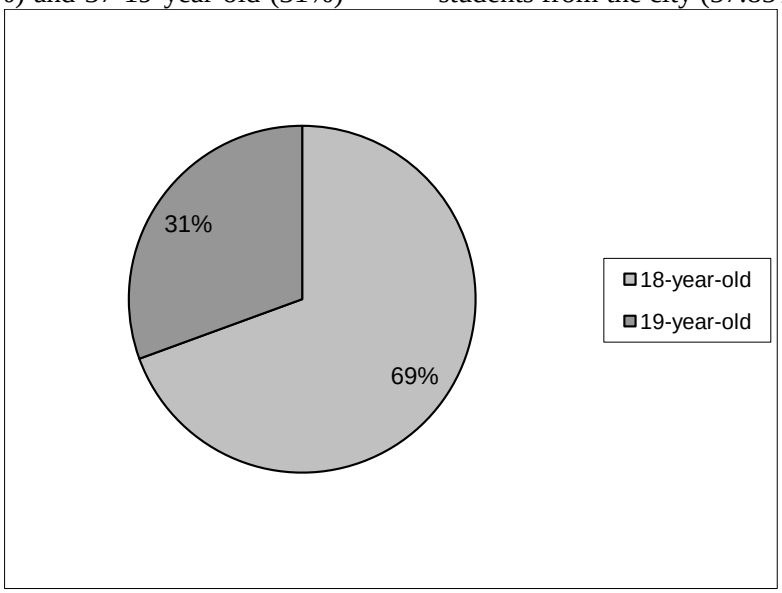

Figure 1. Percentage of respondents according to age [own elaboration]

Knowledge about the incidence of cervical cancer in Poland had 21.49% of the respondents, the rest of respondents have given the wrong answer (45,46%) and marked the answer 'do not know' (33.05%). Similarly, only 16.53% knew the percent of mortality of this disease in our country, while 38,84% of high school girls admitted that they did not know, and a further 44.63% gave a wrong answer.

According to the respondents most frequently mentioned risk factors for cervical cancer were: HPV infection (79.34%), untreated chronic inflammation of the genital tract (71.07%), a large number of sexual partners (55.37%), while less pointed were: cigarette smoking (23.14%), early sexual initiation (37.19%), a large number of births (13.22%), long-term use of oral contraception (20.66%) or poor diet (4.96%) (Table 1).

Table 1. Knowledge of the risk factors for cervical cancer

Determinant	n	%
tobacco smoking	28	23,14
untreated chronic inflammation of the genital tract	86	71,07
early sexual initiation	45	37,19
HPV infection	96	79,34
large number of sexual partners	67	55,37
case of cervical cancer in family	83	68,60
large number of births	16	13,22
long-term use of oral contraception	25	20,66
poor diet (poor in fruits and vegetables)	6	4,96

* The values do not add up to 100% due to the ability to select multiple answers

Source: Own elaboration

Most of the respondents knew that cervical cancer was the most common among women aged 35-59 years (n=107; 88%), only 12% of teens reported the wrong answer or did not know.

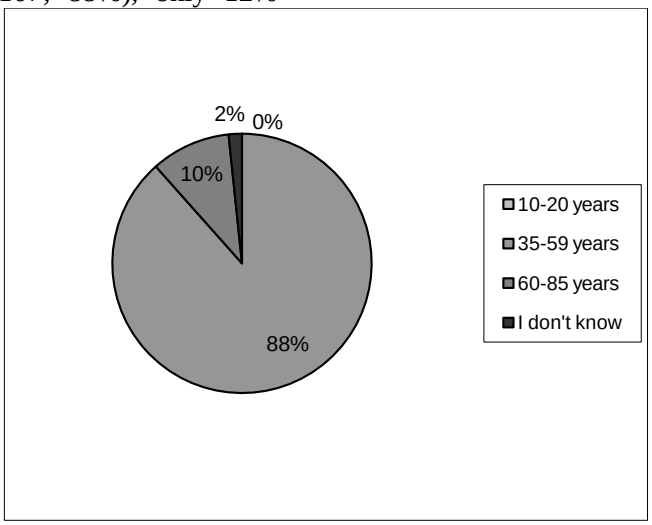

Figure 2. Percentage of respondents with regard to knowledge about the age at which women frequently suffer from cervical cancer [own elaboration]

78.51% of teenagers was oriented that the diagnostic test in the prevention of cervical cancer is the Pap test, while 15.72% of respondents answered incorrectly, that angiography (5.8%), mammography (4.96%) or marked ' I do not know " (4.96%). Only 14.88%of respondents correctly indicated that the Pap smear should be done every 3 years, while 0.93% of them felt that once a month, 55.37%, that once a year and 28.92%, twice a year. The results are shown in Table 2.

Table 2. Knowledge about methods of diagnosis of cervical cancer

Answers	N	%
The basic test to detect cervical cancer in your opinion is		
Pap smear test	102	78,51
Mammography	6	4,96
Angiography	7	5,8
I do not know	6	4,96
How often women should perform Pap smear?		
Once per 3 years	18	14,88
Once a month	1	0,93
Once a year	67	55,37
Twice a year	35	28,92

Source: Own elaboration

Factors that prevent cervical cancer most often chosen by respondents were: vacci-nation against HPV (81.82%), regular visits to the gynecologist (85.12%), cervical smear tests (79.34%) and the treatment of erosions (42.98%). Subsequently, teenagers indicated vitamins supplementation (6.61%), proper diet (8.26%) and the reduction of sexual contacts (27.27%) (Table 3).

Table 3. Knowledge about cervical cancer prevention

Answers	n	%
Vitamin supplementation	8	6,61
HPV vaccination	99	81,82
Use of condoms	51	42,15
Balanced diet	10	8,26
Regular checkups at the gynecologist	103	85,12
Regular cervical smear tests	96	79,34
Treatment of cervical erosions	52	42,98
Reduction of sexual contacts	33	27,27
I do not know	3	2,48

* The values do not add up to 100% due to the ability to select multiple answers
Source: Own elaboration

Information about cervical cancer schoolgirls primarily acquired from TV, radio, newspapers (66.94%) or the Internet (47.11%), and less frequently from the doctor (14.88%), family / friends (29.75%) and very occasionally from the school nurse (4.96%) (table 4).

Table 4. Sources of knowledge about cervical cancer

Sources	n	%
fromTV/ radio/newspapers	81	66,94
from a doctor	18	14,88
from a nurse/midwife	6	4,96
from Internet	57	47,11
from a family/ friends	36	29,75
from other sources	1	0,83

* The values do not add up to 100% due to the ability to select multiple answers
Source: Own elaboration

The results showed that 51.24% of respondents expect education programs or talks on the prevention of cervical cancer from the school nurse, while another 26.45% seeking for informative meetings with patients with cervical cancer or some said they did not expect anything (22.31%).

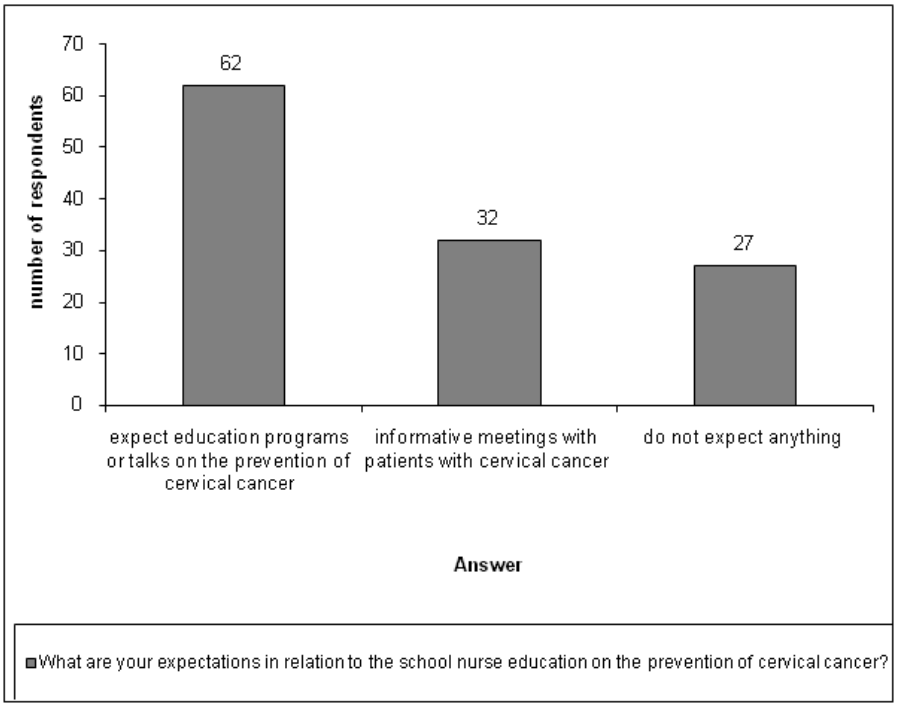

Figure 3. Expectations of school nurses about the prevention of cervical cancer [own elaboration]

6. Discussion

The analysis of the collected research data shows that the respondents do not have any knowledge about epidemiological data concerning cervical neoplasms. Only as little as 21.5% of respondents did know what the incidence of cervical cancer was. As few as 16.5% of respondents provided correct answers to questions about the cervical cancer mortality rate in Poland.

Female secondary school students did not have complete knowledge of factors that contribute to the development of cervical cancer. The study shows that the most frequent risk factors listed by female teenagers included HPV infection (79.3%), chronic untreated infections of the genital track (71%), and a large number of sexual partners (55.4%). The respondents did not know that risk factors include also smoking,

early sexual initiation, large number of childbirths, long-term use of contraceptive pills, and bad diet. A large proportion of respondents (68.6%) believed family history of cervical cancer to be a risk factor. A study conducted by Baay et al. showed that female students and mature women believed the most important determinants of cervical cancer to be genetic predispositions and recurring bacterial infections. Only 3.1% of those women considered the role of HPV in the progression of cervical neoplasms [17]. In addition, a recent analysis has confirmed that the knowledge of young women on the development and prevention of this neoplasm is rudimentary. As few as 51% of students were familiar with the corresponding recommendations [18].

In our study, the most frequently mentioned factors that prevented cervical cancer included vaccines against HPV (81.8%), regular gynaecological check-ups (85.1%), cytology (79.3%) and treatment of erosions (43%). It is important to note the fact that female secondary school children also knew that early sexual initiation and large number of sexual partners could contribute to the development cervical cancer (59.5%) and that human papillomavirus infection could be transmitted during intercourse (88.4%).

Similar results were obtained in a study by Iwanowicz-Palus et al., where 52.5% of the female subjects had knowledge of the correlations between early sexual initiation and incidence of cervical cancer. In addition, more than a half of those subjects (57%) did know that there is a relationship between frequent change of sexual partners and increased risk of developing cervical cancer [19].

The most effective method for the prevention of cervical cancer is health education. It should be provided for both girls and boys, already during their teenage years, before their sexual initiation. It is crucial that women develop a habit of taking care of their own health, thus increasing the likelihood of their participation in cytological tests as part of screening programmes. Cytological screening tests are complemented by colposcopy, which verifies any diagnosed irregularities. The Polish Gynaecological Society recommends that cervical cancer prevention programmes cover mainly women aged 25 to 59 [20, 21].

Education is helpful, yet insufficient, to completely prevent human papillomavirus infection due to its high prevalence. Preventive vaccination against HPV is much more effective, but not infallible. Vaccines against HPV are to be used by persons who have not been infected by human papillomavirus infection, or who have not been sexually active yet. These vaccines are preventive in nature and do not eliminate any pre-existing infections or lesions. Neither do they provide protection against other virus types. Moreover, vaccinated women should still undergo screening tests recommended by the Polish Gynaecological Society [22]. The results of this study show that 66.9% of respondents obtained their knowledge about cervical cancer prevention from TV, radio and newspapers. Moreover, 47.1% of respondents acquired it from the Internet, and only 4.9% gained it from their school nurse.

A study by Iwanowicz-Palus et al. showed that the main sources of knowledge about cervical cancer included gynaecologist (25.9%), the media (20%) and literature (21.8%) [19]. In international analyses, patients obtained information about cervical cancer from their doctors or medical websites, which was associated with their greater awareness of the possible prevention measures in this area [23].

The development of female awareness and broadly defined cervical cancer prevention measures relies heavily on the role of nurses. Individual interactions between nurses and female patients improve the latter's motivation for taking care of their own health, mainly by providing them with knowledge about prevention and health promotion, basic information about neoplasms, and the development of appropriate health behaviour. By listening attentively, sharing information and helping patients solve their disease and treatment-related problems, nurses provide support and determine the outcomes of treatment and rehabilitation. Concerns, fear and stress experienced by women are usually due to their ignorance, uncertainty and confusion. Women who have knowledge about their own bodies, and are aware of the risks, are likely to look after their health, aware of the fact that it is largely up to themselves to prevent the disease. The role of a nurse is to contribute to the efforts for the primary prevention of neoplasms by providing support to women in adopting appropriate health behaviours. They should engage in secondary prevention measures and efforts to detect neoplasms early on, by providing information about the immense importance of programmes focusing on screening tests and opportunities for undergoing such tests, and also by encouraging women to undergo such tests [24].

7. Conclusions

The main sources of knowledge about cervical cancer are mass media.

Teenagers expect to extend existing general, screening preventive methods on educational programs (talks, meetings with women affected by this cancer).

Respondents have basic knowledge about the prevention of cervical cancer, however there is the lack of knowledge of the nature of the disease.

Acknowledgments

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Protein kinase 2 (CK2) in carcinogenesis

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Abstract: The human kinase CK2 plays an important role in the physiological functioning of an eukaryotic cell, which is related to the regulation of the cell cycle, transcription, the DNA synthesis and repair, the mammalian circadian cycle and apoptosis. The activity of CK2 also involves the formation of pathological conditions in cells, which results, among others, with the involvement of the enzyme in the process of tumorigenesis. CK2 is a heterotetramer composed of two catalytic subunits such as CK2α and/or CK2α' and two regulatory subunits CK2β. It is significant that the subunits are functionally independent from the holoenzyme, showing the structural and functional diversity at the same time and other catalytic properties. The increased level of the enzyme activity was confirmed in all types of tumors and promotes tumor cell growth in several respects: stabilizes oncokinom, counteracts the effectiveness of anticancer drugs, promotes neovascularization and most importantly generates a wide spectrum of survival signals of cell. This is the reason of growing interest in the possibility of regulating its activity towards the design and synthesis of specific and potent inhibitors, what may result in obtaining specific antitumor drugs in the future.
Keywords: CK2; cancer; apoptosis, signaling pathways; anticancer therapy

1. Introduction

Phosphorylation is one of the most intensive post-translational protein modification processes. It is estimated that about 30% of the proteins in eukaryotic cells undergo reversible phosphorylation that can alter their functions, interactions, activity, localization, stability and affect to the key cellular regulation mechanisms such as cell cycle, p53 protein activity such as tumor suppressor, mammalian circadian rhythm and apoptosis [1÷5].

Due to the huge importance of protein phosphorylation in cells, the occurrence of protein kinases encoded by one of the largest eukaryotic gene families (about 2% of the genome) is common. In recent years 122 protein kinases genes have been identified in yeast cells, 540 in mice and 518 genes in the human genome, although according to estimates their number may be as high as 2000 [6÷9].

Kinase activity is precisely controlled and abnormalities in their functioning caused by i.a. mutations disturb the functioning of the whole signaling networks, leading to generation pathological and disease states. On the basis of the comparison of human chromosomal maps with identified disease *loci*, a direct contribution of 164 kinases to tumor formation was confirmed [10, 11].

2. General characterization of protein kinase II

The main element of regulatory and signaling networks based on protein phospho-rylation in the eukaryotic cell is the CK2 protein kinase (casein kinase 2 or II). It is a serine / threonine kinase that uses both ATP and GTP as phosphate residue donors. It is present in human cells most commonly in the form of a holoenzyme, a hetero-tetramer of approximately 130 kDa, consisting of two catalytic subunits α (42-44 kDa) and α '(38 kDa) and two regulatory subunits β (26 kDa), so it can exist in configu-rations α α'β_2, $\alpha_2\beta_2$ or $\alpha'_2\beta_2$ (fig.1) [12].

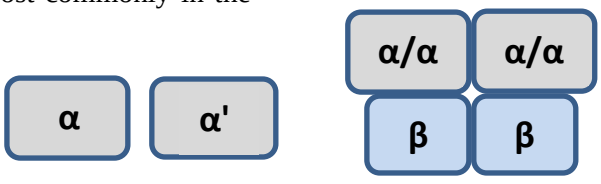


Figure 1. CK2 kinases can function as monomeric kinases and in a tetrameric complex [own elaboration].

The CK2 catalytic subunit consists of two characteristic domains: the smaller N-terminal and the larger C-terminal, which are connected by a single polypeptide chain (linker region), allowing free domain rotation upon binding of ATP and / or substrate. The phosphate donor binding site placed between the two main domains, interacts with the enzyme via

hydrogen bonds. The ATP binding site is characterized by the presence of five specific regions: three hydrophobic (adenine region, hydrophobic pocket I and II) and two hydrophilic (ribose region and phosphate binding region) that can be 4used to bind different chemical groups of inhibitors competing with ATP [13÷15].

The CK2β regulatory subunit in mammalian organisms is encoded by one gene and does not show sequential similarity to any other regulatory protein. Like the catalytic subunit, CK2β also consists of two domains. The N-terminal region represents a larger domain and has a α-helical character. This domain is associated with a smaller β structure (domain II), that contains a characteristic zinc ion group. The C-terminal loop (so-called tail) is directly involved in the holoenzyme formation. Catalytic subunits are attached to the regulatory subunits dimer and do not interact with each other. Each of the α subunits interacts with two β subunits [12, 14, 16, 17].

3. Cellular location, substrates and physiological importance of CK2

CK2 protein kinase is ubiquitous, pleiotropic and highly conserved enzyme in cells. It is widely located in *Metazoa*: plants, animals and humans. It has been also identified in primitive protozoa and in fungi representants [12, 18, 19].

In mammals, CK2 activity has been demonstrated in most tissues and almost in every cellular compartment, mainly in the nucleus, cytoplasm, cell membrane as well as mitochondrium membrane, mitochondrial matrix, endoplasmic reticulum, cytoskeleton, centrosome, Golgi apparatus and ribosomes [12, 19]. CK2 enzymatic activity in different cell compartments is variable and regulated in response to a variety of signals and impulse associated with cell cycle progression or cellular stress [20]. Moreover, nu-merous studies confirm the existence of catalytic and regulatory subunits independently of each other. Each of them can function separately, and their roles often remain different from the functions they perform in holoenzyme. Catalytic subunits preserve enzymatic activity and often is differ in substrate specificity. There is evidence of the dynamic localization of individual subunits and the independent displacement of α and β within the cell [12].

The presence of CK2 kinase in various cellular compartments is associated with the phosphorylation of many substrates and consequently the involvement of the enzyme in regulation of important cellular processes such as differentiation, mobility, cytoskeletal reorganization, proliferation, RNA synthesis, apoptosis and transformation [21].

So far, hundreds of physiological kinase substrates have been identified, and among them transcription factors (~ 60 proteins), proteins regulating the functions of nucleic acids and protein synthesis (~50), signaling proteins leaving out transcription factors (~90), cytoskeletal and structural proteins (~14). Approximately 40 substrates of CK2 were also identified among viral proteins [22].

Apart from the enzyme-substrate type connection, a number of CK2 interactions with non-substrate proteins have been described, which comprise one of the elements of CK2 activity regulation. This regulation takes place by the principle of interactions with the α (e.g. Pin1, APC, IRS-1, CKIP-1, PP2A, Grp94) or β subunit (e.g. $p21^{WAF1}$, p53, TNP-1, FGF-2) or both (e.g. Nopp140, eIF2β), the consequence of this is modulation of activity the subunits themselves or the whole holoenzyme [23].

Among the substrate proteins, both inhibitors (nucleic acid phosphoprotein Nopp140, translation initiation factor eIF2β, tumor suppressor p53), and activators of CK2 (e.g. HSP90 heat shock protein, nucleolin) were identified. Interactions with proteins regulating kinase activity may result in CK2 targeting to specific cellular structures, or modulating specificity for protein substrates (e.g. Pin1-reducing specificity for topoisomerase IIα, FACT – enhancing specificity for p53). So far, the contribution of 68 proteins to direct modification of CK2 activity has been confirmed [23÷25].

CK2 is an important element of every cell cycle step. In mammalian cells, its activity is crucial in the transition and progression G0/G1, G1/S and G2/M [26, 27]. Important elements of the cell cycle control, that remain under the control of CK2 kinase are among others: CAK kinase, p53 protein, SSRP1 proteins, FACT elongation factor element, MDM-2, $p21^{WAF1/CIP1}$, $p27^{KIP1}$, β-tubuline, Cdc25B, tau protein, PP2A, topoisomerase II, Chk1 kinase, CCdc34, Cdk1, Six1 and proteins associated with microtubules 1A and 1B [28]. The contribution of CK2 kinase to the Wee1 degradation pathway has also been

confirmed, and consequently its influence on the initiation of cell division [29].

CK2 is an important element of transcription control. The effects of the kinase activity involve multiple levels of regulation and a multitude of substrates. In particular, this regulation applies to the basic elements of the transcription mechanism, i.e the RNAP I, RNAP II and RNAP III polymerases [28].

Regulation of activity by phosphorylation or on the basis of other interactions with CK2 concerns also transcription factors: NFκB, STAT1, CREB, IRF-1 and IRF-2, ATF1, SRF, Max and protooncogenes: c-Jun, c-Fos, c-Myc and c-Myb [28].

Recently, the role of CK2 kinase in cellular processes that decide about cell entering the apoptosis pathway and the pro-life character of the kinase is the subject of intensive research. Overexpression of CK2 protects against drug-induced apoptosis and vice versa, kinase overexpression is often observed in cell lines that are resistant to apoptosis-inducing drugs. It is an enzyme directly involved in both types of pro-grammed cell death: extrinsic and intrinsic, induced by DNA damage, and inhibition of its activity induces apoptosis in tumor cells, which provides a promising aspect in cancer therapy [30÷32].

Many of the apoptosis signaling pathway proteins are direct substrates of CK2, others are regulated at the level of expression. One of the proteins is survivin, belonging to the group of apoptosis inhibiting proteins known as IAPs (Inhibitory Apoptosis Proteins), activity of which is inhibited by CK2 overexpression [33]. In turn, the phosphorylation of the Bid protein, a pro-apoptotic member of the Bcl protein family, protects it against caspase-8 activity, inhibiting mitochondrial apoptosis mechanism [34]. This type of regulation, where protein phosphorylation prevents caspase activity, indicates an evident antiapoptotic role of CK2. This phenomenon is determined by a similar sequence, rich in aspartate residues, recognized as a caspase cleavage site and simultaneously phosphorylated by CK2 [12]. A similar regulatory mechanism applies also to other caspase substrates: Max protein, HS1, presenilin-2, conexin 45,6 and PTEN [28]. CK2 also regulates the activity of caspases themselves. It has been proven that the caspase-9 phosphorylation in mice model protects it against caspase-8 activity, as well as the inhibitory effect of CK2 on caspase-2 dimerization and thereby its inactivation has been shown. Furthermore, the ARC protein, inhibiting caspase-8 activity, also remains under CK2 control [35÷37].

4. CK2 contribution in tumor processes

In addition to basic and key physiological functions, CK2 kinase is involved in the generation of many diseases, including neurodegenerative, viral, parasitic, inflammatory conditions and in many types of cancer.

CK2 level in cells remain at a constant characteristic level. It is relatively high in some organs such as in the brain or in the testicles, which represents a normal physiological condition. It rises during cell proliferation, whereupon it reaches a stable level that is crucial for cell homeostasis. Instead, in tumor cells elevated kinase activity is observed in the cell nuclei and deregulation of the kinase activity is observed in the disease intensification states, and it even serves as a prognostic indicator. Increased levels of the kinase activity is observed in all known types of tumors, including head and neck, kidney, colon, lung, prostate and breast cancer [38÷43].

High CK2 activity promotes tumor cell growth in a number of respects: a) improves transformation potential of oncogenes, b) stabilizes oncokinom by activation of co-chaperone CDC37, which is crucial for the maintenance of the active conformation of kinases with oncogenic potential, c) counteracts the effectiveness of anti-neoplastic drugs, especially imatinib and melphalan, d) promotes neovascularization, and most importantly e) generates a broad spectrum of pro-life cell signals [44].

Table 1. Known mechanisms by which CK2 plays a global role as a pro-survival and anti-apoptotic agent

CK2
Potentiates the Akt pathway
Promotes I κ B degradation and activates NF-kB
Stabilizes Dvl and β-catenin upregulating the Wnt pathway
Generates caspase resistant sites in Max, Bid, HS1, PTEN, connexin 45, caspase 9 etc.
Phosphorylates and activates the caspase inhibitor protein ARC
Promotes rRNA and tRNA biogenesis
Promotes the degradation of tumor suppressor PML
Facilitates DNA repair

Source: [44]

Many of these CK2 functions, particularly growth signals maintenance, apoptosis inhibition, involvement in angiogenesis lead to the changes in cell physiology characteristic for carcinogenesis.

Table 1 presents known mechanisms in which CK2 is involved, favoring the formation and maintenance of a neoplastic cell phenotype. In addition to the basic functions described in the previous chapter, i.e. participation in growth and proliferation regulation, rRNA and tRNA biogenesis, DNA repair, caspase inactivation, CK2 kinase also affects regulation of anti-apoptotic proteins and pathways, i.e. NF-κB, PI3'K / Akt and Wnt (Fig. 2, 3 and 4) [44].

NF-κB is a transcription factor involved in expression of cellular cytokines, cyclins D1, anti-apoptotic proteins (BcL-xI and IAP). It is usually located in the cytosol, where the interaction with an inhibitor IκB inhibits its activity. Degradation of IκB in the SCF-B-TrCP proteasome pathway releases the NF-κB factor, targeting it to the cell nucleus. CK2 works on several stages of this process. First and foremost, it activates IκB proteolysis, which constitutes an alternative pathway, alongside the basic IKK kinase-dependent pathway, expression of which is also under the control of CK2. Moreover, the p65 subunit of the NF-κB factor undergoes phosphorylation, which in turn increases its activity (fig. 2) [45, 46].

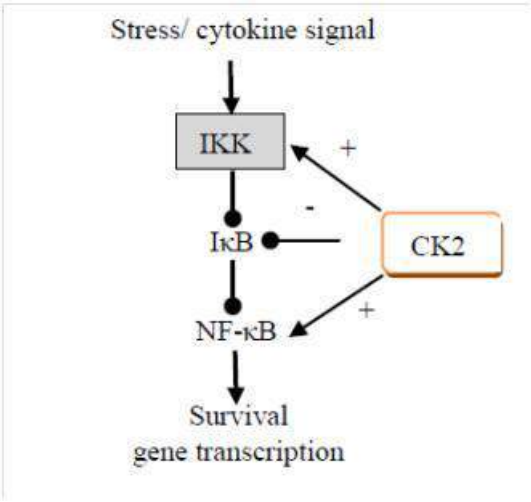


Figure 2. CK2-dependent multisite regulation of NF-κB. A negative effect (−) is indicated by a dot-arrow, and means inhibition or increased degradation, while a positive effect (+), indicated by a normal arrow, means enhanced stability and/or activity [44].

The Wnt signaling pathway plays an important role in embryogenesis, while its activity in adult individuals promotes transformation and carcinogenesis [47]. CK2 is involved in the reactivation of the pathway, which has been observed in case of colorectal cancer. The Wnt pathway regulates cell proliferation by maintaining a high level of β-catenin, which is a cofactor for a group of TCF / LEF transcription factors involved in expression of pro-life signals: c-Myc, c-Jun and cyclin D1. Phosphorylation of β-catenin by CK2 is a key element of the stabilization of this protein and of the protection against

proteasome degradation. The reverse effect is induced by phosphorylation of β-catenin by the GSKβ pathway kinase, which leads to ubiquitination and degradation of the protein. CK2 has also been shown to be involved in the phosphorylation of UBC3 and UBC3B proteins, which interact with the F-box, an element of the B-TrCP proteasome complex (fig. 3) [48, 49].

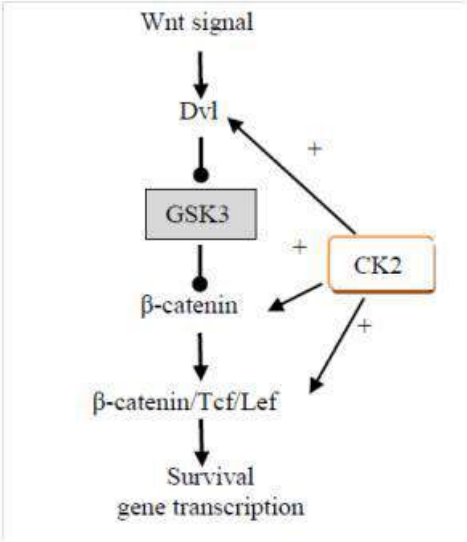


Figure 3. CK2-dependent multisite regulation of β-catenin. A negative effect (−) is indicated by a dot-arrow, and means inhibition or increased degradation, while a positive effect (+), indicated by a normal arrow, means enhanced stability and/or activity [44].

The contribution of CK2 in the stabilization and regulation of β-catenin level in the Wnt pathway also affects phosphorylation of Dvl proteins. These proteins are responsible for GSKβ activity regulation by blocking the ability of β-catenin phosphorylation, which in turn enables phosphorylation by CK2. As a result, dissociation of the protein from APC and Axin proteins, translocation to the nucleus and activation of pro-life signals take place [48].

The tumor suppressor – APC protein is a negative regulator of Wnt signaling, simultaneously interacting with CK2 via the α subunit and as a consequence it inhibits the activity mainly of the holoenzyme of the kinase. This effect achieves the highest level in G2 / M phase [40].

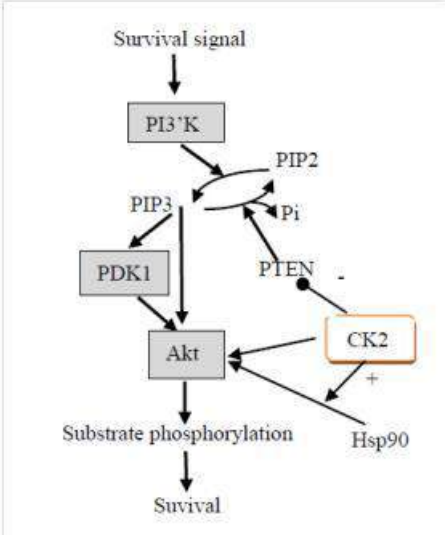


Figure 4. CK2-dependent multisite regulation of Akt. A negative effect (−) is indicated by a dot-arrow, and means inhibition or increased degradation, while a positive effect (+), indicated by a normal arrow, means enhanced stability and/or activity [44].

The effect of CK2 on cell survival is particularly visible in PI3'K / Akt pathway. The progression of the pathway is inhibited by PTEN phosphatase which dephosphorylates

phosphatidyl inositol 3,4,5-triphosphate (PIP3), acting antagonistic towards PI3'K kinase. The CK2-mediated PTEN phosphorylation inactivates this enzyme, which in turn stimulates Akt-dependent signaling. Interestingly, in most tumor cells PTEN activity is lost, whereas in T-ALL primary cells a high level of PTEN is maintained compared to normal T-lymphocytes precursors, which is simultaneously associated with high CK2 expression. Thus, the constitutive activity of the PI3'K / Akt pathway is possible not only in the inhibition of PTEN expression, but also in the inhibition of phosphatase activity by high CK2 level [44, 47, 50].

The direct effect of CK2 on Akt activity was also demonstrated by Thr-308 phosphorylation in the catalytic domain and Ser-473 in the C-terminal domain, as well as Ser-129 phosphorylation, which generates a constitutive kinase activity. CK2 contributes to maintaining a high level of Thr-308 phosphorylation, providing a stable connection with Hsp90 that protects Akt against dephosphorylation [51÷53].

Another mechanism by which CK2 affects the activity of tumor suppressor proteins is based on regulation of vulnerability to proteasome degradation. This type of regulation applies to i.a. PML protein involved in the control of many pathways responsible for growth inhibition, apoptosis or cell ageing. The loss of PML activity is observed in many cancers and correlates with the tumor progression. CK2 phospho-rylates PML at Ser-517, which is critical for directing the protein to degradation, and consequently protects cells against apoptosis [50].

The CK2 implication in signal cascades is often untypical in comparison with other kinases, primarily because it is not a component of hierarchical dependence, remains beyond the molecular regulatory mechanisms, simultanously integrates and consoli-dates the various connections and pathways. Hence deregulation of CK2 activity fosters such profound and diversified changes in cell biology.

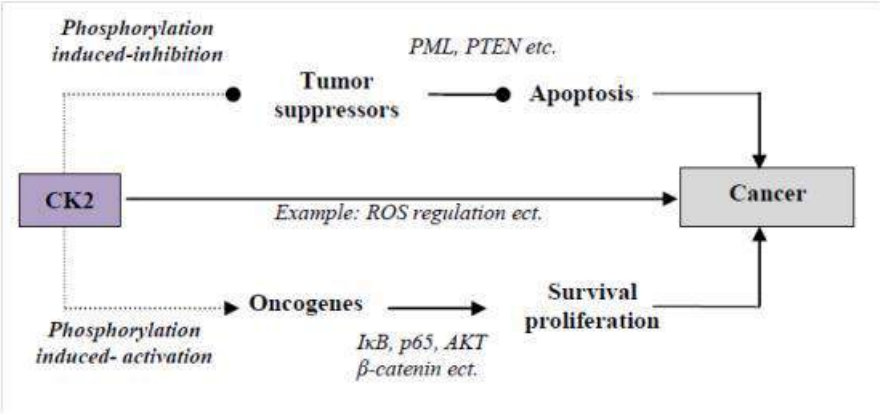


Figure 5. A schematic model for the role of CK2 in carcinogenesis. High levels of CK2 expression and activity have been illustrated in a variety of cancers [54].

Inhibition of CK2 activity by antisense RNAi, overexpression of inactive kinase form or chemical inhibition sensitizes cancer cells to induction of apoptosis by chemo- or radiotherapy. This dependence was confirmed and used i.a. in chemical induction of apoptosis in T-lymphoblastic cells or reactive oxygen species (ROS) dependent apoptosis in leukemia cells. The application of small interference RNAs in order to silence DNA expression supports therapy of rhabdomyosarcoma and colorectal cancer cells, making them sensitive to TNF-related apoptosis-inducing ligand (TRAIL) [54÷55].

Undoubtedly CK2 plays a significant role as an antiapoptotic and pro-life factor at many levels of its activity, as summarized in the figure 5.

5. Inhibitors of CK2

CK2 kinase is involved in many fundamental aspects of the physiological functioning of the cell, on the other hand promotes conditions conducive to carcinogenesis and other pathological conditions. Hence the growing interest in the possibility of regulation of its activity through the design and synthesis of specific and strong inhibitors, which in the

future may result in the acquisition of specific drugs.

5.1. ATP-competitive inhibitors

The human genome encodes more than 500 protein kinases, which are characterized by high conservative ATP binding site. However, there are some structural differences, mainly in proximally located regions, which generate the selectivity of ATP-competitive inhibitors. In *in vivo* studies, inhibitors often show a limited membrane permeability and a poor physiological effect. It is not without significance, that the cells exhibit high levels of ATP (1-10 mM), which is important primarily in inhibiting constitutively active kinases. In case of CK2, the high affinity of the enzyme to ATP also remains problematic [56].

There are several groups of chemicals that exhibit differentiated efficacy and specificity towards CK2. These are, i.a. the compounds of natural origin:

- flavonoids, eg. apigenin, quercetin, myricetin and fisetin;
- coumarins, eg. DBC (3,8-dibromo-7-hydroxy-4-methylchrom-2);
- antrachinons and xanthenones, eg. emodin (1,3,8-trihydroxy-6-methyl-antra-quinone), 1,3,8-trihydroxy-4-nitro-anthraquinone (MNA), 1,8-dihydroxy-4-nitro-xanthan-9-one (MNX) and 1,4-diamino-5,8-dihydroxyanthraquinone (DAA), quinalizarine (1,2,5,8-tetrahydroxy anthraquinone);
- ellagic acid;
- rezorufin [57÷59].

A significant group of competitive inhibitors against ATP are synthetic compounds:

- halogenated benzimidazole and benzotriazole derivatives, eg. DRB (5,6-dichloro-1-β-D-furanosyl-benzimidazole), TBB (4,5,6,7-tetrabromo-1*H*-benzotriazole) and its 2-dimethylamino derivative (DMAT), TBI (4,5,6,7-tetrabromo-1*H*-benzi-midazole), TIBI (4,5,6,7-tetraiodo-1*H*-benzimidazole);
- pyrazole-thiazine derivatives (according to PDB labeled 3BE9, 2PVH, 2PVJ, 2PVK, 2PVL, 2PVN);
- fluorenone derivatives such as FL12 (2,7-dihydroxy-3,6-dinitro-fluoren-9-one) and benzonaphthon derivatives eg. THN (tetrahydroxy-benzonaphthon);
- carboxylic acid derivatives, eg. IQA (5-oxo-5,6-dihydroindole- (1,2-a) -quina-zolin-7-yl-acetic acid), tetrabromic cinnamic acid derivative (TBCA), tribromic benzoic acid derivative and tetraiodic propionic acid derivative TID46;
- 3-carboxy-4-(1H)-quinolones, eg. 5,6,8-trichloro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid;
- antimonic acid derivatives, eg, (*E*)-3-(3-antimonophenyl)-prop-2-enoic acid (IC_{50} = 0,15 μM);
- xanthene derivatives with negatively charged carboxyl or sulfonic groups, eg. 2,3,4,5-tetrabromo-6-6-hydroxy-3-oxo-3*H*-xanthen-9-yl-benzoic acid [57÷59].

Structures of selected inhibitors are shown in the figure 6.

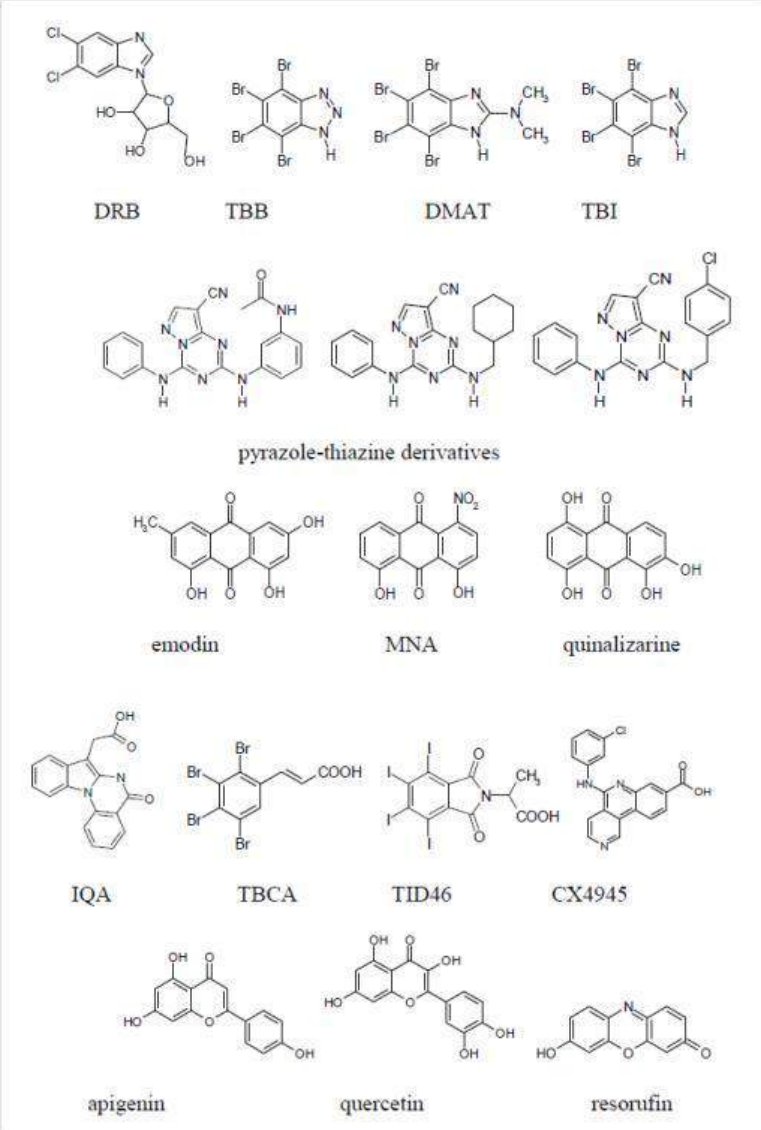


Figure 6. Chemical structures of selected ATP-competitive inhibitors of CK2 [own elaboration]

It is worth emphasizing that to this class of compounds belongs the first CK2 inhibitor which has successfully passed phase I of clinical trials, i.e. CX4945 (5-(3-chloro-phenylamino)-benzo-naphthyridine-8-carboxylic acid), also known as Sil-mitasertib. Activity tests performed on more than 145 kinases have confirmed its high selectivity for CK2. It shows a wide spectrum of antiproliferative activity on various cancer cell lines such as lung, breast cancer cells and prostate cancer. It has been demonstrated that the mechanism of antitumor activity of this compound is based on the inhibition of the processes in which CK2 kinase is involved, directly related to the maintenance of the tumor cell phenotype. This is an inhibition of the PI3K / Akt pathway by suppression of phosphorylation of Akt kinase and other key mediators such as p21 protein. Moreover, it selectively induces apoptosis in cancer cells and exhibits antiproliferative and anti-angiogenic effect. It is effective in the treatment of solid tumors and multiple myeloma, showing promising pharmacodynamic and pharmacokinetic properties. Currently CX-4945 is in phase I/II clinical trials in the United States, South Korea, and Taiwan for the treatment of cholangiocarcinoma in combination with gemcitabine and cisplatin (NCT02128282). The aim of this trial is to determine its maximum tolerable dose in patients followed by a randomized phase II assessment using CX-4945 in combination with gemcitabine and cisplatin versus the standard of care [60÷63].

5.2. Non-competitive inhibitors towards ATP

An alternative to ATP-competitive inhibitors are compounds that do not compete directly with ATP for active site, and inhibition of enzyme activity is based on a variety of mechanisms using structural and functional characteristics specific to CK2. These compounds generate less side effects and higher specificity of action mainly because of their targeting to less structurally conservative CK2 regions. Due to the fact, that they do not compete with cellular ATP, they can be used in concentrations close to biochemical K_i value. On the other hand, they are characterized by limit inhibition power resulting from low affinity and intracellular instability [64]. Examples of such inhibitors together with the mechanisms of their action are listed below:

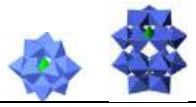
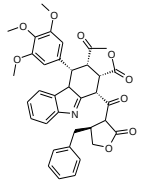
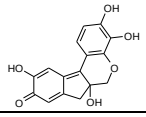
- polyanionic inhibitors (eg. heparin and other acid polysaccharides, eg. poly-glutamic acid and pseudo-substitutive peptides) interacting with a substrate binding site rich in base residues [59];
- CIGB-300 (phage cyclic P15 peptide formed after fusion with the cell penetrating Tat peptide), interacting with the phospho-receptor site of CK2 substrates, especially with B23 oncogene/ nucleoplasmin [65];
- inorganic ionic transition metal complexes, mainly tungsten, molybdenum and vanadium in the form of oxoligands (POM). The mechanism of action of these compounds is connected with the impact on the key structural elements of the enzyme, and in this case, the activation site blocks CK2 in an inactive conformation [66];
- peptide (P1) that interacts with the N-terminal domain of CK2β and blocks interactions between CK2β and specific ligands [67].

Structures of these inhibitors are shown in the table 2.

CIGB-300 is a second CK2 inhibitor tested in I/II Phase of clinical trials. In the clinical ground, this synthetic peptide has proved to be safe and well tolerated in a First-in-Human trial in women with cervical malignancies who also experienced signs of clinical benefit. In a second Phase I clinical trial in women with cervical cancer stage IB2/II, the MTD and DLT have been also identified in the clinical setting. Interestingly, in cervical tumors the B23/nucleophosmin protein levels were significantly reduced after CIGB-300 treatment at the nucleus compartment [68]. It should be noted that CIGB-300 modulates several CK2-dependent signaling pathways. In NSCLC models (non-small cell lung cancer), CIGB-300 induced an anti-proliferative response. This effect was accompanied by the inhibition of the NF-κB pathway, which was associated with an enhanced proteasome activity. Moreover, the NF-κB pathway appeared to be critically involved in the cisplatin-resistance of A549-cispR cells, which became more sensitive to CIGB-300 treatment [69]. Knowable data suggest a potential use of CIGB-300 as a novel therapeutic agent against lung cancer, because this peptide markedly decreased lung colonization and metastasis development of murine 3LL cells in mouse models and significantly reduced tumor cell-driven neovascularization [70].

CK2 is a very interested candidate for targeted therapy, with two inhibitors in ongoing clinical trials. CX-4945 is a bioavailable small-molecule ATP-competitive inhibitor targeting its active site, and CIGB-300 is a cell-permeable cyclic peptide that prevents phosphorylation of the E7 protein of HPV16 by CK2. In preclinical models, either of these inhibitors exhibit antitumor efficacy. Furthermore, in combinations with chemo-therapeutics such as cisplatin or gemcitabine, either CX-4945 or CIGB-300 promote synergistic induction of apoptosis [71].

Table 2. Non-competitive inhibitors with the mechanisms of their action.

Type of interaction	Type of molecule	Examples
Allosteric inhibitors	Inorganic compounds	POM 
CK2β binding inhibitors	Peptides	Peptide P1 GKMNGVLPLAWPSLYLRL
Inhibitors inhibiting interactions CK2α/CK2β	Cyclic peptide Organic compounds	Peptide Pc GCRLYGFKIHGCG W16 
Inhibitors inhibiting the attachment of a protein substrate	Cyclic peptide Organic compounds	Peptide P15 CWMSPRHLGTC Hematein 

Source: own elaboration

6. Conclusion

There is strong evidence that CK2 plays a role in the pathogenesis of cancer. CK2 is overexpressed in many cancers and often overexpression is associated with worse prognosis. CK2 is involved in many key aspects of cancer including inhibition of apoptosis, modulation of signaling pathways, DNA damage response, and cell cycle regulation. This enzyme has the ability to regulate signal transduction pathways, which may vary in different cancers, such as Wnt signaling, JAK/STAT, NF-κB, and PTEN/PI3K/Akt-PKB. Furthermore CK2 can be used as a diagnostic and prognostic marker in certain malignancies, such as prostate cancer.

The ability of CK2 to promote tumors causes the CK2 has emerged as a potential anticancer target. The wide range of cell-permeable chemical CK2 inhibitors have been developed. The most frequently used are TBB, quinalizarin, hematein, TBCA, CIGB-300, CX-4945, DRB, apigenin, DMAT, and emodin. Two of these CX-4945 and CIGB-300 have made into preclinical and clinical trials. These inhibitors are already used in phase I/II trials in certain malignancies like lung, head and neck cancer, cholangiocarcinoma, cervical cancer and multiple myeloma with promising results for the future.

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Targeting G-coupled estrogen receptor signaling in melanoma

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Abstract: Melanoma is the most common malignancy diagnosed in pregnant woman. Some clinical data suggest the rapid progression of melanoma disease during the pregnancy.
It is well known that melanoma is the most immuno-dependent malignancy and during the pregnancy the immune system undergoes changes that can impact on tumor surveillance. However, clinical data on melanoma indicate the potential impact of sex hormones (especially estrogens) on progression of the melanoma disease and it is suggested that melanoma can be hormone dependent tumor.
In this paper, based on our experimental results, we focus on the role of G-protein coupled specific estrogen receptor (GPER) in melanoma. GPER receptor is the third type estrogen receptor with poorly understood function in melanoma. We show that GPER is expressed in MeWo melanoma cells. We also describe the effects of agonist G-1 and antagonist G15 of GPER as well as GPER siRNA silencing on proliferation of MeWo human melanoma cells. Proliferation of MeWo melanoma cells is reduced under activation of GPER dependent signaling. The presented data show a novel impact of GPER dependent signaling on the surveillance of melanoma.
Keywords: GPER, agonist G-1, antagonist G-15, melanoma, MeWo

1. Introduction

Melanoma malignant has been considered to be a hormone-related cancer since 1950s. Then, the potential influence of pregnancy on melanoma progression was mentioned for the first time [1]. Results of only few studies regarding cancer incidence during pregnancy are available and according to them melanoma is one of the most commonly diagnosed malignancies during pregnancy [2, 3]. Interestingly melanoma is also much more frequent in women than in men in age group from 20 to 50 years old, during the period when sex hormone level differ greatly between females and males [4]. The hypothesis that melanoma cells benefit from hormonal changes e.g. from increased serum plasma levels of estradiol was taken into account and although many studies on influence of estradiol on melanoma growth were performed, results of them are contradictory. Some of researchers showed that estradiol has no influence on melanoma proliferation [5], few of them report that estrogens inhibit melanoma growth [6] and the other suggest that estradiol induces proliferation of melanoma cells *in vitro* [7]. Thus, the role of estradiol in melanoma progression appears to be controversial. It is assumed that differences in melanoma response to estradiol depend on a presence or absence of estrogen receptors. Presence of intracellular receptors for estrogens ERα and ERβ in human primary melanomas were reported by few investigators [8÷9]. To our knowledge there are no available data from the studies on a presence of another estrogen receptor, a membrane G-protein coupled estrogen receptor (GPER formerly GPR30) in melanoma cells. GPER mediates rapid estradiol-induced nongenomic signaling and participates in the proliferative response in various cancer cell lines including endometrial, breast or ovarian cancer cell lines [10÷12]. In the present study we aimed to find out if the G-protein coupled receptor was present in the MeWo melanoma cells. We also studied influence of the GPER specific agonist G-1 and antagonist G-15 on melanoma cells viability.

2. Material and methods

2.1. Reagents

G-1 and G-15 were purchased from Tocris Bioscience and dissolved in DMSO as 10 mg/ml stock solution. 17-β estradiol was purchased from SIGMA Aldrich and dissolved as 5 mg/ml stock solution. Rabbit IgG polyclonal anti GPER antibody was purchased from Santa Cruz Biotechnology.

2.2. Cell lines and culture conditions

The human skin melanoma cell line MeWo and the human cervical cancer cell line HeLa was purchased from ATCC.

Both cell lines were maintained in vitro as monolayer cultures in RPMI 1640 medium (Biochrom, Germany) supplemented with 10% fetal calf serum (FCS, Biochrom, Germany) and antibiotic/antimycotic solution (Sigma-Aldrich) at 37°C in a humidified atmosphere of 5% CO_2. Cells were passaged every three days after washing with PBS and detached with trypsin/EDTA solution (all from Biochrom, Germany)

2.3. Transfection

For the siRNA transfection, cells were cultured in a six well culture plate until 80% of confluence was attained. Then the cells were incubated for 7 hours in previously prepared Transfection Reagent and Transfection Medium mixture (Santa Cruz) containing siRNA against GPER or control siRNA (Santa Cruz). Afterward cells were cultured for three days in normal growth medium containing antibiotics and 10% FCS and then used for further experiments including cells viability assays and immuno-fluorescence.

2.4. Western Blot

MeWo cell line was cultured in media enriched in 17-β estradiol at concentration 100 pg/ml for 72 hours or in media with vehicle (dimethylosulfoxide, DMSO). HeLa cell line which served as positive control was cultured in growth medium. Then lysates of cells were prepared using RIPA buffer (Sigma Aldrich) supplemented with protease and phosphatase inhibitors (Complete, Roche). Total protein concentration of each lysate sample was determined using BCA assay (Sigma-Aldrich). Equal amounts of protein was mixed with sample buffer and then heated at 95°C for 5 minutes. Electrophoresis was conducted on 0.1% SDS-10% polyacrylamide gel for one hour and next separated proteins were transferred to PFVD membrane (BioRad). Membrane was blocked with 3% skimmed milk for one hour in room temperature and incubated overnight at 4°C with diluted (1:200) anti GPER antibody or anti Rab11 rabbit polyclonal antibody (Santa Cruz). Membrane was washed tree times with TBS and incubated in 5% blocking buffer containing diluted goat anti rabbit HRP-conjugated antibodies for one hour. Signals were detected on X- ray films by means of chemi-luminescence. Relative bands density measurements was performed with ImageJ software.

2.5. Immunofluorescence

For the immunofluorescence experiments, cultured cells were seeded in microscope chamber slides and allowed to attach for 24 h. Then the cells were fixed with buffered 4% paraformaldehyde, permeabilized with PBS 0.2% triton X-100 and incubated in bovine serum albumin 5% to block nonspecific binding. Immunocytochemistry staining was performed using rabbit polyclonal anti-GPER antibody (Santa Cruz, dilution 1:500) Incubation with the primary antibody was performed at 4°C overnight. After washing 3-times in PBS the chamber-slides were incubated in the secondary anti-rabbit antibody Alexa Fluor 488 (Invitrogen, dilution 1:1000) for one hour, washed 3-times in PBS and mounted in Vectashield containing4′,6-diamidino-2-phenylindole (DAPI) (Vectorlabs, USA). Images were captured by Leica confocal microscope and analyzed by means of Las AF software (Leica, Germany).

2.6. Proliferation assays

For proliferation assays we used cells cultured in regular media or cells transfected with siRNA silencing GPER gene. 5 000 cells per well were seeded on 96 well plates and left to attach. After 24 hours media in wells were replaced with media enriched with G-1 or G-15 at concentrations from 1 nM to 10 μM. Cells treated with vehicle (DMSO) were used as a controls. After 48 hours cells viability assays using Presto Blue reagent according to manufacturer protocol were conducted to determine cell counts.

3. Results

3.1. GPER receptor is expressed in human melanoma cell line MeWo cells and is silenced by si-RNA

The expression of GPER protein in MeWo cell line was demonstrated by means of western blot using anti GPER antibody (Santa Cruz) (Figure 1 a). The densitometric analysis by ImageJ software showed that GPER is expressed in MeWo melanoma cells at lower level than in HeLa human cervical cancer cells, which served us as positive control (Figure 1 b).

We also analyzed the influence of 17-β-estradiol (E2) on GPER expression. As it is shown in Fig. 1b., the relative density of GPER band is similar in the presence and absence of 17-β estradiol both, suggesting no significant effect of 17-β estradiol on GPER expression.

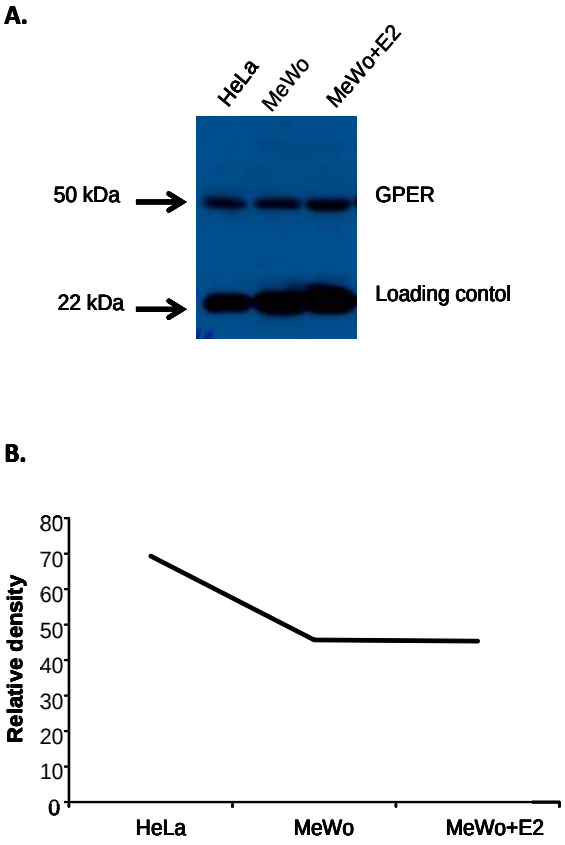

Figure 1. The protein expression of GPER was demonstrated by using Western blotting. A, Western blotting bands. MeWo cells were treated with vehicle or E2 (100pg/ml). HeLa cell line treated previously with vehicle served as positive control. B, Optical bands density analysis. Each sample was normalized to loading control content. GPER is expressed at lower level in MeWo cells than in HeLa cells. The expression is not changed by the E2 stimulation

Moreover, we also performed immuno-fluorescence staining to confirm GPER expression in MeWo melanoma cells.

The MeWo cells are positive for GPER and its immunoreactivity give strong signal in all of the cells. The signal was detected in the cytoplasm confirming its intercellular membrane localization (mainly in ER membranes) as well as at the cell surface (Figure 2). Moreover, the intensity of the observed GPER signal was reduced in cells under siRNA silencing of GPER (data not shown). This observation confirms the specificity of the used antibodies.

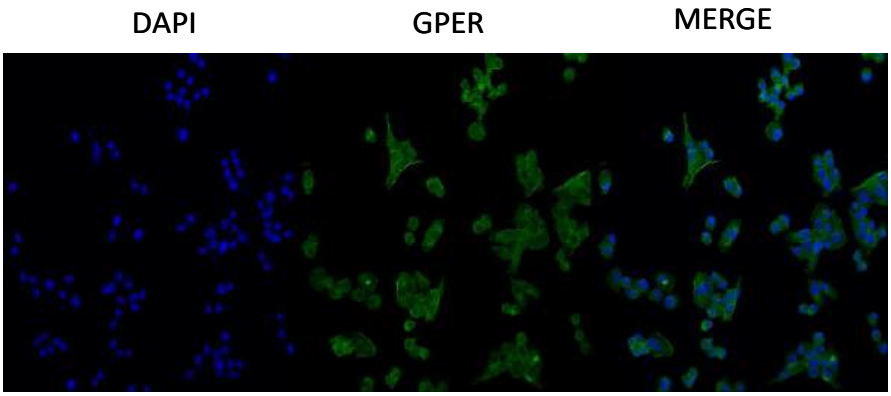

Figure 2. MeWo cells were positive for GPER (green).

Immunofluorescence staining of MeWo cells. Left panel: cell nuclei stained with DAPI. Middle panel: GPER expression at cell surface and within the cytoplasm. Right panel: overlay of DAPI and GPER signals

3.2. GPER agonist reduce the number of viable MeWo melanoma cells, while antagonist had no such effect

We studied the effect of GPER agonist and antagonist on MeWo melanoma cells viability.

The GPER activation or inhibition was performed using its agonist G-1 and antagonist G-15 and the influence of them on MeWo melanoma cells was investigated.

For GPER activation or inhibition its agonist and antagonist were used and the viability was assessed after 48 hours of incubation with agonist or antagonist.

After treatment with G1 agonist (in concentrations from 1- to 10 μM) the number of viable cells was significantly lower as it is shown in Figure 3.

In the group treated with G-1 antagonist at 1μM the number of viable cells was 63% ($p<0.05$) in comparison to the control. Treatment with G-1 antagonist at concentration of 10 μm caused maximum reduction of cells viability almost to 48% of control ($p<0.05$).

The GPER antagonist G15 alone did not reduce the number of melanoma cells significantly when compared to the control (data not shown).

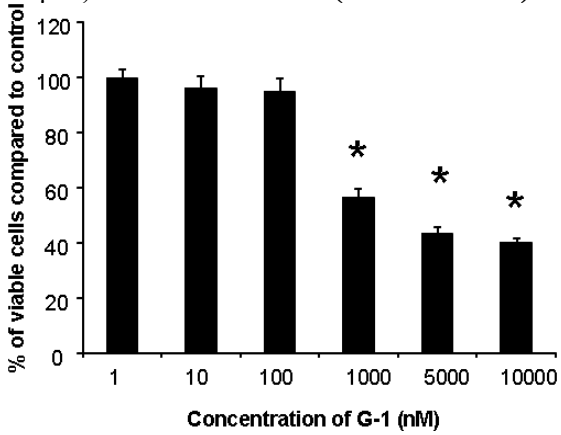

Figure 3. G-1 reduced the viability of MeWo cells. MeWo cells were treated with G-1 (1nM-10μM) or vehicle (DMSO) and after 48 hours of exposure the viability was determined by Presto Blue assay. Results are expressed as mean ± SEM and statistical significance ($p\leq0.05$) was assessed by Student T-test

4. Discussion and review

4.1. GPER – general data

Based on epidemiological data there exist a hypothesis that melanoma is a hormone, esp. estrogen, dependent cancer. However, there are only few reports in the literature regarding the presence of the estrogen receptors in melanoma cells and most of them refer to nuclear receptors ERα and ERβ alone [5, 6]. There is no specific information available on the presence of the estrogen membrane receptor- GPER in melanoma. However the upregulation of GPER was shown to stimulate melanin production and melanogenesis by protein kinase A (PKA) pathway [13].

Thus our paper focuses on surface receptor for estrogen (GPER) that mediates immediate estrogen effect. In the present study we demonstrated for the first time that GPER is expressed at protein level in human melanoma cell line – MeWo.

4.2. GPER – receptor

Membrane estrogen receptor named as GPER mediates rapid, nongenomic reaction of cells that are estrogen sensitive. The previous name for this receptor was GPR30. This receptor belongs to the seven domain transmembrane receptor family and is coupled with G-protein. Identification of GPER agonists and antagonists enabled studies on estrogen dependent signaling in physiology and pathology of sensitive cells and tissues [13÷15].

4.3. GPER – localization

Membrane bounded receptor can be localized in the cell membrane, or in any intracellular membrane-enclosed compartment. Also receptor recirculation from compartment to compartment is possible. Based on the literature, GPER receptor undergoes also its specific translocation.

First it was published that GPER is localized in the ER membranes [16], however another investigators reported its cell membrane localization [17].

The GPER receptor was shown to be localized in the cell membrane in HEK-293 cells, hippocampal CA-2 cells, ovaries of *Micropogonias undulates*, murine oocytes, myometrium [18÷22].

While cytoplasmatic/ER/Golgi localization has been shown for: COS7, CHO, HEK-293, HEC-50, MDA-MB231, H-38, hippocampal neurons, rat spermatocytes [23÷28].

Some authors suggest also the possibility of nuclear GPER localization in HUVEC and CAF cells [29÷32].

Our data show both cell membrane and cytoplasmatic GPER localization (Figure 2). The stronger signal was observed form the plasma membrane, the slighter came from cytoplasmatic structures. This observation can be easily explained by the well known fact that cell membranes circulate constantly between ER and plasma membrane in vesicles transporting phospholipids and proteins to the plasma membrane. The suggested ER localization give many unsolved questions about signaling cascade esp. localization of G-protein (inner or outer face), thus plasma membrane localization seems to be more physiological. Moreover, despite the fact of different cellular localizations of GPER it is still unknown in which of then the receptor is functionally active.

4.4. GPER receptor signaling

In the classical estrogen signaling, receptors for ligands are localized within the cell and act as transcription factors. However, this signal transduction mechanism does not explain rapid changes in cell metabolism after estrogen exposition.

The nongenomic action of estrogens is mediated by membrane bound receptor (GPER) discovered in year 2000 [33]. GPER is a G-coupled receptor and transduces signal using G-protein. In classical pathway it activates adenylyl cyclase and thereby protein kinase A [15, 17, 19, 34÷35]. PKA activation causes both fast metabolic effect as well as long term transcriptional gene activation by cAMP response element binding protein (CREB) activation [36÷38].

It was shown that GPER activates also MAP kinase pathway (MAPK), ERK (extracellular signal-regulated kinase) by Ras/Raf pathway as well and phosphoinositide 3-kinase (PI3K), enabling Akt (known as protein kinase B-PKB) kinase activation. In addition, activation of phospholipase C (PLC) leads to IP3/Calcium dependent signaling [16, 19, 39÷42].

The Ras/Raf/Erk, PI3K/Akt, and MAPK pathways are the main cellular regulators of cell growth and proliferation, and Raf is a common molecular target for melanoma treatment nowadays [43]. Thereby activation of GPER in melanoma triggers the critical pathways for cell surveillance and can be potentially good candidate for drug targeting.

In the contrast to direct G-protein dependent PKA and PLC signaling, the activation of these pathways recruits cross-activation of epidermal growth factor receptor (EGFR), that takes place by activation of Src kinase leading to MMP dependent releasing of heparin-binding EGF-like growth factor (HB-EGF) form its membrane bound form. HB-EGF binds to EGF receptor activating mainly Ras/Raf/Erk pathway, however PI3K/Akt and MAPK pathways can be also triggered [33, 41].

It was also shown that GPER stimulation by 17β-estradiol leads to activation of another pathway closely related to cell growth and differentiation – Notch [44].

Summarizing the GPER dependent signaling is very wide and involves many crucial pathways for melanoma cell surveillance.

4.5. GPER – and tumor

In our study we show for the first time the antiproliferative effect of GPER agonist G1 in melanoma MeWo cells. G1 agonist demonstrates antimelanoma activity at concentration starting from 1 μM. Recently, G1 agonist was also shown to reduce the viability of prostate cancer cells [45]. Moreover, the deficiency of GPER resulted in increased tumorigenesis in the liver [46].

The best characterized in the literature is the effect of GPER stimulation in ovarian, breast and prostate cancer that represent hormone-dependent malignances.

Activation of GPER in prostate cancer cells leads to growth inhibition and G2/M cell cycle arrest acting by Erk1/2 pathway and p21 upregulation. The inhibitory effect was observed in both androgen-dependent and androgen-independent prostate cancer cells *in vitro* and *in vivo* [45, 47].

In ovarian cancer cells GPER agonist G1 was shown to block tubulin poly-merization [48] and to inhibit cell proliferation by inducing G2/M cell cycle arrest and to initiate caspase-dependent apoptosis [49]. Moreover, in the same study Ignatov et al. show that GPER expression in ovarian cancers clinically correlates with higher 2-year disease-free survival of patients (28.6% for GPER-1 negative and 59.2% for GPER-1 positive cases by $p = 0.002$). This observation makes GPER positive ovarian cancer potential candidate for GPER targeted therapy.

Also in breast cancer cells G1 GPER agonist displayed anticancer activity inducing G2/M cell cycle arrest [50]. However, the data on the effect of GPER activation/ inhibition in breast cancer cells are contradictory, it was also shown that inhibition of this receptor by natural estriol (not synthetic G1 agonist) inhibits the growth of triple-negative breast cancer cells [51]. Moreover, Albanito et al. demonstrated that G-1 can potentially simulate the proliferation of ovarian and breast cancer cell lines [52]. These contradictory observations taken together with postulated non-receptor action of G1 [53] should turn us for a detailed research before potential clinical usage of GPER targeted therapy.

It is worth to mention that antagonists/modulators of canonical estrogen like tamoxifen, commonly used in the treatment of breast cancer was also shown to display GPER agonist activity [14].

G1 GPER agonist acts also on non-hormone dependent cancers and noncancerous cells e.g. it inhibits antiproliferative action of human vascular smooth muscle cells [54] or mediates progression of NSCLC (non small cell lung cancer).

Nowadays, according to GPER discovery in melanoma, the old question of possible estrogen targeting in melanoma is re-opend [55]. Recently, GPER agonist was shown to stimulate melanin production in melanoma cells [13], and here we describe its antimelamona activity. Thus G-1 GPER agonist should be considered as a new promising drug for cancer treatments including the therapy of melanoma.

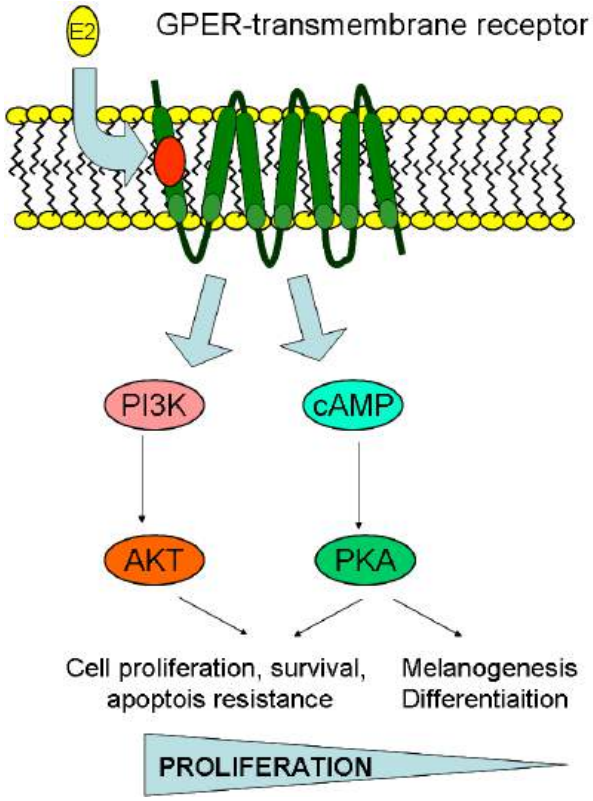

Figure 4. The effects of GPER activation on the proliferation of melanoma cells depends on balance in signaling pathways. E2 – estrogen as ligand, PI3K – Phosphoinositide 3-kinase, AKT – known also protein kinase B, cAMP – cyclic adenosine monophosphate, PKA – protein kinase A. Based on [14÷15].

5. Summary

In our study we showed for the first time that GPER receptor is expressed in human melanoma cells and GPER stimulation by its G-1 agonist reduces viability of melanoma cells. Thus, we propose GPER receptor to be a good candidate for research and targeting in antimelanoma strategies.

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Targeting the PD-1 signaling pathway in cancer immunotherapy

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Abstract: Blocking the PD-1 signaling pathway is one of the intensively investigated technique of cancer immunotherapy. This approach consists of stimulation the patient's immune system to successfully recognize and destroy cancer cells. Anti-PD-1/PD-L1 therapy is one of the option for the therapy of relapsed and refractory malignancies. In many currently underway clinical trials various regimens of anti-PD-1 and anti-PD-L1 monoclonal antibodies are evaluated. It was proved that their application is effective in numerous cancer types treatment and some of them were already approved for clinical practice. This review summarize the latest, selected clinical trials of anti-PD-1 and anti-PD-L1 monoclonal antibodies in solid tumors.
Keywords: PD-1, PD-L1, monoclonal antibodies, cancer immunotherapy, clinical trials

1. Background

Programmed death 1 (PD-1) protein is an immunoreceptor which undergoes inducible expression on activated T and B cells, monocytes and some of dendritic cells. PD-1 belongs to CD28 molecules family transmitting the costimulatory signal during the lymphocyte activation. PD-1 is a negative immunoreceptor, it acts regulatory by transferring the braking signal into cell interior. The ligands for PD-1 are transmembrane glycoproteins PD-L1 and PD-L2. Their expression is varied, PD-L1 is found on B and T lymphocytes, monocytes and dendritic cells, its amount increases with the activation of these cells. PD-L1 molecule is also present on many types of non-hematopoetic cells, including vascular endothelial cells, pancreatic islets, neurons, astrocytes or trophoblast cells of the placenta. The PD-L2 expression is limited to dendritic cells and monocytes, however PD-L2 mRNA is also synthesized in human heart, liver, placenta and pancreatic cells. The interaction of PD-1 with its ligands is an example of the escape of tumor cells from immune surveillance by strengthening the negative signal transmitted to tumor infiltrating lymphocytes, which impairs their function. The PD-L1 widespread expression indicates the importance of this molecule in the modulation of the immune response. The presence of PD-L1 and PD-L2 was found also in many types of malignancies. High expression of these factors was correlated with more aggressive course of the disease and unfavorable prognosis for patients. Currently, many clinical trials underway to verify the effectiveness of anti-PD-1 and anti-PD-L1 antibodies and fusion proteins in cancers therapy. Conducted research allow to suppose that therapeutic use of PD-1/PD-L pathway inhibitors will increase in the future [1÷4]. The aim of this review is to summarize selected, latest clinical trials of solid tumors, that evaluate the efficiacy of anti-PD-1 and anti-PD-L1 antibodies in cancers therapy.

2. PD-1/PD-L1 blockade in clinical application

Many clinical studies have demonstrated that PD-L1 overexpression is connected with a poor prognosis in several tumor types, including esophageal cancer, gastric cancer, hepatocellular carcinoma, pancreatic cancer, renal-cell carcinoma, bladder cancer and ovarian cancer. These studies suggested that inhibition of PD-1 signaling may improve clinical outcomes of patients with these neoplasms [5].

2.1. Anti-PD-1 mAbs

The PD-1 blocking approach has unique mechanism compared to conventional therapies. In general, standard chemotherapies target certain molecule in tumor cells. Malignant cells can avoid such therapy through mutations of target molecules, which lead to rapid regression. Differently, a PD-1 blockade induces a response for a longer period because it stimulates an anti-tumor immune system to target mutated proteins, and therefore is applied in various types of cancers. What is important, PD-1 blocking exhibits significantly lower rate of high-grade toxicities than other immunotherapies or conventional therapies due to the fact that the anti-tumor immunity preferentially recognizes and combats tumor-derived antigens, not self-antigens [5÷6].

At least 500 clinical trials of PD-1 signal inhibitors were carried out so far, assessing 9 types of antibodies from 8 pharmaceutical companies in at least 20 different types of solid and hematological malignancies [5, 7].

2.1.1. Nivolumab

Nivolumab is the first PD-1 blocking antibody approved for clinical practice worldwide [8]. It is a fully humanized monoclonal antibody (mAb), directed against PD-1. Nivolumab is also known as MDX-1106, ONO4538 or BMS-936558. It was first produced using genetically modified mice possessing human immunoglobulins encoding loci. Nivolumab is IgG4 isotype, what minimizes antibody-dependent cell-mediated cytotoxicity (ADCC) and complement activity. This antibody contains a serine-to-proline substitution at 228 position, which significantly reduces ADCC effect against activated T cells. Clinical trials with nivolumab have started in 2006 in the United States. The phase 1 large study of nivolumab (NCT00730639) reported cumulative response rates of 28% for melanoma, 27% for renal carcinoma and 18% for non-small-cell lung cancer (NSCLC). Grade 3 or 4 drug-related adverse events were reported in 14% of patients. Importantly, nivolumab has exhibited durable clinical effectiveness as a single agent and significantly fewer side effects than ipilimumab – mAb against another immune checkpoint CTLA-4. A clinical trial of anti-PD-L1 mAb demonstrated relatively low response rates in comparison with anti-PD-1 mAb (NCT00729664) [5, 9÷10].

In a phase 1 trial of nivolumab (NCT00730639) in 17 patients with kidney cancer, objective responses were seen in 24% and 31% of patients at 1 mg/kg and 10 mg/kg dose, respectively. Stabilization of the disease was seen in other 27% of patients. Five patients had a durable response remaining more than 1 year [9, 11].

Nivolumab was also assessed in a phase 1, dose-escalation trial in 17 patients with castrate-resistant prostate cancer (CRPC) (NCT00730639). However, no objective responses were seen; thus, nivolumab was not examined in phase 2 or 3 studies in prostate cancer [9, 11].

In ongoing phase 1/2 study nivolumab is being evaluated in advanced hepatocellular carcinoma (HCC) patients who can not be treated with sorafenib (NCT01658878). It was observed that responses were independent of the PD-L1 expression on tumor cells. Based on promising preliminary data from this trial, a phase 3 study is currently ongoing com-paring nivolumab versus sorafenib in patients with advanced HCC (NCT02576509) [12].

In the first-in-human phase 1 study of nivolumab (NCT00441337) in treatment-refractory solid cancers, only one patient in the trial group reached a durable CR. It was demonstrated, that this was a patient with microsatellite instability and high T cell infiltrated colorectal cancer (MSI-Hi CRC). The rest of 19 CRC patients did not exhibit any tumor response, it was checked that all of them had microsatellite-stable (MSS) disease [12÷13].

In randomized, phase 3 study, nivolumab was compared to everolimus (immuno-suppressant) in previously treated patients with advanced renal cell carcinoma (NCT01668784). The median overall survival (OS) was 25 months with nivolumab in comparison with 19.6 with everolimus. The median progression free survival (PFS) was similar and amounted to 4.6 months with nivolumab and 4.4 months with everolimus. The overall response rate (ORR) was statistically higher with nivolumab and reached 25% versus 5% with everolimus. Interestingly, the use of nivolumab did not improve PFS, although ORR and OS were significantly superior with nivolumab in comparison with everolimus [11, 14].

In a phase 3 study, which compared nivolumab to docetaxel (the plant alkaloid chemotherapeutic) in patients with advanced squamous-cell NSCLC (NCT01642004), the response rate (RR) with nivolumab was 20% versus 9% with docetaxel. The ORR after 1 year was 42% with nivolumab versus 24% with docetaxel. The frequency of occurrence grade 3 or 4 treatment-related adverse events was greatly lower in nivo-lumab group (7%) compared to docetaxel group (55%) [5, 15].

In a phase 2 clinical study of nivolumab (UMIN000005714) in 20 patients with platinum-resistant recurrent ovarian cancer, the objective RR was 20%, including two cases of complete response (CR). For all patients the RR was 15% and the durable CR (DCR) was 45%. The median progression-free survival (PFS) and OS were 3.5 and 20 months,

respectively. After completing 1-year nivolumab treatment, two patients who reached CR survived over 2 years without any antitumor treatment. The phase 2 trial currently underways, comparing nivolumab versus standard 2nd-line chemotherapy in ovarian cancer (JapicCTI-153004) [5, 16].

Immunotherapeutic strategy of targeting PD-1/PD-L1 is also investigated in the treatment of pancreatic cancer. In ongoing phase 2 study the combination of pancreatic cancer vaccine GVAX/CRS-207 with or without nivolumab is comparing in previously treated patients with metastatic pancreatic cancer (NCT02243371). An another pilot study is evaluating nivolumab combined with dendritic cell vaccine in patients with metastatic pancreatic cancer. In the cohort of 7 patients treated until data cutoff, 2 of them had PR so far [12, 17].

In ongoing phase 1/2 study, also combination of anti-CTLA-4 and anti-PD-1 mAbs together is being explored (NCT01928394). This trial compares the activity of nivo-lumab alone or together with ipilimumab, in resistant to chemotherapy, advanced solid cancers. The three treatment arms involve nivolumab 3 mg/kg, nivolumab 3 mg/kg with ipilimumab 1 mg/kg, and nivolumab 1 mg/kg with ipilimumab 3 mg/kg. The early results of the gastric cancer cohort revealed an ORR of 14, 10, and 26% in treatment arms, respectively. Nivolumab was assessed in combination with ipilimumab also in another phase 2 study (NCT02060188). In this trial patients with metastatic CRC were enrolled into two cohorts, nivolumab with or without ipilimumab. The preliminary results show that the combination of this two agents is associated with favorable clinical activity, particularly in the MMR-deficient subgroup, where 4-month PFS was 80% and 5-month OS was 100% [12, 18].

The U.S. Food and Drug Administration (FDA) approved nivolumab for patients with unresectable or metastatic melanoma in 2014, metastatic NSCLC in 2015, classical Hodgkin's lymphoma, advanced RCC and for recurrent/metastatic squamos cell carcinoma of the head and neck in 2016. In 2015 FDA approved also combined therapy nivolumab with ipilimumab for unresectable or metastatic melanoma. This combined therapy is now being clinically applied to numerous cancer types, including NSCLC, RCC and ovarian cancer. Many combination therapies with nivolumab and ipilimumab or VEGF tyrosine kinase inhibitors currently underway [5, 8, 11].

2.1.2. Pembrolizumab

Pembrolizumab (MK-3475) is another anti-PD-1 antibody. Pembrolizumab is a highly selective, high-affinity, humanized monoclonal IgG4 antibody that prevents PD-1 binding to its ligands. In several countries pembrolizumab is approved for the treatment of advanced melanoma, NSCLC, and in second-line treatment of head and neck squamous cell carcinoma. Clinical studies have demonstrated encouraging efficacy of pembrolizumab in many advanced cancers, such as gastric and urothelial cancer [8].

One of the first clinical studies of pembrolizumab in PD-L1-positive, previously treated, advanced solid tumors was the multi-cohort, phase 1b trial (NCT01848834). The tumors were classified as PD-L1-positive if this marker was detected on ≥1% of cancer cells or any positive stainig was found in the stroma. In gastric cancer cohort 39 patients were enrolled with PD-L1-positive relapsed or metastatic stomach adenocarcinoma or gastroesophageal junction (GEJ). The ORR was 22% in 36 evaluable patients with advanced gastric cancer. In this trial, no association between response and the level of PD-L1 expression on cancer cells was observed. In another similar phase 1b study pembrolizumab was evaluated as a single agent in PD-L1-positive advanced solid malignancies (NCT02054806). To the esophageal carcinoma cohort 23 patients were enrolled with esophagus squamous cell carcinoma (SqCC) or adenocarcinoma, or GEJ, who had progressed with standard therapies. In this group of heavily pretreated patients the ORR primary endpoint was established at 30%. During data cutoff, four out of seven responses were still continuing and the median duration of response had not been achieved. This promising clinical activity was the basis for phase 2 and 3 trials of anti-PD-L1 regimens in gastric, esophageal and GEJ malignancies. Currently, there is phase 2 multi-cohort study in progress for patients with relapsed or metastatic gastric or GEJ adenocarcinomas (NCT02335411). The three cohorts in this trial comprise pembrolizumab with fluoropyrimidine and cisplatin in previously untreated patients, pembrolizumab as a single

agent in previously treated patients and pembrolizumab monotherapy in treatment-naive patients. Another ongoing phase 2 study (NCT02559687) is assessing pembrolizumab monotherapy in previously treated patients with esophagus advanced adenocarcinoma or SqCC either with GEJ. Many phase 3 trials of anti-PD-L1 therapy in gastric and esophageal cancers are currently ongoing. One of them (NCT02370498) compares pembrolizumab versus paclitaxel in metastatic or unresectable gastric or GEJ adenocarcinoma as a second line treatment. Another study (NCT02494583) is a three-arms study, which compares pembrolizumab monotherapy versus 5-fluorouracil with cisplatin, versus this three agents together in PD-L1-positive advanced gastric or GEJ adenocarcinoma as a first-line treatment. Another large, phase 3 study (NCT02564263) will compare pembrolizumab towards paclitaxel, docetaxel or irinotecan as a single-agent chemotherapy in previously treated advanced SqCC or adenocarcinoma of the esophagus or GEJ patients [12, 19÷21].

In phase 1b trial pembrolizumab was evaluated in patients with metastatic, relapsed urogenital tract cancers (NCT01848834). For this study 33 bladder cancer patients with PD-L1 expression in ≥1% of tumor cells or in stroma were enrolled. The ORR was 24%, with 10% of CR and 14% of partial responses (PR) to pembrolizumab. The 1-year PFS was 19%. Obtained results allow to conclude that pembrolizumab exhibits significant antitumor activity in PD-L1-positive bladder cancer patients. The registra-tion phase 3 trial of pembrolizumab compared to selected regimen (docetaxel, paclitaxel or vinflunine) in advanced or metastatic bladder cancer has finished recently and results are expected (NCT02256436). Several combination therapies with pembrolizumab and cytotoxic agents or targeted therapies as first-line and rescue therapies are presently being verified in clinical studies [11, 22].

Pembrolizumab was also evaluated in single-arm, phase 2 study in patients with CRPC after progression on enzalutamide (an androgen receptor antagonist) (NCT02312557). In 3 out of 10 patients a rapid PSA response was noted and 2 subjects had a partial response. In a retrospective study, PD-L1 expression on testicular germ cell tumors (TGCT) was evaluated. Using immunohistochemistry, PD-L1 expression was noted in 73% of seminomas and in 64% of non-seminomas. In normal testicular tissue no PD-L1 expression was observed at all. In another study it was confirmed that PD-L1 expression in TGCTs was higher than in normal testicular tissue. The highest level of PD-L1 protein was detected in choriocarcinoma, a little less in embryonal carcinoma, teratoma, yolk sac tumor and seminoma. Low-PD-L1 expression was associated with a better PFS. Also OS of patients with low-PD-L1 expression was improved compared to subjects with high-PD-L1 expression. Currently, there is a phase 2 ongoing study, in which the role of pembrolizumab is assessed in patients with relapsed or metastatic, cisplatin resistant germ cell cancer (NCT02499952) [11, 23].

In a phase 2 study the activity of pembrolizumab monotherapy was evaluated in patients with already treated, progressive metastatic cancers, with and without mismatch repair (MMR) deficiency (NCT01876511). The patients were divided into three cohorts: MMR-deficient CRC, MMR-proficient CRC and MMR-deficient non-colorectal cancers. Subjects with MMR-deficient CRCs were found to have favorable responses to pembrolizumab. The ORR and durable CR were 50% and 89% for MMR-deficient CRCs and 0% and 16% for MMR-proficient CRCs, respectively. The median PFS and median OS was not achieved in the MMR-deficient CRC, otherwise than 2.4 months and 6 months in the MMR-proficient CRC cohort, respectively. Based on the obtained results it may be concluded that MMR-deficient CRC patients benefit from anti-PD-L1 treatment. This data was the reason for ongoing phase 2 and 3 studies of pembrolizumab in MMR-deficient advanced CRC (NCT02460198 and NCT02563002) [12, 24].

An ongoing phase 2 study is assessing the effect of combination pembrolizumab with radiotherapy or tumor ablation in MMR-proficient metastatic CRC patients (NCT02437071). Out of 11 subjects in the pembrolizumab plus radiotherapy group, one patient achieved PR in the non-irradiated tumor. No responses were noted in in the eight patients from the pembrolizumab plus ablation group. There is also an ongoing pilot study evaluating the combination of 5-azacitidine (DNA methyltransferases inhibitor) or romidepsin (histone deacetylases inhibitor) or both with

pembrolizumab in patients with MMR-proficient advanced CRC (NCT02512172) [12, 25].

In open-label phase 2 study acalabrutinib (Bruton's tyrosine kinase inhibitor) was assessed in combination with or without pembrolizumab in metastatic pancreatic cancer patients (NCT02362048) and it was noted that this regimen has a favorable clinical activity. In the combination arm, in 23 evaluable patients, 3 of them achieved PR and 5 had the stabilization of the disease [12, 26].

2.2. Anti-PD-L1 mAbs

2.2.1. Atezolizumab

Atezolizumab (MPDL3280A) is an engineered, humanized IgG1 monoclonal anti-PD-L1 antibody which inhibits its interaction with receptors PD-1 and B7-1. Atezolizumab has been approved in USA for the treatment of locally advanced or metastatic urothelial carcinoma and metastatic NSCLC [8].

Atezolizumab was evaluated in phase 1 trial in patients with urothelial bladder cancer. Neoplasms were differentiated based on PD-L1 expression defined as ≥5% of tumor-infiltrating immune cells or tumor cells in immunohistochemistry (IHC) staining. For 67 assessed patients, ORR was 43% in PD-L1-positive group and 11% in PD-L1-negative group. 7% of patients in PD-L1-positive cohort reached a CR including several patients with durable responses. On the basis of these results, atezolizumab was granted by the FDA a breakthrough status in bladder cancer. Long-term results reported that the median OS was 28.9 months and median PFS was 5.6 months. Altogether, atezolizumab was well tolerated and an increased baseline effector T cell to regulatory T cell ratio was connected with better response [11, 27].

In a large, phase 2 study atezolizumab was examined in patients with platinum resistant locally advanced or metastatic urothelial carcinoma (NCT02108652). PD-L1 expression on tumor-infiltrating immune cells (IC) was evaluated by IHC and classified according to staining intensity. ≥5% of staining was stratified as IC2/3, ≥1-4% as IC1 and <1% of staining as IC0. Patients in IC2/3 had a median OS of 11.4 months, in IC1 6.7 months and in IC0 6.5 months. In all patients median PFS was 2.1 months and ORR was 15%. The ORR was significantly higher for all patients in comparison with historical control, where ORR was 10%. Obtained results reveal that responders had an elevated median mutational load compared to non-responders. Differently than in lung cancer, smoking was not connected with higher mutational load and did not foresee response to atezolizumab. Higher response rates were observed in the luminal II subtype of bladder cancer, which is associated with the inherence of activated T cells in cancer site. In view all of this, atezolizumab was approved by the FDA for the use in platinum-resistant advanced or metastatic urothelial carcinoma patients. At present, a large phase 3 trial comparing atezolizumab to chemotherapy in urothelial bladder cancer progressed during or following a platinum-based regimen is underway (NCT02302807) [11, 28].

Atezolizumab was evaluated in phase 1 study in 70 patients with metastatic RCC (NCT01375842). Median OS of patients with clear-cell RCC (62 subjects) was 28.9 months, median PFS was 5.6 months, and ORR was 15%. Response to atezolizumab was associated with decrease in circulating plasma markers and acute phase proteins, the increase of baseline effector T cell to regulatory T cell gene expression ratio was also reported [11, 27].

It was demonstrated that the inhibition of MAPK/ERK kinase (MEK) is associated with enhanced anti-PD-L1 activity. Thus, in phase 1b trial the combination of atezolizumab with cobimetinib – MEK inhibitor, was evaluated in patients with advanced solid cancers (NCT1988896). The early results of 23 CRC patients who have been administered this combination revealed that 4 subjects, including 3 MMR-proficient patients, reached a PR and another 5 had SD. These results contributed to the design of phase 3 study which is analysing cobimetinib plus atezolizumab combination in patients with MMR-proficient advanced CRCs [12, 29].

2.2.2. Durvalumab

Durvalumab (MED14736) is another anti-PD-L1 antibody. It was assessed in a phase 1/2 study in patients with advanced urothelial cancer (NCT01693562). For this trial, 61 patients with advanced or metastatic transitional cell carcinoma of the bladder were enrolled. The ORR was 31% in 42 assessed patients. PD-L1 positivity was established if ≥25% of tumor or immune cells expressed PD-L1. With this qualification of PD-L1 positivity, the ORR was 46% for the PD-L1-positive group and 0% for PD-L1-negative group [11, 30].

In ongoing phase 2 study durvalumab is evaluated in combination with or without tremelimumab (anti-CTLA-4 mAb) in patients with previously treated metastatic pancreatic cancer (NCT02558894) [12].

2.2.3. Avelumab

Avelumab (MSB0010718C) is a fully human anti-PD-L1 monoclonal antibody of IgG1 isotype. It has been approved recently by FDA for the treatment of metastatic Merkel cell carcinoma [8, 31].

Avelumab was evaluated in phase 1b trial in advanced gastric or GEJ malignancies as a first-line maintenance or second-line treatment (NCT01772004). The preliminary ORR was 9%, including 2 CR and 6 PR in the first-line maintenance arm and 10%, including 6 PR in the second-line therapy arm. The disease control rate (DCR) was 57% and 29% and median PFS was 12 and 6 weeks in this two treatment arms, respectively. Because of favorable activity of avelumab, two large phase 3 clinical trials of this mAb in gastric cancer have been started (NCT02625623 and NCT02625610) [12, 32].

3. Conclusions

Immunotherapy has enriched the treatment options accessible for patients with different types of malignancies. The application of PD-1 or PD-L1 inhibitors induced durable responses in many patients, who had limited treatment alternatives before. A number of checkpoint inhibitors trials are currently ongoing, which are aimed at development a new treatment regimens that will provide better outcomes for cancer patients.

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