NATIONAL REPORT ON SITUATION/STRATEGIES/SUPPORT INSTITUTIONS AND SPECIALISTS/BEST PRACTICES OF THE GENETIC CONSULTANCY/ ONCOGENETICS IN BULGARIA

Report created in the framework of the project Promoters of advanced oncogenetics open online training and multimedia raise awareness on multidisciplinary assessment of patients and their families at risk of hereditary or familial cancer, Reference number 2018-1-RO01-KA202-049189, Strategic Partnerships for vocational education and training Erasmus+ programme

1. Current epidemiological situation of cancer, and of hereditary cancer in particular (breast, ovarian, colorectal and other types) in Bulgaria.

Organization of cancer registration in Bulgaria

Cancer registration has been compulsory in Bulgaria since 1952. In 1951/1952 work began on the development of a specialized oncology network, consisting of 13 regional oncological dispensaries and the Cancer Research Institute in Sofia. In the regional oncological dispensaries there were departments which dealt with the registration of all newly diagnosed cancer patients. At the same time, in the Cancer Research Institute, a department for the statistical and epidemiological analysis of cancer cases began its work. The current structure of the cancer registration network is based on the relationships established more than 60 years ago between the country's oncology hospitals. It consists of the Bulgarian National Cancer Registry (BNCR), which is a structural unit of the National Hospital of Oncology (former Cancer Research Institute) in Sofia, and the 13 Regional Cancer Registries (RCR), within the regional oncology centres.

The Bulgarian National Cancer Registry is population based and covers the whole country. The data is provided by the RCRs, which collect information on patients diagnosed and treated in hospitals within their region. Each RCR is responsible for one, two or three districts – Fig. 1.

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Fig.1. Map of the Regional Cancer Registries and the territory that they cover

The staff of the RCR is trained to extract, code and record data for patients with cancer and carcinoma in situ in a specialized registration information system which complies with international classifications and standards [1-5].

The main sources of information are the hospital discharge records of patients, pathology and other laboratory results, medical journals, cancer notification forms , protocols from oncology committees, reports from hospital information systems and from regional health insurance funds, and others. Two methods of data collection are used in combination – passive (hospitals send the necessary documents on cases to the RCR for registration) and active, in which RCR staff visit hospitals within the territory they serve and collect the sources of information.

Based on official agreements, the BNCR regularly receives information from the Civil Registration System (GRAO) and the National Statistical Institute (NSI). GRAO provides data on all deceased persons for each quarter and again at the beginning of the year for the entire previous year. This information is used to update the vital status of registered patients. Until the end of 2011, GRAO has provided information on causes of death, which has allowed for the identification of cancer deaths which had not been recorded in the database and had thus required a search for additional information. Since 2012, the NSI provides information on causes of death as well as population and mortality data and life tables.

The RCRs maintain regional databases containing information on registered patients from their region. Regularly these regional databases are sent to the BNCR to be combined into a national database. BNCR staff checks for completeness, accuracy and consistency of the data. Where there is doubt, the case is sent to the RCR for further verification, correction or adjustment.

The BNCR collects all invasive and in situ cancers with ICD-10 codes C00-C96 and D00-D09. [5] Cases

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with borderline behaviour (D37 – D48), which in ICD-O-3 [6] are classified as malignant, are also collected. The information on each case is structured in more than 85 fields with the following characteristics:

- Patient information – place of residence, personal identification number, name, sex, age, date of birth, marital status, occupation, social group, vital status, date of death or loss of contact, etc.
- Cancer characteristics – date of diagnosis, basis of diagnosis, topography and morphology, TNM, stage, site of metastases, grade, Clark, Breslow, Gleason, estrogen and progesterone receptors, HER2, multiple primary, etc.
- Data for the treatment and progression of the disease – surgery, radiotherapy, chemotherapy, targeted therapy, hormone therapy, other systemic therapy, relapse and recurrence of the disease (since 2011).

Structured in this way, information from the BNCR can be used not only to calculate incidence, prevalence, and survival, but also for more detailed statistical analysis and epidemiological research projects in oncology as well as for public health purposes. As of 30.09.2017 the BNCR database contains 770 452 records of cancer cases diagnosed in the period 1993 – 2017.

The topography and morphology of cancer cases are coded according to the International Classification of Diseases (ICD). The most recent, tenth revision, has been applied since 01.01.2005. Previously, from the initial creation of the registry, ICD-7 (to 1968) [7], ICD-8 (from 1969 to 1979) [8] and ICD-9 (from 1980 to 2004) [9] had been used. The morphology of tumors (ICD-9) has been coded since 1991. With the introduction of ICD-10, all records in the database were automatically converted from ICD-9 to ICD-10. Data to be presented to inter- national institutions or in projects according to ICD-O-3 is converted using standard programs [10].

The date of diagnosis is taken to be the incidence date. Until 01.01.2008 the rule had been that the incidence date was the earliest date from the medical records of the patient. To ensure better comparability of data, the BNCR decided that for cases diagnosed after 01.01.2008, ENCR rules would be applied [1].

The basis of diagnosis is classified by using ENCR recommendations. Priority, however, is given to microscopic methods [1].

The BNCR collects information on all cases of malignant disease in the same patient (multiple primary cases). They are reported according to the IARC rules [3].

The stage of malignant tumors is recorded according to the TNM – classification (where applicable). [4, 12] Information about T, N and M has been collected since 1991. When TNM is not applicable, other internationally accepted classifications are used. In these cases, cancer registrars record the stage, as documented in the medical history of the patient.

The quality of data at the BNCR has been evaluated for comparability, completeness, and validity [13].

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A comprehensive assessment for the period 1993 – 2010 showed that the registration of cases is according to the guidelines recommended for European cancer registries, as far as the coding of topography and morphology, date of diagnosis, multiple primaries and stage are concerned. Cancer incidence in Bulgaria is lower than in other countries of Southeast Europe, but the trends in incidence by cancer sites are similar, the curves of age- specific incidence are identical and childhood cancer incidence is within the expected range. The mortality to incidence ratio is one of the lowest in the region, with a steadily decreasing trend, which indicates a high level of completeness of the data. In 2013 the direct assessment methods showed 94.5% – 96.0% completeness of registration for men and 91.1% – 93.6% for women.

The analysis of the validity indicators for the data showed a trend towards a constant proportion of morphologically confirmed cases and an increase in cases registered from death certificates only and in cases with an unspecified stage. The indicators are within the defined range of the validity of the data in international studies [14].

The population of Bulgaria in 2015

The population figure at the end of 2015 is based on the census results as of 01.02.2011 and the respective calculations of the natural and migration increases, taken from the demographic statistics. [15]

As of 31 December 2015 the Bulgarian population is 7 153 784 persons representing 1.4% of the population of the European Union. This is by 48 414 persons less than in 2014. The male population is 3 477 177 (48.6%) and the female population – 3 676 607 (51.4%). Aging of population continues and is expressed by a decrease in the proportion of children up to the age of 15 years and an increase in the proportion of the population aged 65 and over. As of 31.12.2015 the proportion of persons aged 65 and over is 20.4% (1 461 786) of the total population. This represents an increase of 0.4 percentage points compared with 2014 and of 3.5 percentage points, compared with 2001. Children up to 15 years of age represent 14.0% (998 206) of the total population. Their number increased by 0.1 percentage points compared with 2014.

Population aging leads to an increase in the population mean age. In Bulgaria it increased from 40.4 years in 2001 to 41.2 years in 2005 and to 43.3 years by the end of 2015. Population ageing is observed both in urban and rural areas, but the mean age of the rural popula- tion is 3.8 years higher than the urban population; in urban areas the mean age of the population is 42.3 years, compared with 46.1 years in rural areas. The urban population is 5 227 187 or 73.1% of the to- tal population and the rural population is 1 926 602 or 26.9%. There are seven towns, whose population is more than 100 000 and 34.3% of the population lives in these towns. The district of Vidin has the smallest population – 91 235 persons or 1.3% of the total population while Sofia, the capital, has the largest population – 1 319 804 persons (18.4%).

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Life expectancy in the country, calculated for the period 2013 – 2015 is 74.5 years.

In 2015, 29 470 persons declared a change of address, having moved from Bulgaria abroad. Of these 52.1% were male. 43.6% of the emi- grants are aged 20 to 39 years, while emigrants aged 40 to 59 years accounted for 25.3%. The youngest emigrants (those under 20 years) accounted for 17.5% and the emigrants over 60 years of age, 13.5%. In the same year 25 223 changed their address abroad with an address in Bulgaria – 54.5% male and 45.5% female.

Among those who came to live in the country 35.4% were in the age group 20 to 39 years and 29.9% were aged 40 to 59 years. The youngest immigrants (those under 20 years) accounted for 17.2% and the oldest, those over 60 years of age, 17.5%.

The population of Bulgaria decreased by 44 167 in 2015 due to the negative natural increase.

The average annual population in 2015 was 7 177 991, 3 489 596 of whom were men and 3 688 395 women. Compared with the population 20 years ago there was a decrease of 1 228 076 people (-14.6%), with the greatest reduction of 43.4% in the age group 10 to 14. While in the youngest age groups (up to 19) the population decreased by 38.4%, in the elderly (over 70) there was an increase of 22.3%. Changes in the age structure of the population are related to the patterns of cancer incidence and should therefore be considered when interpreting the data.

Data used in this report:

- The number of new cancer cases diagnosed in 2015 and registered in BNCR as of 01.06.2017 by sex, age, cancer site (ICD-10) and place of residence;
- The average annual population in 2015 by sex, age and place of residence provided by the National Statistical Institute (NSI);
- The number of cancer deaths in 2015 by sex, age, cause of death (ICD-10) provided by the NSI.

Incidence and mortality refer to the number of new cases and deaths, respectively. Both measures can be expressed as the absolute number of cases (or deaths), or as the crude incidence (or mortality) rate, taking into account the size of the population at risk. In this report, cancer incidence and mortality are presented as both numbers and crude rates per 100 000 of the population.

1.1. Incidence

It is observe the upward statistically significant trend in the number of new cancer cases for all types. Over the past 20 years, the number of newly registered cancer cases has risen by an average of 678.81 cases per year (Fig. 2).

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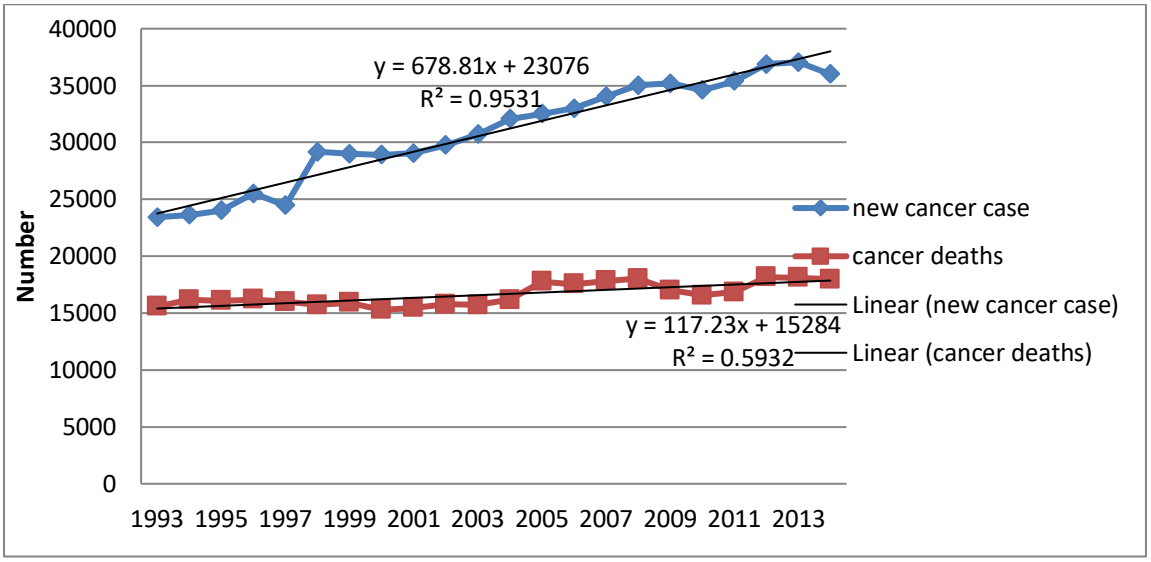

Fig.2. Number of new cancer cases and deaths in Bulgaria (1993-2014)

According to BNCR data for cancer incidence in Bulgaria in 2015, the number of new cancer cases were 35265 and the crude incidence rate was 491.3 per 100 000 inhabitants. In men the prevalence was higher - 530/100 000, whereas in women it was 454.7/100 000 inhabitants.

The newly diagnosed breast cancer cases in females were 3988 with a crude incidence rate of 108.1 per 100 000; for ovarian cancer - 825 new cases with a crude incidence rate of 22.4/100000.

In colon cancer the crude incidence rate in males was higher as compared to that of females - 1541 cases, or 44.2/100 000 *vs* 1202 cases, or 32.6/100 000, respectively. The total crude incidence rate for colon cancer in Bulgaria was 38.2/100 000 for year 2015.

Breast cancer is the most common malignant disease among women. It represents 26.8 % of all malignant diseases in females. Colon cancer comes third in incidence (8.1%), ovarian cancer is fifth (5.5%) (Fig.3). Colon cancer is third in incidence in males as well (9.5%) (Fig.4).

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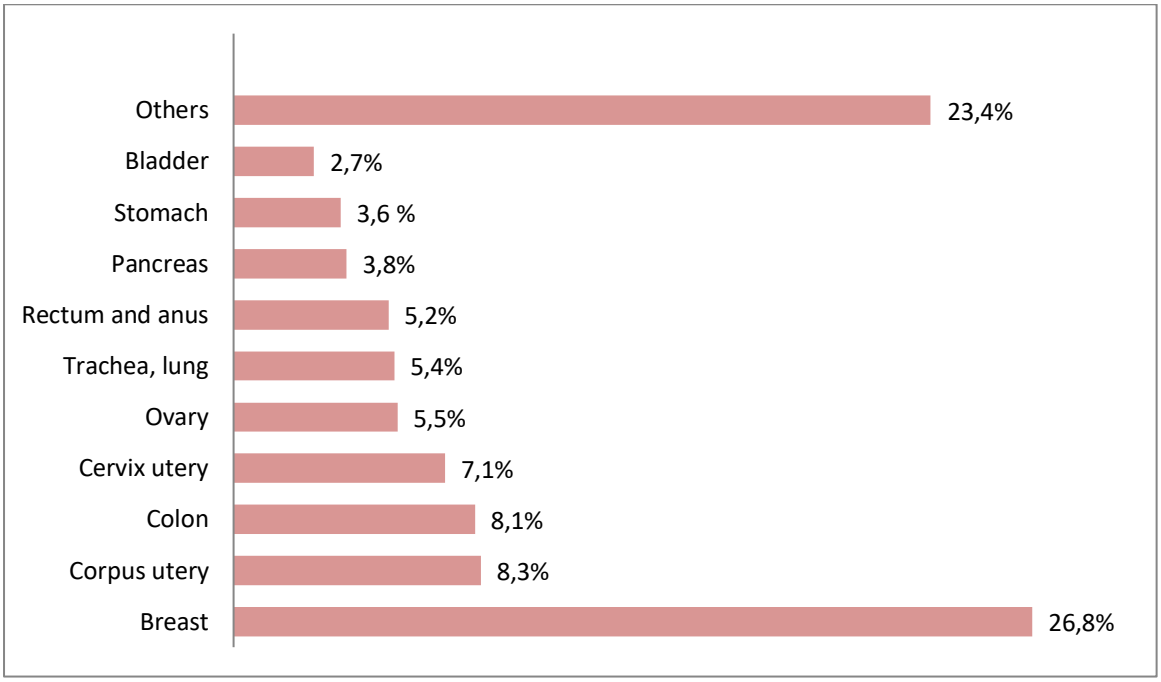

Fig.5. Percentage distribution of the most common cancers in females in Bulgaria, 2015

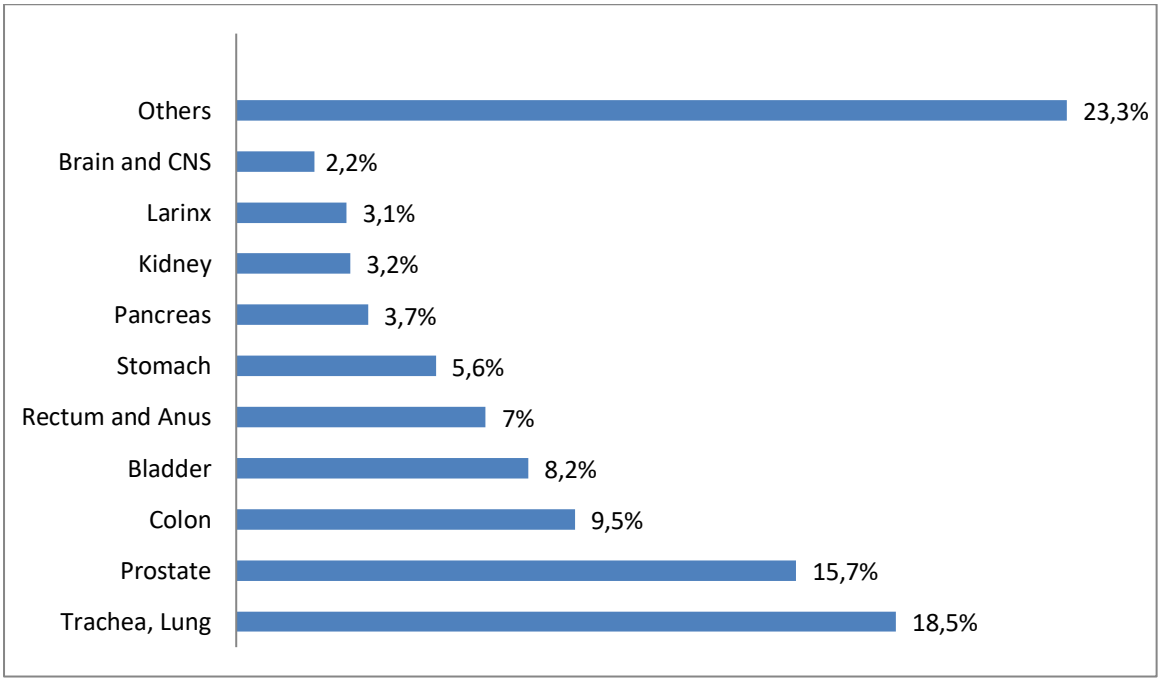

Fig.6. Percentage distribution of the most common cancers in males in Bulgaria, 2015

Disturbing is the fact that a great part of the newly diagnosed cases were in an advanced stage. The distribution of the incidence of breast, ovarian and colon cancer by stage at diagnosis is presented in Fig.7.

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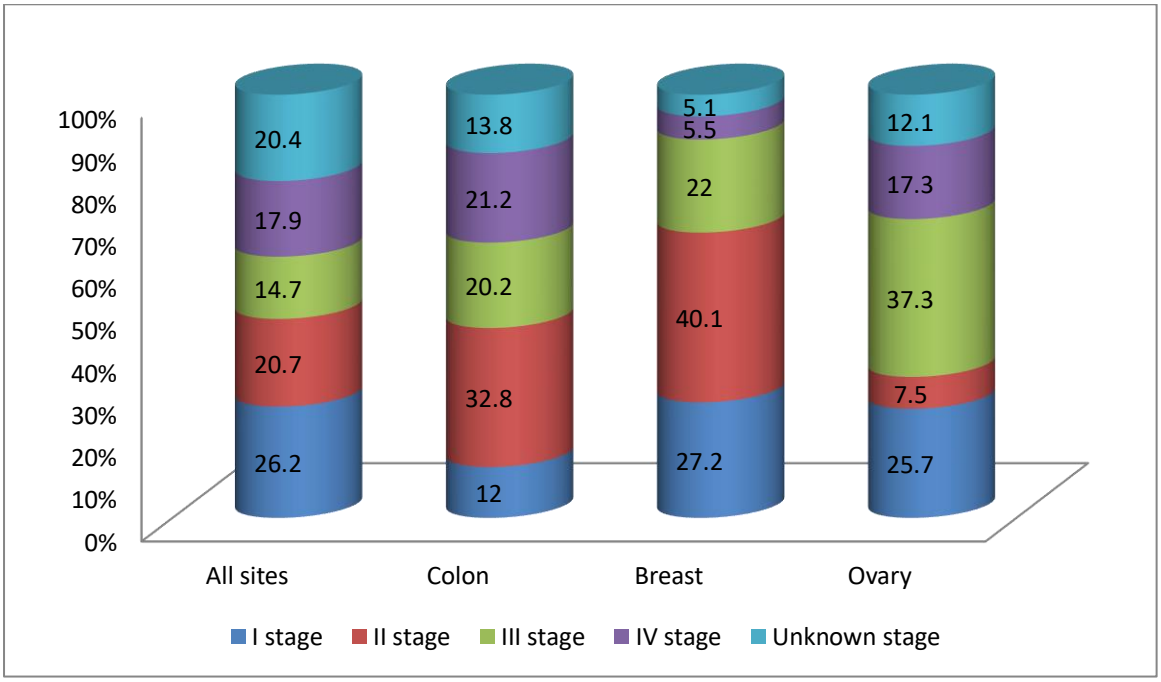

Fig.7. Distribution of the newly diagnosed cases of all sites, breast, ovarian and colon cancer by stage (2015)

1.2. Prevalence

The number of cancer patients (all sites) registered in Bulgaria was 284 355 by 2015, which represented a crude prevalence rate 3961.5 per 100 000, i.e. 4% - 4 people out of every 100 were diagnosed with cancer. In females the crude prevalence rate was higher as compared to males - 170 860 and 113 495 cases, respectively, or 4632.4 /100 000 *vs* 3252.4/100 000. The data for crude prevalence rate for colon cancer, breast cancer and ovarian cancer by sex are presented in Table 1.

Table 1. Crude prevalence rate for colon cancer, breast cancer and ovarian cancer by sex in Bulgaria (2015)

	Male		Female		Total	
	Cases	Crude prevalence rate /100 000	Cases	Crude prevalence rate /100 000	Cases	Crude prevalence rate /100 000
Colon cancer	9249	265	9398	254.8	18647	259.8
Breast cancer	506	14,5	51108	1385.6	51614	719.1
Ovarian cancer			7465	202.4	7465	202.4

1.3. Risk factors (see text below)

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1.4. Mortality

BNCR data show that the number of cancer deaths in 2015 was 17932, which represented a crude mortality rate of 249.8/100 000 people. In men this index was higher - 10412 cases, or 298.4/100000, as compared to that of women – 7520 cases, or 203.9/ 100 000. Mortality due to colon cancer was higher in males – 954 cases, 27.3/ 100 000 as compared to females – 738 cases, 20/100 000 inhabitants. A considerably higher mortality rate was observed in breast cancer of women - 1312 cases, or 35.6/100 000. Mortality due to ovarian cancer was lower - 438 cases, or 11.9/100 000 .

The five-year relative survival for breast, ovarian and colon cancer patients >15 years of age diagnosed in the period 2000-2007 in Bulgaria are presented in Table 2 according to EUROCARE-5 data (last update 5 March, 2014).

Table 2. Five-year relative survival (%) for breast, ovarian and colon cancer patients by sex

	Number of cases		5-year relative survival			Difference from the European average
	Males	Females	Males	Females	All	
All sites	62313	59218	29.4	51.2	40.2	-14.4
Colon cancer	5266	4605	45.0	45.3	45.2	-10.4
Breast cancer	176	16802	57.2	72.8	72.6	-11.1
Ovarian cancer	-	3777	-	40.6	40.6	-0.2

2. Current situation of the National Strategies in models of genetic risk assessment for hereditary cancer (HBOC, CRC and other types) in Bulgaria

Organization of Genetic Research in Bulgaria

The Bulgarian population consists mainly of 3 ethnic groups. In the last few years these groups have been presented among the newborns as follows: 69% Bulgarians, 22% Romani and 9% Turkish. Because of historical and geographical causes Bulgarians are very heterogeneous. The same refers to the Turkish population, whereas the Romani ethnic group (about 800 000 inhabitants) is somewhat of a genetic isolate.

According to the healthcare strategy in Bulgaria the basic healthcare structures are the following: approximately 5200 general practitioners (GP), approximately 500 Diagnostic and Consultation Centres, approximately 300 hospitals (100 of which with maternity wards) and

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26 University Clinics and 5 Medical Universities in Sofia, Plovdiv, Pleven, Varna and Stara Zagora. There are also more than 500 private medical practices and several private Diagnostic and Consultation Centres.

On the basis of the current financial and personnel resources providing the infrastructure of the Bulgarian healthcare system and having in mind the existing trends in economic development, a **National Programme for Diagnosis and Prevention of Hereditary Diseases, Predispositions and Congenital Abnormalities** was started in year 2000. The Programme was financed by the Ministry of Health for a period of 5 years. Its main principles were as follows:

- Centralized organization of activities requiring highly specialized medical staff and laboratory equipment;
- Providing the basic healthcare structures (e.g. GPs, Diagnostic and Consultation Centres and hospitals) with sufficient information regarding clinical discussion and referral of patients.

Structures of primary importance to the National Programme are the five already existing Medical Genetics Laboratories (MGL), located in the University Hospitals in Sofia, Plovdiv, Pleven, Stara Zagora and Varna. A MGL is a functional organization consisting of various units: specialized laboratories, trained groups of specialists (geneticists, obstetricians, gynaecologists, neonatologists, pediatricians, etc.). Each MGL has strictly defined aim and functions. For instance, each MGL in Plovdiv, Pleven, Stara Zagora and Varna provides proper genetic consultation and postnatal cytogenetic tests covering the needs of 1 – 1.5 million inhabitants. Apart from the above-mentioned activities, the laboratory in Plovdiv also provides prenatal cytogenetic diagnosis and risk assessment for Down syndrome by means of a prenatal serum screening.

MGL in Sofia is the main executive structure of the National Programme. This genetic centre is located in the *Maichin Dom* University Hospital of Obstetrics and Gynaecology. The co-ordinating unit is the Molecular Pathology Laboratory consisting of two departments: Biochemical Genetics and Molecular Genetics. This MGL also includes the Medical Genetics Department (routine and molecular cytogenetics) and the Clinic of Foetal Medicine (providing trained teams of obstetricians, gynaecologists and pediatricians). The MGL provides day hospital care and genetic consultations.

MGLs in Bulgaria are involved in performing and providing the following:

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- Mass and selective screening programmes
- Postnatal biochemical and DNA diagnosis
- Prenatal and pre-implantation biochemical and DNA diagnosis
- Regular follow-up and treatment control of patients identified by screening programmes
- Establishment of a national DNA bank
- Genetic consultation of patients identified by prevention programs
- Quality control of laboratory activities and results
- Study of the molecular basis and characteristics of the most common hereditary monogenic diseases in Bulgaria
- Establishment and maintenance of a computerized genetic registry.

The neonatal screening programme for congenital hypothyroidism was started in 1993 with the help of the Swiss government and was included in a National Programme for the period 2001-2005. This mass screening programme was carried out by an independent screening laboratory – the Endocrinology Laboratory at the University Hospital of Paediatrics in Sofia. Hypothyroidism screening was based on material used for neonatal screening of phenylketonuria [16].

Hereditary ovarian and familial colorectal carcinoma

The mutant genes BRACA 1 and BRACA 2 are responsible for 48% and 27% of the cases of ovarian Ca, respectively, and other genes are involved in 25% of these cases. Twenty-five per cent of the cases of ovarian Ca are considered to be due to a hereditary mutation, most frequently described as 1. hereditary breast-ovarian cancer syndrome, and 2. Lynch syndrome.

The hereditary breast-ovarian cancer syndrome is associated with a mutation of BRCA1 and BRCA2 (BRCA1/2) genes and is the most common cause of hereditary ovarian cancer, including fallopian tube carcinoma and primary peritoneal carcinoma. About 0.1-0.2% of the total population, carry the BRCA1 and BRCA2 mutation, whereas the incidence of this mutation is 15% in women with ovarian carcinoma. Its incidence is particularly high in some ethnic groups (Jewish women of the Ashkenazi family) where it can reach 30-40%. For this reason many professional organizations recommend genetic consultation for women with ovarian carcinoma; test sensitivity is 90%.

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Twelve genes have been studied which are associated with hereditary ovarian cancer, and some of them are considered to have bearing on hereditary breast cancer as well. It is advisable to study a panel of genes associated with the repair mechanism of the damaged DNA molecule (repair is inherent to normal cells). The genes in question are the following:

1. Category 1 – genes functionally related to BRCA 1 and BRCA 2 (ATM, BARD1, CHEK2, MRE11A, NBN, RAD50, RAD51D)
   • ATM (ataxia telangiectasia mutant)
   • BARD1 (BRCA1-associated RING domain 1)
   • CHEK2 (cell cycle checkpoint kinase 2)
   • MRE11A
   • NBN (nibrin; aka NBS1)
   • RAD50
   • RAD51D

2. Category 2 – other genes, characteristic of Fanconi's anaemia, which increase breast cancer risk (BRIP1, PALB2, RAD51C)
   • BRIP1 (BRCA-interacting protein C-terminal helicase 1; FANCJ)
   • PALB2 (partner and localizer of BRCA2; FANCN)
   • RAD51C (FANCO)

The risk for breast cancer is not completely clear in genes category 1 and 2.

3. Category 3 – genes responsible for hereditary colorectal carcinoma (MLH1, MSH2, MSH6, PMS2, EPCAM, MYH)

For women diagnosed with breast and ovarian cancer or a family history of breast and ovarian cancer, the National Comprehensive Cancer Network (NCCN) provides recommendations for genetic consultations and tests. The term *first-degree relatives* includes parents, siblings and children. The second-degree relatives include aunts, uncles, grandparents, grandchildren, nephews, nieces and half-siblings. Third-degree relatives include first cousins, great-grandparents and great-grandchildren.

Genetic testing is recommended if a person or a family meet at least one of the criteria mentioned below:

1. A member of the family has a certain mutation in the BRCA 1 or BRCA 2 genes, or other genes associated with a risk for breast cancer.

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2. Personal history of breast cancer plus one of the following:

- Diagnosis of triple negative breast cancer at age 60 or less
- Diagnosis of breast cancer at age 45 or less
- Diagnosis of a second breast cancer (in the same or the other breast)
- One or more first, second or third-degree relatives (on the side of one parent) diagnosed with breast cancer.

According to metaanalyses, the risk of developing a carcinoma in cases of BRCA1 and BRCA2 mutation is 40 and 20%, respectively. An area has been identified in the exon 11 of BRCA2 gene, associated with a particularly high carcinogenic risk, called ovarian cancer cluster region. The average age of ovarian carcinoma occurrence in carriers of BRCA1 mutation is 49-53, in carriers of BRCA2 mutation it is 55-58, versus 63 years of age for the general population. However, BRCA1 or BRCA2 positive carcinomas are most responsive to treatment with poly(ADP-ribose) polymerase (PARP) inhibitors.

Women carrying BRCA1/2 mutant genes should be identified because they are subject to screening (early diagnosis of carcinoma) or prevention before they have developed the cancer itself. It is controversial which of the two strategies is to be preferred. The National Comprehensive Cancer Network recommends a risk-reducing salpingo-oophorectomy at age 35-40, after which women do not usually plan to have children. If the woman foregoes surgery, she should be followed-up (should undergo screening) by means of transvaginal ultrasound and CA 125 tumour marker after age 30. In carriers of BRCA1/2 mutation, surgery reduces the risk of ovarian carcinoma by 80-95% and breast cancer by 50%. Surgical prevention has an advantage over screening – the latter may fail to detect an early stage carcinoma. Prevention can also be achieved by administration of oral contraceptives. They reduce the risk of ovarian carcinoma by 50%, but it is yet unclear to what extent they may increase the risk of breast cancer. A combination of data should be considered – age, family history, genetic testing.

Lynch Syndrome

This syndrome is the most common hereditary form of colorectal carcinoma representing 2-3% of the colorectal carcinomas. It is associated with a mutation of the genes responsible for the repair of the damaged DNA molecule - MLH1, MSH2, MSH6, PMS2. It

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is characterized by a comparatively early-onset colorectal, uterine or other carcinomas (e.g. gastric, intestinal, pancreatic, ovarian, urinary carcinoma, and rarely brain tumours).

3. Current practical situation in Genetic Testing Availability for HBOC and CRC in Bulgaria.

Currently, genetic tests for hereditary colon cancer, ovarian cancer and breast cancer in Bulgaria are available in a number of private laboratories and licensed intermediaries. Most of these tests are performed abroad and the time required to obtain results varies from several weeks to a few months. The setback is that not all patients receive genetic counseling from a physician specialized in medical genetics when their results are given. At present, research on hereditary colon cancer, ovarian cancer and breast cancer in Bulgaria is performed at the Center for Molecular Medicine, Sofia.

4. The situation of the current Research Programs on mutation screening and founder mutation detection for HBOC and CRC in Bulgaria.

At present, research on hereditary colon cancer, ovarian cancer and breast cancer in Bulgaria are performed at the Center for Molecular Medicine (CMM), *Maichin dom* University Hospital of Obstetrics and Gynaecology.

Basic structural units of the Center for Molecular Medicine (CMM) are the research groups - multidisciplinary teams from various departments of Medical University – Sofia, CMM, the National Genetic Laboratory and various national specialized clinical centres and hospitals working on specific collaborative projects with an access to common equipments and management. One of the main CMM research groups is the Oncogenetics Research Group. It studies the molecular basis of cancer in search of new diagnostic and prognostic markers [17-45]. Apart from that, the Oncogenetics Research Group studies the influence of the environmental factors and the way of life, their interaction with the genes responsible for cancer development. The group includes surgeons, pathologists, oncologists, chemo- and radiotherapists, molecular biologists and geneticists.

Research focuses on several tumour types:

- Colorectal carcinoma (CRC)
- Endometrial carcinoma (EC)

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- Breast cancer (BC)
- Prostate cancer (PC)
- Brain tumours (BT)
- Laryngeal carcinoma (LC)
- Chronic Obstructive Pulmonary Disease (COPD) and Pulmonary carcinoma (PC)
- Neuro-endocrine lung tumours

Currently activities are carried out within the framework of the following projects:

Colorectal Cancer (CRC)

For several years the Oncogenetics Research Group has been working on projects related to Hereditary Non-Polyposis Colorectal Cancer (HNPCC) syndrome that accounts for approximately 3% of all colorectal cancer cases.

The increased risk of developing cancer in HNPCC syndrome is nearly always due to an inherited mutation in a mismatch repair (MMR) gene. In HNPCC kindreds the most common cancers are colorectal and endometrial. The research group has established methodology for microsatellite instability (MSI) testing and screening for germline mutations in DNA mismatch-repair genes of patients with HNPCC. A National Registry for colorectal cancer was instituted in the *Tzaritza Yoanna* University Hospital, Sofia and more than 400 patients were selected for MSI and loss of heterozygosity (LOH) analysis. The MSI positive tumours were further analyzed for hMLH1 and hMSH2 mutations, hMLH1 promoter hypermethylation and MLH1 protein expression. A significant association has been found between the epigenetic change and MSI/LOH sporadic cases. Additionally, they have investigated the role of the hotspot V600E somatic mutation in BRAF gene and its application in the HNPCC screening selection criteria.

The mutation screening detected some known and two novel mutations, in both hMLH1 and hMSH2 genes. They have developed an efficient diagnostic strategy for HNPCC testing. The association between CRC and polymorphisms in estrogen receptor-alpha (ER-alpha), Vitamin D receptor (VDR) and CHEK2 genes has also been investigated. Screening for K-ras mutations has been performed. Recently MMC is expanding its abilities by the use of MLPA (Multiplex Ligation-dependent Probe Amplification) in search of large genomic deletions and duplications.

Breast Cancer (BC)

According to a recent survey of the National Oncological Center, in Bulgaria breast cancer is the most common female malignancy. Therefore, the research group has focused

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their research interest in studying the influence of mutations in genes BRCA1 and BRCA2 on development of BC in Bulgarian population. Recently the successful application of the microarray technology gave the opportunity to distinguish between different subtypes of cancer and define their specific expression profiles. Different mechanisms and pathways are related to the pathogenesis of BC, including DNA damage repair pathway; androgen and estrogen homeostasis, cell signalling and epigenetic regulation of gene expression. By focused analysis of key participants in these pathways, they expected to identify genomic and epigenetic alternations associated with these types of cancer in Bulgarian population. The elucidation of the impact of the individual genes/ variants included in this study and estimation of their cumulative risk for cancer development, aggressiveness and survival rate will assist the discovery of new diagnostic and prognostic biomarkers. Collaboration with the aim to study circular tumor cells (CTC) in patients with BC has been established. The main goal of the initiative is to define the relationship between the levels of CTC (determined by the levels of expression in the peripheral blood samples of three genes: hMAM, CK-19 and CD44) and the objective response, time to progression, survival rates and overall survival in patients with metastatic breast cancer using Real Time PCR.

The Oncogenetics Research Group has worked on a number of scientific projects which are as follows:

1. "Analysis of mutations in BRCA1 gene in Bulgarian patients with triple negative breast cancer", 2011-2012.
2. "Promoter hypermethylation in laryngeal carcinogenesis", 2011-2012.
3. "Genetic analysis of mutations in exon 11 of BRCA2 gene in Bulgarian patients with hereditary breast cancer", 2011-2012.
4. "Analysis of mutations in IDH1, IDH2 and TP53 genes and 1p/19q deletions in Bulgarian patients with malignant glial tumours", 2011-2012.
5. "Association study of polymorphic variants in locus 11p15 and their role in prostate carcinogenesis", 2011-2012.
6. "Follow up of circulating tumor cells (CTC) levels in patients with metastatic breast carcinoma - prognostic and predictive meaning", 2010-2012.
7. "Analysis of clinical and molecular characteristics of laryngeal squamous cell carcinoma and their application for prediction of prognosis and treatment response", 2010-2012.

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8. "Analysis of polymorphisms in low-penetrance genes associated with high risk of breast cancer ", 2010-2011.
9. "Genetic markers with prognostic value in high-grade gliomas", 2010-2011.
10. "Analysis of promoter hypermethylation of biomarkers in urine of patients with prostate cancer - validation of their diagnostic value in Bulgarian population", 2010-2011.
11. “Clinical and molecular markers with predictive value for survival, quality of life and treatment response in high-grade gliomas”, 2009-2011.
12. “Study of the genetic variations, associated with increased risk and identification of diagnostics and prognostics markers for prostate cancer”, 2009-2011.
13. “Analysis of big deletions, insertions and rearrangements in BRCA1 and BRCA2”, 2009-2010.
14. “Analysis of polymorphic variants in candidate genes associated with prostate cancer”, 2009-2010.
15. “Determination of mutation frequencies in MSH6 gene between patients with endometrial carcinoma which biopsy materials show inactivating mutations in KRAS gene”, 2009-2010.
16. “Squamous cell carcinoma of the larynx and the hypopharynx. Genetic defects associated with carcinogenesis and biological behaviour of the tumor”, 2009-2010.
17. “Molecular pathways involved in mammary gland cancer”, 2008-2010.
18. “Evaluation of the relevance of the CTNNB1 gene mutation analysis for patients with endometrial cancer”, 2008-2009.
19. “Analysis of big genome deletions, insertions and rearrangements in MLH1 and MLH2 genes in Bulgarian patients with colorectal cancer”, 2008-2009.
20. “Association study of polymorphic variants in regulatory genes of androgen biosynthesis (CYP1B1, CYP19 and SRD5A2) with increased risk for development of prostate cancer in Bulgaria”, 2008-2009.
21. “Association studies on the role of I157T polymorphism in CHEK2 gene for development of colorectal and endometrial cancer in Bulgarian patients”, 2007-2008.
22. “Mutations in BRCA1 and BRCA2 cancer susceptible genes and their association with the development of breast and prostate cancer in Bulgaria” 2007-2008.

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23. “Study on the Role of Polymorphisms in Genes XRCC1 and XRCC3 for Development of Early Radiation Reactions after Radiation Therapy of Cervical and Endometrial Carcinomas”, 2006-2007.
24. “Prognostic and Predicting Value of the Timidilat Sintetase Expression in Patients with Colorectal Cancer”, 2006-2007.
25. “Investigation Study of the molecular basis of the endometrial cancer in Bulgaria ”, 2005-2007.
26. “Study of the molecular genetic and clinical profiles of the hereditary and sporadic colorectal cancers in Bulgaria”, 2005-2006.
27. “Expression profiling of miRNAs in Bulgarian patients with prostate cancer and development of a panel of biomarkers for diagnosis and prognosis”, 2013-2015

Currently Department of Medical Genetics in Plovdiv carried out activities within the framework of the **Project No BG05M2OP001-1.002-0005-C 01 Center for Competence "Personalized Innovative Medicine (PERIMED)" Building a Competence Center under the "Science and Education for Intelligent Growth" Operational Program 2014-2020, with the financial support of the European Regional Development Fund.**

The project is in the field of oncology, genetics, personalized medicine and pharmacogenetics. The project will contribute to an earlier and more accurate diagnosis of cancer by minimal invasive approaches, will lead to accurate personalized therapy and prognosis, and monitoring for minimal residual disease for each individual patient. This project will contribute to the precise choice and dosing of the therapeutic agent used individually in each patient. Additionally, the frequency and type of the studied polymorphisms will be determined for the Bulgarian population. The study of minimal residual disease by digital droplet PCR as a criterion for the effect of treatment in cancer patients is extremely necessary in view of the current trends in predicting the individual risk of relapse and limiting the toxic effects of chemotherapy.

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5. List of institutions, cancer centers, etc. that provide genetic testing and counseling services in Bulgaria [46-57]

Genetic laboratories in Bulgaria, as well as the other laboratories, are two types: in hospitals or outpatient care facilities. The latter are mainly private genetic laboratories. The Genetic Laboratories, which are located in the University Hospitals in Varna, Pleven, Stara Zagora, Plovdiv and Sofia have been assigned under a specific regulation of the Ministry of Health to perform certain genetic studies. If a genetic test is not included in a clinical pathway or is not funded by other mechanisms, it should be paid by the patient at rates set by the laboratory. Prices of genetic research vary from a few hundred to several thousand levs. In fact, the lack of regulated funding often restricts access to genetic testing for those who need it.

The structures performing genetic counseling and / or diagnosis of oncological diseases in Bulgaria are the following:

A. **The *Maichin Dom* University Hospital of Obstetrics and Gynecology, National Genetics Laboratory (Clinical Genetics Laboratory)** is a center for diagnosis, prevention and control of the treatment of more than 120 rare genetic diseases and genetic predispositions in Bulgaria.

The National Genetic Lab is the coordinator and main contractor of the National Program for Rare Diseases, funded by the Ministry of Health (2009 - 2013). It maintains the National DNA Bank and the National Genetic Registry. Performs oncogenetic studies related to:
Treatment of colorectal carcinoma - KRAS and NRAS mutations in tumor tissue;
Treatment of Non-Small Cell Carcinoma of the Lung - ALK Mutations in Tumor Tissue;
Treatment of Melanoma - BRAF Mutations in Tumor Tissue.

B. National Specialized Hospital for Active Treatment of Hematological Diseases - EAD, Sofia, Laboratory of Cytogenetics and Molecular Genetics

The laboratory is the only one in the country to provide a comprehensive, highly specialized diagnosis of the various chromosomal and molecular disorders in patients with malignant blood disorders, using different, complementary methods: direct and indirect chromosomal band analysis, qualitative and quantitative polymerase reaction (PCR) and fluorescence hybridization (FISH). The presence of modern equipment and trained staff with years of experience in genetic diagnostics in oncohematology is a prerequisite for the

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laboratory to be a leading structure where genetic testing of patients from all over the country is concentrated. The results of the Complex Genetic Diagnosis of Chromosomal and Molecular Disorders conducted in the Laboratory, according to the latest revision of the WHO Classification of Hematopoietic and Lymphoid Tumors of the World Health Organization (2008), are crucial to confirm the diagnosis of the disease, its precise subclassification , determination of prognosis and risk selection of adapted therapeutic protocols as well as follow-up of treatment performance and control of "minimal residual disease" with high sensitivity.

C. Laboratory of Genomic Diagnostics, Center of Molecular Medicine, Department of Medical Chemistry and Biochemistry, Medical Faculty, Medical University - Sofia

Genetic and genomic diagnostic tests, genetic diagnosis, carrier assignment and risk assessment for affected individuals and other family members with breast, ovarian, colon, lung, and other type of cancers; Prognostic and predictive biomarkers for oncological diseases; Pharmacogenetic studies.

D. *St. George* University General Hospital - Plovdiv

The Department of Medical Genetics (DMG) is a specialized unit in which genetic counseling and diagnosis are provided and genetic research is carried out in the field of cytogenetics, molecular cytogenetics and biochemical genetics. The highly specialized activities involve patients with polymalformative syndromes, pregnant women, people with reproductive problems, oncohematological patients. Collection of genetic material is provided, as well as diagnosis of gene-related disorders associated with oncologic disorders.

E. *St. Marina* University General Hospital EAD Varna

The Laboratory of Medical Genetics (LMG) is a genetic center that performs highly specialized activities and is an essential element of medical (clinical) genetics. It performs hospital and mainly pre-hospital genetic support and is the only unit to provide this type of care for the population of North-East Bulgaria, covering 23% of the total population of the country. It includes two sectors: a) laboratory genetics, which offers diagnostic genetic studies (at the cellular, subcellular and molecular level); and b) genetic counseling with registration and dispensarisation. The main activity of the genetic structure can be defined as "integrated

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clinical and laboratory services for disorders with significant genetic component (inherited or sporadic)".

LMG is actively involved in the implementation of National Program for Service of Oncohematological Patients.

F. "Dr. Georgi Stranski" EAD, Pleven

Laboratory of Medical Genetics

The Laboratory of Medical Genetics is a highly specialized diagnostic and consulting unit, performing hospital and pre-hospital clinical and laboratory diagnostics as well as genetic counseling of patients with congenital and hereditary diseases, families with reproductive failure, pregnant women and others.

The Laboratory of Medical Genetics provides:

- Cytogenetic diagnosis of specific chromosomal aberrations in bone marrow in patients with oncohematological diseases;
- Obtaining and sending genetic material and providing molecular-genetic diagnosis of genetic disorders

G. *Genika* Genetic Medical Diagnostic Laboratory Ltd.

Performs DNA and RNA diagnostics of oncological diseases:

Colorectal carcinoma - KRAS & NRAS status in target therapy, Melanoma, Astrocytoma, Hirschsprung disease, Glioblastoma, Hereditary non-colorectal / colorectal carcinoma (Lynch syndrome), Oligodendroglioma, Papillary renal carcinoma, Papillary thyroid carcinoma, Peutz-Jeghers Syndrome, Sipple Syndrome, Familial Adenomatous Polyposis (Gardner's Syndrome).

H. NUTRIGEN LTD

Performs DNA diagnosis of hereditary oncologic diseases: Hereditary Breast Cancer and Ovarian Cancer; Familial adenomatouspoliposis; Multiple endocrine neoplasia; Hereditary nonpolyposis colorectal carcinoma (Lynch syndrome); Li-Fraumen syndrome; Kauden's syndrome; Piotz-Jaegers syndrome; Von Hipel-Lindau disease; Neurofibromatosis type 1 and 2; Tuberous sclerosis type 1 and 2; Xeroderma pigmetnosum; Fanconi anemia; Malignant haematological diseases.

I. NM Genomics

The company's activity is focused on integrating the Bulgarian market with world-class genetic research in the field of prenatal follow-up, follow-up studies and evaluation of the effectiveness of therapy in patients with oncological diseases according to the individual

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profile of the disease as well as psychiatric studies on the basis of which physicians can choose the most appropriate therapy and optimize the regimens of patients with mental disorders.

J. *Nadezhda* Hospital, Sofia

MY BRCA

Research genes - BRCA1, BRCA2

It takes 3 to 4 weeks to receive the results from the Veritas Genetics Laboratory, Boston, USA.

MY BRCA HIRISK

Analysis of 26 genes * (including BRCA genes 1 and 2) associated with a high risk of developing breast cancer, ovarian cancer and other inherited oncological diseases.

Private genetic laboratories in the country offer a wide range of oncogenetic tests, but a great part these tests are performed abroad, and the time required to obtain results ranges from weeks to months and not always genetic counseling is provided when the patients receive their results.

6. The situation of Education Programs regarding Oncogenetics:

6.1. The only educational program regarding Oncogenetics in Bulgaria is "The Multidisciplinary Oncology Talks and Extracts" (in bulgarian the abbreviation is **MORE**) [58]. This multidisciplinary conference is organized by the Bulgarian Oncological Scientific Society. It takes place every year and is designed /a kind of educational course/ for pathologists, molecular biologists, medical geneticists, medical oncologists, urologists, radiation therapists, radiologists, radiologists, clinical lab doctors, medical oncologists and all other medical specialists involved in the diagnosis, treatment and prevention of cancer.

On this conference the National Expert Board (a multidisciplinary team including experts from several medical specialties and their national branch organizations) discusses and accepts the National Guide for clinical practice for cancer patients. This document is based on evidence-based clinical practice guideline. It provides advanced algorithms for molecular diagnostic testing and clinical use of validated predictive biomarkers for the treatment of malignant solid tumors and haematological neoplasias according to their localization.

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6.2 Postgraduated course for medical doctors in medical genetic

The duration of the specialty training for graduate doctors (with no specialty) is four years, including teaching, placements and leave. During the education there is a module "oncogenetics", including :

- knowledge of the basic molecular mechanisms, diagnostic options, international guidelines in clinical care.
- ability to construct and analyse pedigree,
- knowledge of the principles and methods for obtaining diagnostic information;
- ability to plan and coordinate diagnostic tests ;
- ability to talk to families taking due account of psychological and social barriers and other features of such discussions, as well as the principles of non-directive counselling;
- ability to formulate risks and explain them comprehensibly on the basis of the assessment, diagnosis, knowledge of the nature of the disease and other relevant factors;
- provide genetic counselling, in particular concerning the disease's characteristics, the possibilities and limitations of treatment and rehabilitation, and preventive-diagnosis procedures for potentially affected family members;
- ability to provide and/or supervise prospective care for the affected family in matters pertaining to the genetic nature of the disease;
- ability to keep genetic counselling records in accordance with professional standards and applicable law;
- ability to interpret screening results for the purposes of: genetic counselling, identifying ability to interpret legal provisions on the diagnosis of cancer and genetic counselling; knowledge of legal provisions and welfare arrangements affecting the quality of life of cancer people and their families

State Specialty Examination: Speciality training in clinical genetics ends with the sitting of the theoretical and practical parts of State Specialty Examination.

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6.3 Postgraduated course for medical doctors in medical oncology.

The duration of the specialty training for graduate doctors (with no specialty) is five years, including teaching, placements and leave.

After completing the speciality training, a doctor should be able to demonstrate the following skills concerning oncogenetics:

- knowledge of the biology of malignant neoplasms, the principles of the therapy, the accepted therapeutic algorithm and the interpretation of the clinical research.
- knowledge of what genetic markers are appropriate for determining the stage of the disease (staging and therapeutic follow – up effect) and the benefit and restrictions on use of such markers.

7. The identification of the practical needs (infrastructure, human, and financial resources) in order to establish and to develop an Oncogenetic Network at the level of each country involved as partner in HOPE project.

In Bulgaria there is need of oncogenetic centers (situated in the medical universities) regarding care for cancer patients. In these centers a multidisciplinary team will be available to the cancer patients. These centers will organize training programs for specialists dealing with cancer patients, also for patients and their families at risk for hereditary cancer and for General Public.

7.1 Requirements for carrying out the activity in oncogenetic lab.

Genetic studies with diagnostic, prognostic, preventive and predictive value are highly specialized studies and are not performed in primary outpatient medical care.

7.1.1.Requirements for carrying out the activity in specialized oncogenetic tests

7.1.2.Requirements for the structure of the activity.

7.1.2.1. Equipment and equipment of the Cabinet for conducting medical-genetic counseling.

Required minimum equipment:

-Doctor’s desk, chair;

-Suitable medical document cabinet;

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-Coach for consultants;

-Sink with running hot and cold water;

-Telephone, computer and printer with internet access.

7.1.2.2. Equipment for laboratories. At least the following functionally separate work and service spaces are required:

1. A waiting room with access to a bathroom;

2. Premises for sample registration, archiving and storage of results equipped with desks, computers with Internet access, cabinets;

3. Premises for preparation, processing and analysis of biological samples with equipment and furnishing corresponding to the type of activity performed;

4. Warehouses;

7.1.2.3. Requirements for provision of medical equipment, equipment and other conditions for carrying out the activity:

When conducting molecular oncogenetic diagnostics, the laboratory must have the following equipment:

1. Thermostat, 37 ° -50 ° C;

2. PCR for the replication of DNA/RNA;

3. Apparatus for electrophoretic separation of nucleic acids;

4. Centrifuge with flying rotor for volumes up to 25 ml up to 3000 g / min;

5. Refrigerator at 4 ° C with chamber at -20 ° C;

6. Tabletop high-speed centrifuge with volumes of 0.2 to 2 ml up to 14000 g / min;

7. Spectrophotometer for DNA/ RNA / protein measurement;

8. Computer with a printer;

9. Sequencer with specialized software for fragment analysis and sequencing

10. Real-time PCR apparatus;

11. ddPCR apparatus;

12. Laminar Boxing/UV box;

13. Freezer for bio-banking;

14. Adjustable micropipettes, multi-channel micropipettes, adjustable volume dispensers

7.1.3. Requirements for the personnel carrying out the professional activity in the oncogenetic lab are as follows:

Total number of doctors (Specialists of Medical Genetics) – 2-3

Other specialists / persons (in the laboratory or in the medical facility) - 2 (biologist /

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chemist), 1 midwife / nurse / laboratory, 1 cleaner.

7.1.4. Financial resources

Financial needs estimate:

- Equipment: EUR 500.000-600.000
- Reagents: EUR 1000-3000 /index case sample and EUR 100 / mutation verification in relatives sample

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