MEDICAL UNIVERSITY
”PROF.DR. PARASLEV STOYANOV”-VARNA
FACULTY OF MEDICINE
DEPARTMENT OF ONCOLOGY

DESSERTATION SUMMARY

For awarding the educational and scientific degree “PhD”

of

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PREVALENCE OF PIK3CA-MUTATIONS AND RESPONSE TO FIRST-LINE ENDOCRINE THERAPY IN A POPULATION OF BULGARIAN PATIENTS WITH HR(+) HER2(-) MATASTATIC BREAST CANCER

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The dissertation is presented in volume of 115 pages and is illustrated with 6 tables and 29 figures. The literature review contains 266 titles, all in Latin.

The dissertation has been discussed, approved and forwarded the public thesis defense at meeting of the Departmental Council of the Department of Oncology at the Faculty of Medicine on 08.01.2026 at the Medical University –Varna and according to the order of the Rector of MU Varna, a scientific jury has been selected consisting of:

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Dr. Mila Petrova, MD, to my colleagues from MHAT"Nadezhda" and from the Oncology Clinic at UMHAT"St. Marina" and to my family.

The public thesis defense will take place on May 12, 2026, in electronic environment during an open session of the Scientific Jury.
The materials are available at the library of the Medical University-Varna and on the University's website (mu-varna.bg).

CONTENTS

Used abbreviations:

AKT1 - protein kinase B
AJCC - American Joint Committee on Cancer
ATM - Ataxia-telangiectasia mutated
BRCA1/2 - Breast Cancer gene 1/2
CDH1 - Cadherin-1
CDK4/6 - Cyclin-dependent Kinase 4/6
CHEK - checkpoint kinase
DCIS - Ductal carcinoma in-situ
DFS - Disease Free Survival
DNA - Deoxyribonucleic Acid
ECOG PS – Eastern Cooperative Oncology Group Performance Status
ESR1 - Estrogen Receptor 1
FFPE - Formalin-fixed paraffin-embedded
GLOBOCAN - Global Cancer Statistics
HER2 - Human Epidermal Receptor
HR - Hormone Receptor
HR - Hazard ratios
mTOR - Mammalian target of rapamycin
NGS - Next generation sequencing
NF1- Neurofibromin
OS - Overall Survival
PALB2 - Partner and localizer of the BRCA2 gene
PARP - Poly (ADP-ribose) Polymerase
PIK3CA - Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PFS - progression free survival
PTEN - Phosphatase and Tensin Homolog
RB1 - retinoblastoma protein
RECIST – response evaluation criteria in solid tumours
RT-PCR - reverse transcription polymerase chain reaction
STK11 - Serine/Threoni ne Kinase 11
TCGA -The Cancer Genome Atlas-USA
TNM - Tumor, Node, Metastasis
TP53 - Tumor protein p53

I. INTRODUCTION

Breast cancer is one of the most common oncological diseases among women worldwide (24.5% of all cancers according to GLOBOCAN data for 2020) which also makes it a socially significant disease. It is diagnosed more frequently at an early stage due to improved diagnostics and organized screening programs among high-risk population groups. In some countries, entire families are included, whose members carry pathological mutations known to increase the risk of developing neoplasms much more than individuals in general population. The most common mutations in this group are BRCA1/2, CHEK, PALB2, PTEN, TP53, STK11, NF1, ATM, CDH1.

Breast carcinoma is a heterogeneous disease, which at present can be divided into several main groups based on the status of hormone receptors and the expression of the HER2 receptor. Additionally, to determine the tumor's biology, its aggressiveness, and response to applied therapies, important roles are played by the histological variant, the degree of differentiation, and the presence of pathogenic mutations (*BRCA1/2, PIK3CA, ESR, RB1*).

Despite improved diagnosis and treatment of this socially significant disease, there are still cases in which the disease is diagnosed at a metastatic stage. The goal for these patients is to extend life while maintaining a good quality of life. Contemporary treatment of metastatic breast cancer aims to use drug molecules that target specific activated pathways or disrupted regulatory mechanisms in malignant cells, so that the applied treatment achieves the maximum therapeutic effect combined with minimal side effects. Such targets include the presence of positive hormone receptors and use of endocrine therapy +/- CDK4/6 inhibitors; in cases of confirmed HER2-positive status – anti HER2 therapy; the presence of BRCA1/2 mutations and, accordingly, the use of PARP inhibitors; activation of the mTOR pathway and the use of mTOR inhibitors; and in the case of PI3K/AKT/mTOR pathway activation – AKT inhibitors, which are still under development.

PI3K inhibitors have recently entered our routine practice. The presence of a *PIK3CA* mutation is considered not only a predictive but also prognostic factor in patients with HR(+), HER2(-) metastatic breast cancer. Detecting it among this patient population would guide us regarding the therapeutic approach to take, as well as potentially predict the response to the ongoing therapy. These patients often develop resistance to the endocrine therapy being administered. Therefore, the *PIK3CA* mutation status should be routinely tested as early as possible after diagnosis of HR(+), HER2(-) breast cancer in metastatic stage.

II. AIM AND OBJECTIVES

1. AIM

The aim of the dissertation is to determine the frequency of the *PIK3CA* mutation in the population of Bulgarian patients with hormone-positive, HER2-negative metastatic breast cancer and to compare it with its prevalence worldwide, as well as to investigate and compare PFS and OS according to the *PIK3CA* mutation status of patients treated with first-line ET, either as monotherapy or in combination with a CDK4/6 inhibitor. An additional goal is to establish the relationship between the main clinicopathological characteristics and *PIK3CA* status.

2. OBJECTIVES

2.1 Selection of patients in the metastatic stage with hormone-positive HER2-negative breast.

2.2 To determine the frequency of the *PIK3CA* mutation among the selected patients by examining tumor tissue from the primary tumor or metastasis. Comparison of the results with those from The Cancer Genome Atlas-USA database.

2.3 To investigate the correlation between mutation status and clinicopathological characteristics such as age, menopausal status, type of metastases, type of adjuvant ET, and type of research material.

2.4 To analyze the relationship between the presence of a *PIK3CA* mutation and the diagnosis of patients at a more advanced stage.

2.5 To determine whether there is connection between the presence of *PIK3CA* mutation and the development of primary endocrine resistence to the applied endocrine therapy as first-line treatment for metastatic disease

2.6 To analyze whether the presence of *PIK3CA* mutation affects the effectiveness of endocrine therapy used as first-line treatment and whether it is related to overall survival.

III. MATERIALS AND METHODS

1. MATERIALS

1.1 Material Base for Conducting the Dissertation

The selection of patients for the dissertation was carried out in tree Oncology Clinics in Bulgaria:

- MHAT"Nadezhda" Sofia
- UMHAT"St.Marina"Varna
- Acibaden City Clinc UMHAT Tokuda Sofia

The preparation of histological specimens for analysis was carried out in the Department of General and Clinical Pathology at USBALO Sofia and in the Department of Clinical Pathology at Acibadem Sity Clinic Tokuda Hospital Sofia.

The genetic analysis was conducted in the Genetics Laboratory at Nadezhda Hospital.

1.2 Patient population

A retrospective, multicenter study was conducted during the period from January 2016 to November 2022. A total of 250 patients with de novo metastatic hormone receptor-positive, HER2 negative breast cancer were included, as well as those who progressed after initially being diagnosed at an early stage of the disease. All patients were treated with first-line endocrine therapy. The distribution by centers was as follows-141 patients from MHAT"Nadezhda" Sofia, 51 patients from UMHAT"St. Marina" Varna, and 58 patients from Acibadem City Clinic Tokuda Hospital Sofia. All patients underwent initial staging with imaging-CT+/-Bone scan or PET/CT.

2. METHODS

2.1 Inclusion Criteria

- Age > 18
- ECOG PS ≤ 2 according AJCC, over 90% of patients was in performance status 0 or 1.
- Histologically confirmed HR(+), HER2(-) metastatic or locally advanced inoperable breast
- Signed informed consent
- Availability of a histological specimen with sufficient tumor tissue for conducting genetic analysis with RT-PCR.
- Initiated therapy for metastatic disease – chemotherapy or endocrine therapy
- Eligible patients were also those who received therapy in an adjuvant or neoadjuvant setting – chemotherapy, radiotherapy, and endocrine therapy.

2.2 Exclusion Criteria

- Age < 18
- ECOG PS >2 according to AJCC
- Patients who have not signed informed consent
- Patients who have not received treatment for metastatic disease

2.3 Demographic and clinical data of the patients included in the medical history

2.3.1 Demographic data

- Names
- Date of birth
- Gender
- Age
- Age at diagnosis
- Date of death, if the patient has died

2.3.2 Clinical data

- Medical history
- Menopausal status
- Comorbidities
- ECOG PS

2.3.3 Medical history of the oncology disease:

- Disease stage at diagnosis – TNM
- Tumor grade (G), Ki-67 value
- Immunohistochemical assessment of the tumor
- Imaging used for staging and restaging – CT, bone scan, PET/CT – evaluation according to RECIST 1.1
- Duration and type of adjuvant endocrine therapy
- Interval between completion of adjuvant ET and disease progression
- Use of adjuvant/ neoadjuvant chemotherapy
- Sites of the metastasis
- Date of initiation of first-line therapy for metastatic disease
- Type, number, and duration of therapies administered for metastatic disease
- Disease-free survival (DFS)
- Progression-free survival (PFS)
- Overall survival (OS)

2.4 Staging of the disease – TNM

The staging of the patients' oncological disease was conducted according to TNM – classification. The classification is developed and mainted by the Union for

International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC). The current version we use for staging solid tumors is the eighth edition from 2017. (Tab.1, 2, 3 and 4)

Table 1 - TNM classification, eighth edition, 2017

	T – primary tumor
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis - DCIS	Ductal carcinoma in situ
Tis - Paget	Paget disease of the nipple NOT associated with invasive carcinoma and/or carcinoma in situ (DCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget disease are categorized on the basis of the size and characteristics of the parenchymal disease, although the presence of Paget disease should still be noted
T1	Tumor ≤ 20 mm in greatest dimension
T1mi	Tumor ≤ 1 mm in greatest dimension
T1a	Tumor > 1 mm but ≤ 5 mm in greatest dimension (round any measurement >1.0-1.9 mm to 2 mm)
T1b	Tumor > 5 mm but ≤ 10 mm in greatest dimension
T1c	Tumor > 10 mm but ≤ 20 mm in greatest dimension
T2	Tumor > 20 mm but ≤ 50 mm in greatest dimension
T3	Tumor > 50 mm in greatest dimension
T4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules), not including invasion of dermis alone
T4a	Extension to chest wall, not including only pectoralis muscle adherence/invasion
T4b	Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma

Table 2 - TNM classification, eighth edition, 2017

	N – regional lymph nodes
NX	Regional lymph nodes cannot be assessed (for example, previously removed, or not removed for pathologic study)

N0		No regional lymph node metastasis identified histologically, or isolated tumor cell clusters (ITCs) only. *Note:* ITCs are defined as small clusters of cells ≤ 0.2 mm, or single tumor cells, or a cluster of < 200 cells in a single histologic cross-section; ITCs may be detected by routine histology or by immunohistochemical (IHC) methods; nodes containing only ITCs are excluded from the total positive node count for purposes of N classification but should be included in the total number of nodes evaluated
	N0(i+)	ITCs only in regional lymph node(s)
	N0(mol+)	Positive molecular findings by RT-PCR; no ITCs detected
N1		Micrometastases; or metastases in 1-3 axillary lymph nodes and/or in internal mammary nodes; and/or in clinically negative internal mammary nodes with micrometastases or macrometastases by sentinel lymph node biopsy
	N1mi	Micrometastases (200 cells, > 0.2 mm but none > 2.0 mm)
	N1a	Metastases in 1-3 axillary lymph nodes (at least 1 metastasis > 2.0 mm)
	N1b	Metastases in ipsilateral internal mammary lymph nodes, excluding ITCs, detected by sentinel lymph node biopsy
	N1c	Metastases in 1-3 axillary lymph nodes and in internal mammary sentinel nodes (ie, pN1a and pN1b combined)
N2		Metastases in 4-9 axillary lymph nodes; or positive ipsilateral internal mammary lymph nodes by imaging in the absence of axillary lymph node metastases
	N2a	Metastases in 4-9 axillary lymph nodes (at least 1 tumor deposit > 2.0 mm)
	N2b	Clinically detected* metastases in internal mammary lymph nodes with or without microscopic confirmation; with pathologically negative axillary lymph nodes
N3		Metastases in ≥ 10 axillary lymph nodes; *or* in infraclavicular (level III axillary) lymph nodes; or positive ipsilateral internal mammary lymph nodes by imaging in the presence of one or more positive level I, II axillary lymph nodes; *or* in > 3 axillary lymph nodes and micrometastases or macrometastases by sentinel lymph node biopsy in clinically negative ipsilateral internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes
	pN3a	Metastases in ≥ 10 axillary lymph nodes (at least 1 tumor deposit > 2.0 mm); *or* metastases to the infraclavicular (level III axillary lymph) nodes
	pN3b	pN1a or pN2a in the presence of cN2b (positive internal mammary nodes by imaging) *or* pN2a in the presence of pN1b

pN3c	Metastases in ipsilateral supraclavicular lymph nodes

Table 3 - TNM classification, eighth edition, 2017

	M– distant metastasis
M0	No clinical or radiographic evidence of distant metastasis
cM0(i+)	No clinical or radiographic evidence of distant metastases in the presence of tumor cells or deposits no larger than 0.2 mm detected microscopically or by molecular techniques in circulating blood, bone marrow, or other nonregional nodal tissue in a patient without symptoms or signs of metastase
cM1	Distant metastases detected by clinical and radiographic means
pM1	Any histologically proven metastases in distant organs; or if in non-regional nodes, metastases > 0.2 mm

Table 4 - TNM classification, eighth edition, 2017

Стадий	**T**	**N**	**M**
0	Tis	N0	M0
I A	T1	N0	M0
I B	T0	N1mi	M0
	T1	N1mi	M0
II A	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
II B	T2	N1	M0
	T3	N0	M0
III A	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
III B	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
III C	Any T	N3	M0
IV	Any T	Any N	M1

2.5 Imaging diagnostics and assessment of therapeutic response

All patients underwent initial staging with chest and abdominal computed tomography +/- bone scan or PET/CT, followed by restaging every 4-6 months. The therapeutic response was assessed according to RECIST 1.1 criteria, which can be complete response, partial response, disease progression, or stable disease.

- Complete response – disappearance of all lesions, pathological lymph nodes should be reduced in size to < 10 mm in short diameter, and there should be no new lesions.
- Partial response – Reduction of at least 30% in the sum of the longest diameters of the target lesions compered to baseline.
- Disease progression – appearance of new lesion or at least a 20% and 5 mm absolute increase in the sum of the longest diameters of the target lesions compared to the smallest sum ever measured in the patient.
- Stable disease – does not meet the criteria for partial response or disease progression.

2.6 Genetic and pathomorphological analysis

To the project, 250 paraffin blocks provided by the patients participating in the project were examined. The histological specimens were from the primary tumor or from metastasis.

The first stage of specimen processing involved micrprodissection of the formalin-fixed specimens and preparation of 5 micrometer thick sections, followed by standard hematoxylin and eosin staining to identify the areas with the highest concentration of tumor cells. The second stage of study included genetic analysis. DNA was isolated from the microdissected specimens using the QIAamp® DSP DNA FFPE tissue kit. The QIAamp DSP DNA FFPE kit uses the proven QIAamp MinElute technology for purifying genomic DNA from formalin-fixed (FFPE) tissues.

The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes ranging from 20 to 200µl. Special lysis conditions are used to release DNA from tissue sections and to overcome inhibitory effects caused by formalin cross-linking of nucleic acids. The procedure consists of 6 steps – deparaffinization, lysis, heating, binding, washing, and elution. The paraffin is dissolved in xylene and removed. The sample is lysed under denaturing conditions with proteinase K digestion. Incubation at 90 °C reverses formalin cross-linking, after which the DNA binds to the membrane and contaminants are washed away. DNA was eluted in ATE buffer and was immediately ready for use in amplification reactions, for which real-time qPCR analysis was used to look for 11 mutations in the *PIK3CA* gene, specifically in exons 7, 9 and 20. The investigated mutations were - p.C420R (екзон7), p.E542K, p.E545A, p.E545D [c.1635G > само T], p.E545G, p.E545K, p.Q546E и p.Q546R (exon 9) and p.H1047L, p.H1047R and p.H1047Y (exon 20). The QMDx 5plex HRM rotary gene was used to automate the detection and amplification process.

2.7 Statistical analysis

2.7.1 The demographic data were presented using descriptive statistical methods – frequencies, percentages, medians, means, and standard deviation.

2.7.2 The associations between the presence of *PIK3CA* mutations and various clinic-pathological parameters of the patients were assessed using the Mann–Whitney U – test and the χ^2-test (Chi-square test). The Mann–Whitney U – test is non-parametric test used to compare two independent groups when the distribution is not normal, while the χ^2-test used to examine the relationship between two qualitative variables.

2.7.3 The survival curves were constructed using the Kaplan–Meier method, and the differences were assessed using the log-rank test. This is a non-parametric test used to compare survival in two or more groups.

2.7.4 Propensity matching score analysis was used to match the two groups being compared when calculating progression-free survival. It aims to reduce the bias in the selection of patients.

2.7.5 For the calculation of HR (Hazard ratios) and 95% CI (confidence intervals), a Cox proportional-hazards regression model was used. This is regression model used to study the relationship between patients' survival time and one or more predictor variables. The p - value indicates whether the null hypothesis is true. A p - value less then 0,05 is considered statistically significant. The data were sorted and analyzed usining SPSS version 23 and EZR.

IV. RESULTS

The study examined 250 patients with metastatic HR(+)/HER2(–) breast cancer, diagnosed and treated between January 2016 and November 2022 at three hospitals in Bulgaria (MHAT “Nadezhda”- Sofia, UMHAT”St. Marina - Varna, MHAT Tokuda – Sofia). The data analysis was retrospective. All patients provided histological samples for examination after signing informed consent. Some of the patients were diagnosed at the metastatic stage, while the others were diagnosed at early stage and later progressed. All patients received first-line therapy

1. Characteristics of the patients included in the study – Table 5

Characteristic	Number	Percent
Number of patients	250	100%
Sex		
men	1	0.4%
women	249	99.6%
Metastatic sites		
Visceral	147	58.8%
Bone-only	103	41.2%
Stage at the initial diagnosis		
Metastatic	117	46.8%
Nonmetastatic	133	53.2%
Sample used for detection of PIK3CA mutation		
Archival tissue	202	80.8%
Most recent biopsy tissue	48	19.2%
CT as adjuvant/neoadjuvant therapy		
Yes	108	43.2%
No	142	56.8%
Type of adjuvant ET		
Tamoxifen	42	34.7%
AI	73	60.3%
Tamoxifen switch to	6	5%
Duration of adjuvant ET		
Progression less than 2 years since the beginning of ET	42	34.7%
Progression between 2 and 5 years since the beginning of ET	33	27.3%
Progression less than 1 year after the end of adjuvant therapy	11	9.1%
More than 1 year after the end of adjuvant therapy	35	28.9%

Table 6 Clinical and pathological data of the patients

The gender distribution favors women, as of the 250 patients included, only one is male, the rest are female (Fig.13)

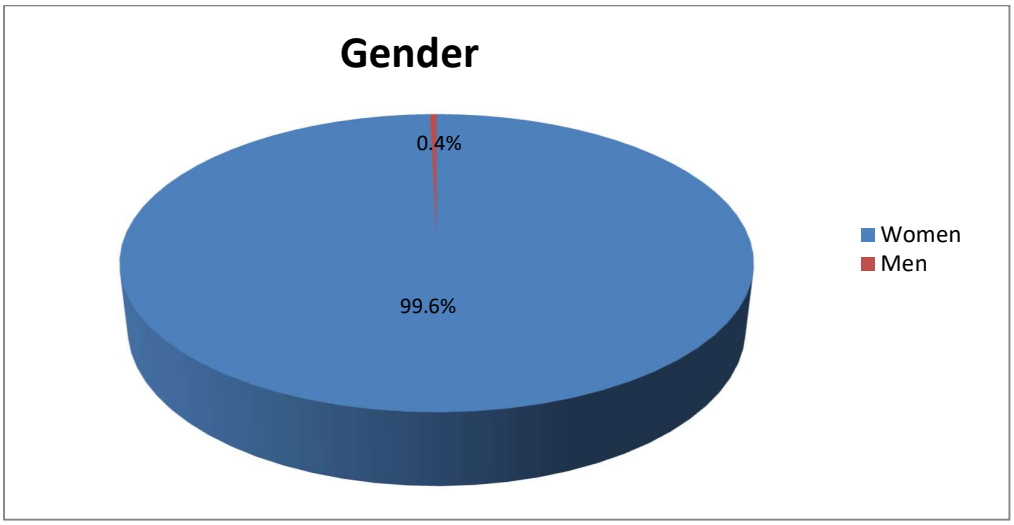


Fig.13 Distribution of the patients by gender

According to the site of metastasis, patients with visceral metastases are predominant, accounting for 58.8% (N=147) compared to those with only bone metastases - 41.2% (N=103). (Fig.14)

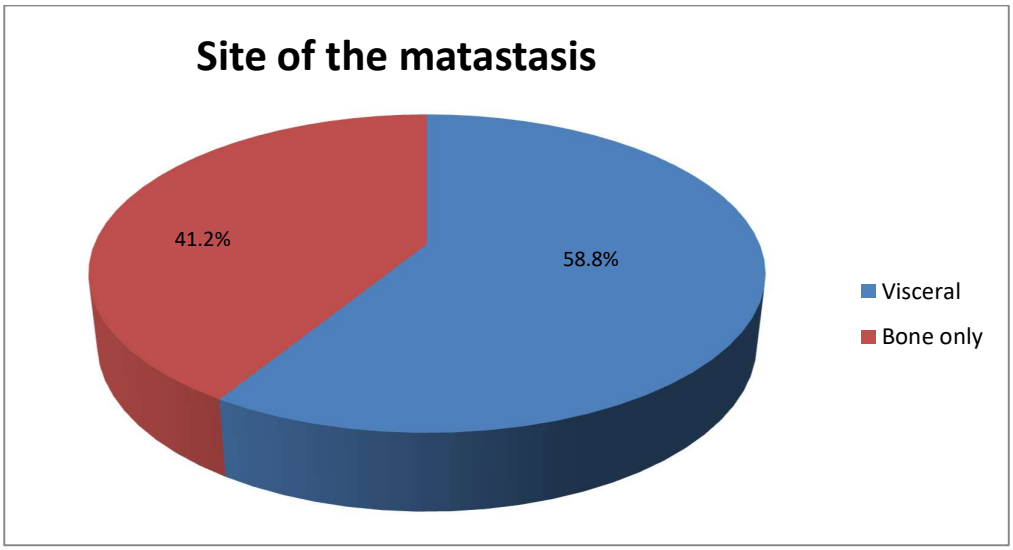


Fig.14 Distribution of the patients by site of metastasis.

The study included only patients in stage IV, but dame of them were in a localized stage at the diagnosis and later progressed with metastases. Of all patients, 46.8% (N=117) had de novo metastatic disease, while 53.2 % (N=133) were initially diagnosed at an early stage and later developed metastases. (Fig.15)

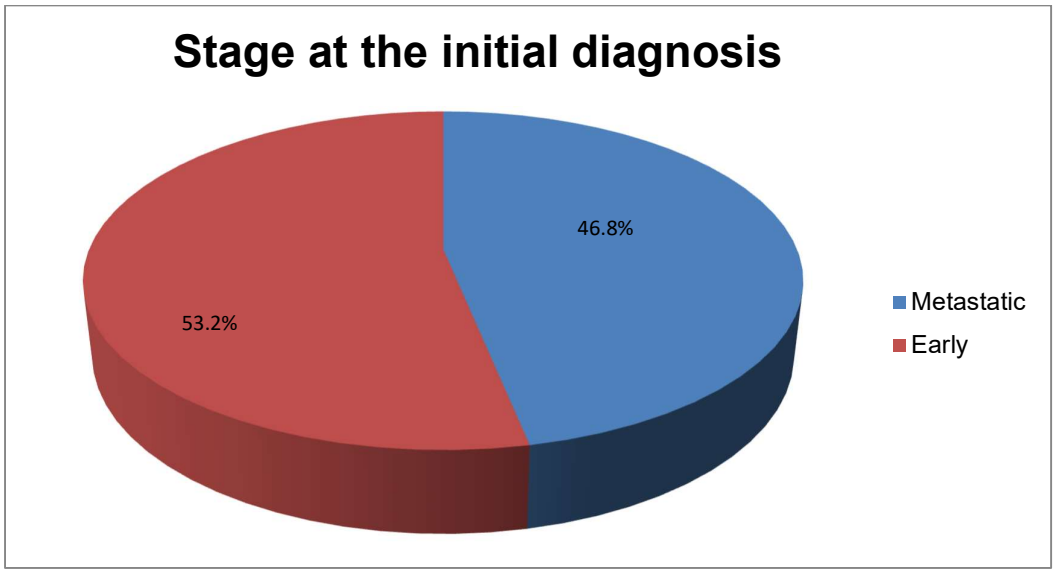


Fig.15 Stage at the initial diagnoses

The histological specimens used were 80.8% (N=202) of archival tissue and 19.2% (N=48) recently taken biopsy. (Tab.6)

According to whether the patients received adjuvant/neoadjuvant chemotherapy, the group that did not receive chemotherapy predominates accounting for 56.8% (N=142), while 43.2% (N=108) received. (Fig.16)

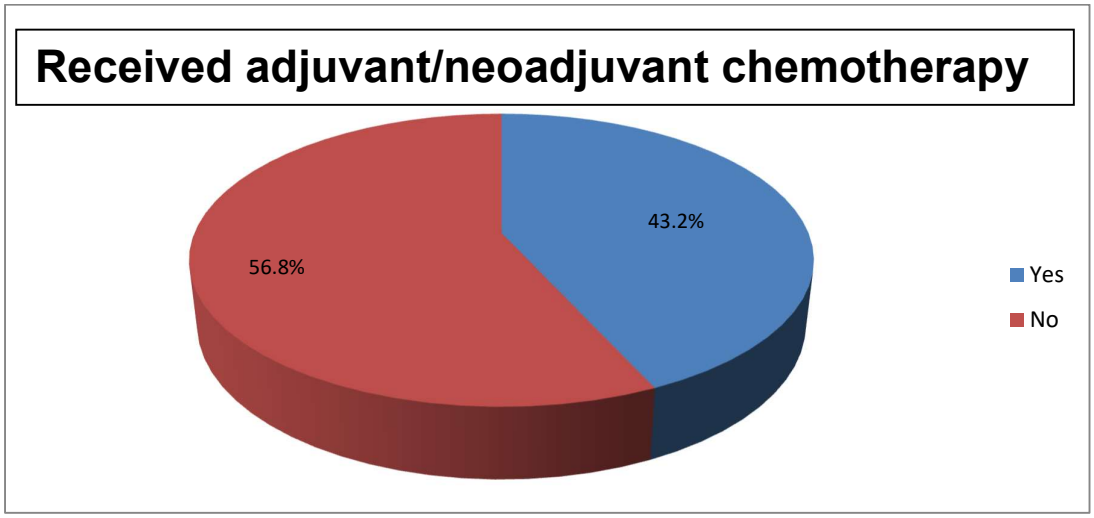


Fig. 16 Distribution of the patients received adjuvant/neoadjuvant chemotherapy.

According of the type of received adjuvant endocrine therapy, patients are divided into three groups – those treated with an aromatase inhibitor 60.3% (N=73), with tamoxifen 34.7% (N=42) and those who received sequential therapy with an aromatase inhibitor and tamoxifen 5% (N=6). (Fig.17)

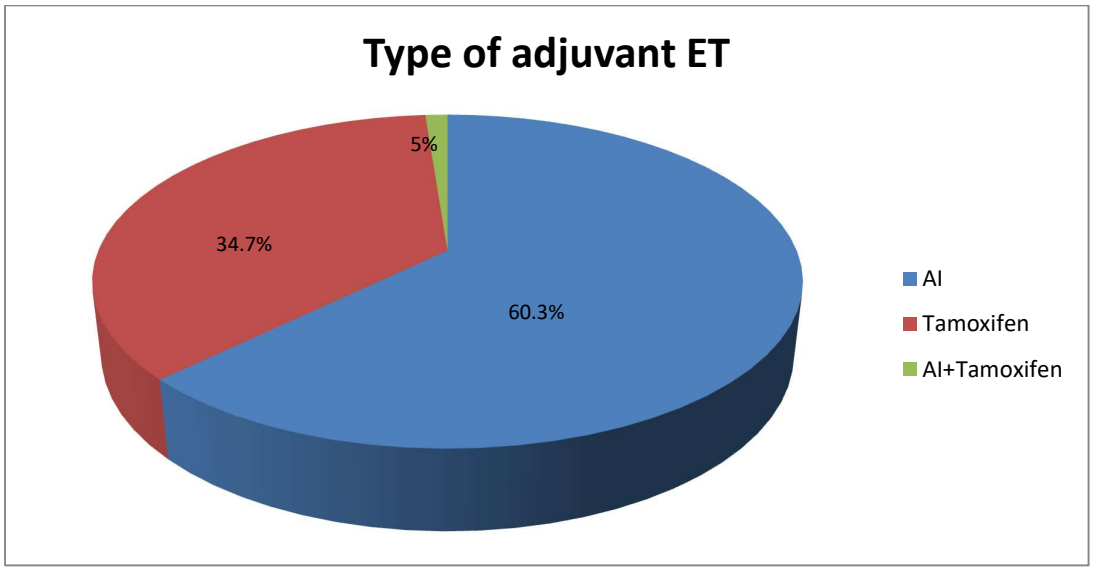


Fig.17 Distribution of the patients according the type of adjuvant ET.

Patients are divided into 4 groups depending on when the disease progression occurred in relation to the administered adjvant endocrine therapy. In 34.7% (N=42) progression was observed before the second year of its initiation 27.3% (N=33) progressed between the second and fifth year, 9.1% (N=11) progression was observed less than a after the completion of ET, and in 28.9% (N=35) progression occurred more than a year after the completion of adjuvant ET. (fig.18)

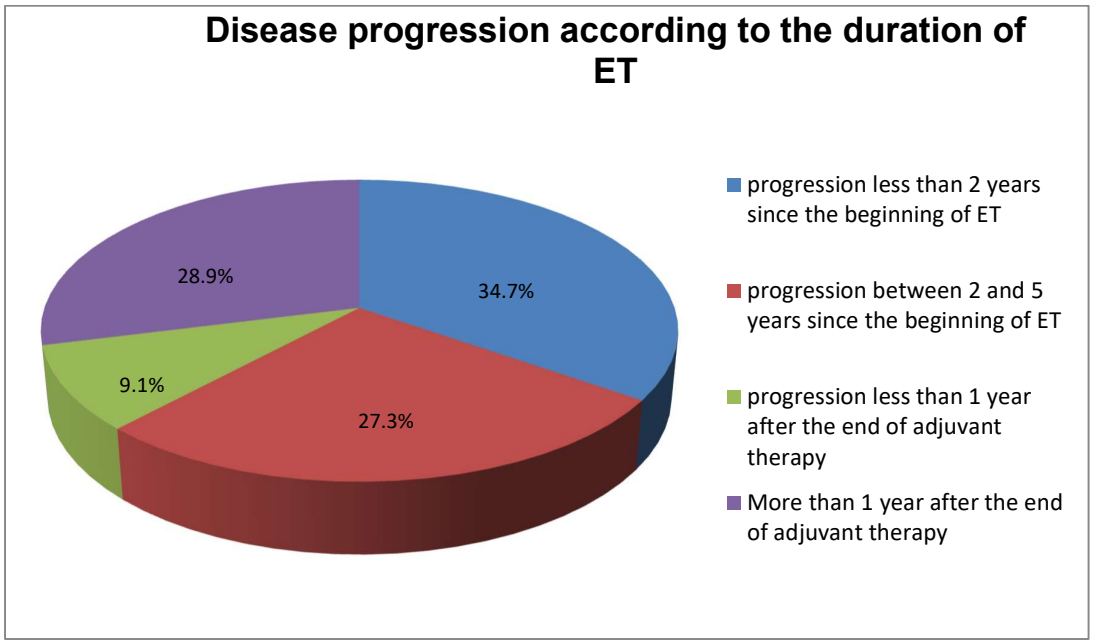


Fig. 18 Distribution of patients according to the time of progression during adjuvant ET

The distribution of patients according to the hospital in which they were treated is as follow: 141 patients at MHAT"Nadezhda"-Sofia, 51 patients at UMHAT "St. Marina"-Varna, 58 patients at MHAT "Tokuda"-Sofia. (Fig.19)

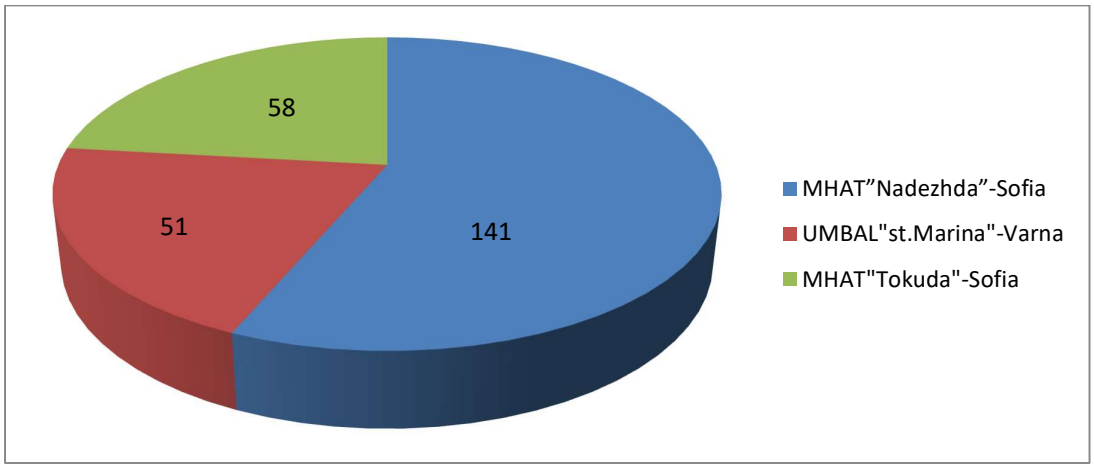


Fig.19 Distribution of patients according to the where were treated.

2. Prevalence of PIK3CA mutation in the studied population and its comparison with that reported by The Cancer Genome Atlas-USA

Among the 250 patients included, the presence of the *PIK3CA* mutation was found in 29.2% (N=73) while 70.2% (N=177) did not have a mutation. The result obtained does not differ from that reported in the literature, where the mutation frequency ranges from 30-40%. Die distribution of mutations according to the exon in which they are located as follows: – 39.7% in exon 9, 54.8% in exon 20 *и* 5.5% in both exons simultaneously. The most common mutation is H1047R in 20 exon, occurring in 46.5% of patients, followed by E545K in 21.9 %, E542K in 15%, H1047L in 6.8%, E545G in 1.4%, H1047Y in 1.4%, and Q546R in 1.4%. The presence of mutations affecting two exons was observed in four patients. (Fig.20)

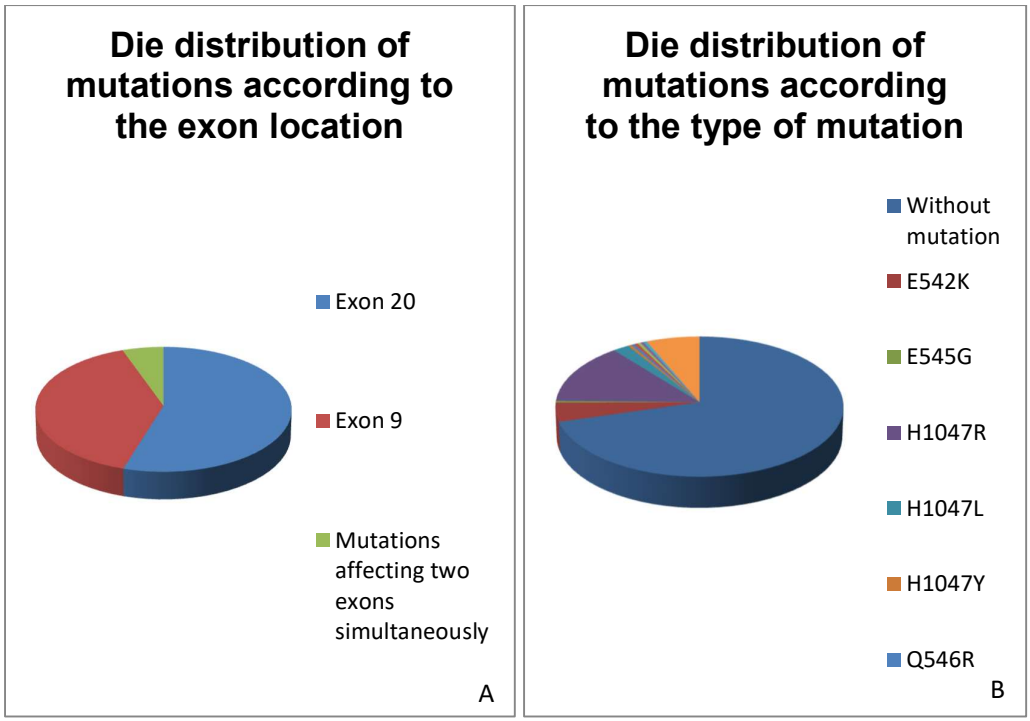


Fig.20 Distribution of mutations according to the exon in which they are located (A) and based on that, whether a mutation is detected and what type of mutation it is. (B).

The results we obtained were compared with TCGA (The Cancer Genome Atlas-USA) data base as of 04.01.2023r. TCGA is a national program launched in 2006, aimed at conducting genetic research on individual mutations up to sequencing the entire human genome through high-tech genetic analysis methods (NGS), collecting databases, and performing statistical processing to improve the diagnosis and treatment of oncological diseases, as well as providing easy access to the information, A total of 933 patients with characteristics similar to those of the patients in our project were selected namely patients with hormone-positive, HER2-negative breast cancer in metastatic stage. When comparing the two patients' cohorts, it was found that there was no statistically significant difference between them. (p=0.10). (Tab.7)

	% (BG) N=250	%(TCGA) N=933	P-value (Chi-squared test)
Prevalence of PIK3CA mutation	29.2	34.8	0.10

Table 7 Chi-squared test for comparing the patients from the project with those from TCGA.

3. *Correlation between the presence of PIK3CA mutation and certain clinico-pathological characteristics of the patients. (Tab.8)*

	***PIK3CA* (+) (N=73)**	***PIK3CA* (-) (N=177)**	**P-value**
Age (mean ± SD)	57.6 ± 11.6	56.5 ± 12.6	0.522
Menopausal status			0.915
Premenopausal, n (%)	11 (15.3)	28 (15.8)	
Postmenopausa, n (%)	61 (84.7)	149 (84.2)	
Metastatic sites			0.794
Visceral, n (%)	42 (57.5)	105 (59.3)	
Bone only, n (%)	31 (42.5)	72 (40.7)	
Stage at the initial diagnosis			0.003
Metastatic, n (%)	45 (61.6)	72 (40.7)	
Nonmetastatic,, n (%)	28 (38.4)	105 (59.3)	
Sample used			0.496
Archival tissue, n (%)	57 (78.1)	145 (81.9)	
Most recent biopsy tissue, n (%)	16 (21.9)	32 (18.1)	
CT as adjuvant/neoadjuvant therapy			0.034
Yes, n (%)	24 (32.9)	84 (47.5)	
No, n (%)	49 (67.1)	93 (52.5)	
Type of ET			0.491
Tamoxifen, n (%)	7 (25.9)	35 (37.2)	
Aromatase inhibitor, n (%)	18 (66.7)	55 (58.5)	
Tamoxifen+Aromatase inhibitor, n (%)	2 (7.4)	4 (4.3)	
Duration of adjuvant ET			0.013
Progression less than 2 years since the beginning of ET n (%)	8 (12.3)	34 (23.1)	
Progression between 2 and 5 years since the beginning of ET n (%)	5 (7.7)	28 (19)	
Progression less than 1 year after the end of adjuvant therapy, n (%)			

More than 1 year after the end of adjuvant therapy, n (%)	2 (3.1) 12 (18.5)	9 (6.1) 23 (15.6)
Declined adjuvant ET n (%)	38 (58.4)	53 (36.2)

Table.8 Relationship between patients' clinic-pathological characteristics and the *PIK3CA* status

The average age in the group *PIK3CA* mutation was 57.6 ± 11.6 which did not differ statistically from that in the group without the mutation, 56.5 ± 12.6 (p= 0.522). Regarding menopausal status, the distribution in the two groups was similar, with premenopausal patients accounting for 15.3% in the mutation group and 15.8% in the non-mutation group, while postmenopausal patients were 84.7% in the 84.7% in the *PIK3CA*-positive group and 84.2% in the other group (p= 0.915). (Tab.8)

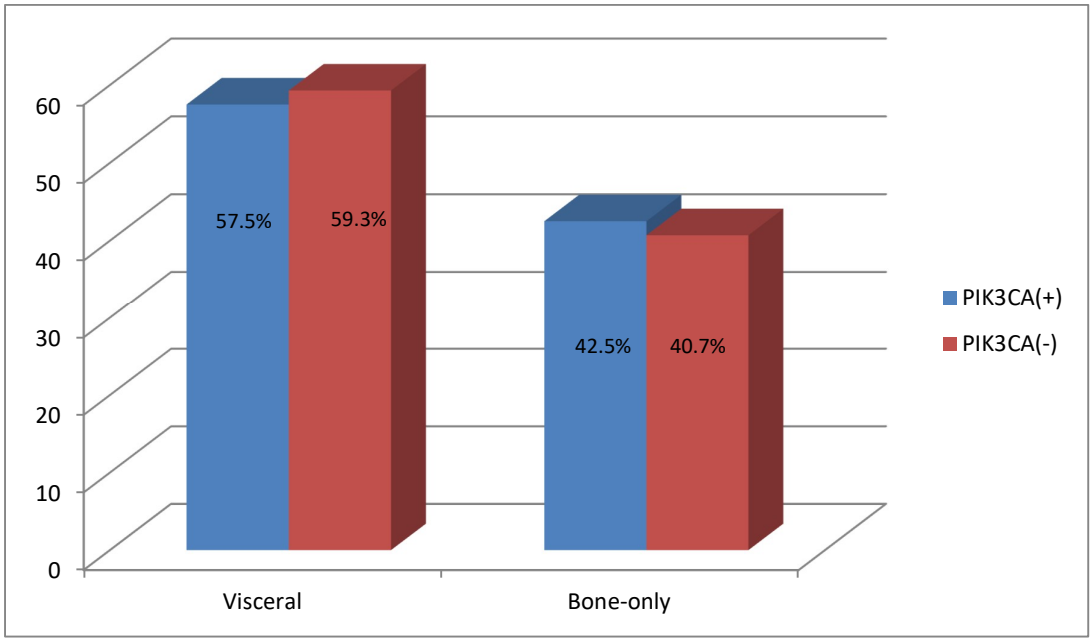


Fig.21 Distribution of patients by site of metastasis

There was no difference in two groups in terms of location of metastases, whether in the bones or visceral organs (p= 0.794). (Fig.21)

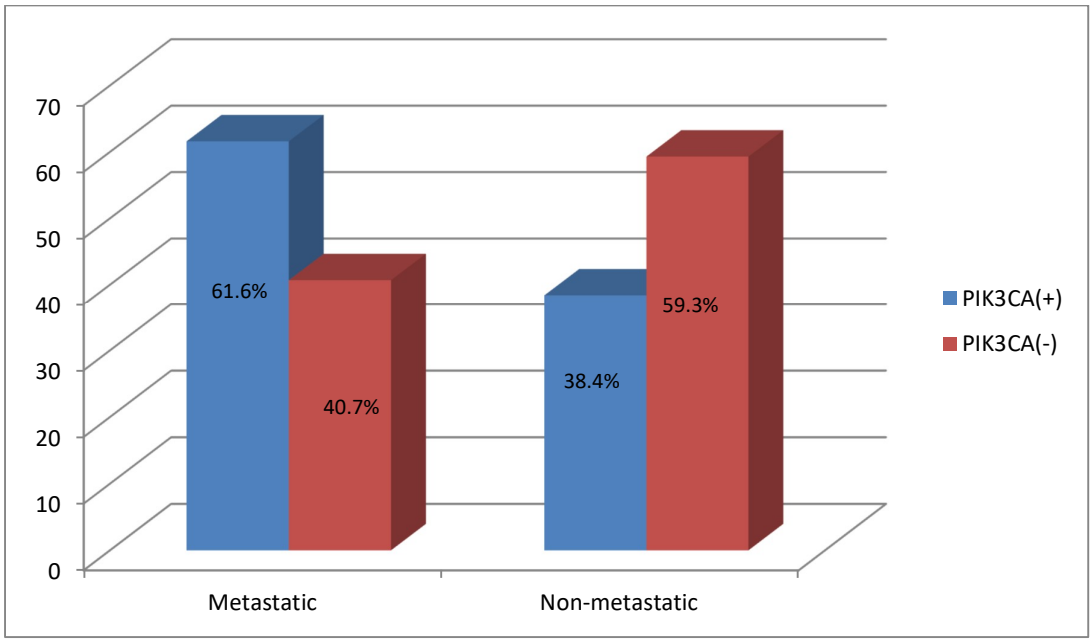


Fig.22 Percentage distribution of patients according to the stage of the disease at diagnosis.

Depending on the stage at which the disease was diagnosed, a statistically significant difference was found between the two groups (p=0.003); specifically, more patients were diagnosed at the metastatic stage, 61.6% in the group with the *PIK3CA* mutation , while 59.3% of the patients without mutation were diagnosed at an early stage. (Fig.22) Regarding the type of histological specimen study (archival tissue or recent biopsy), no statistically significant difference was found between the two groups either (p=0.496). (Tab.8)

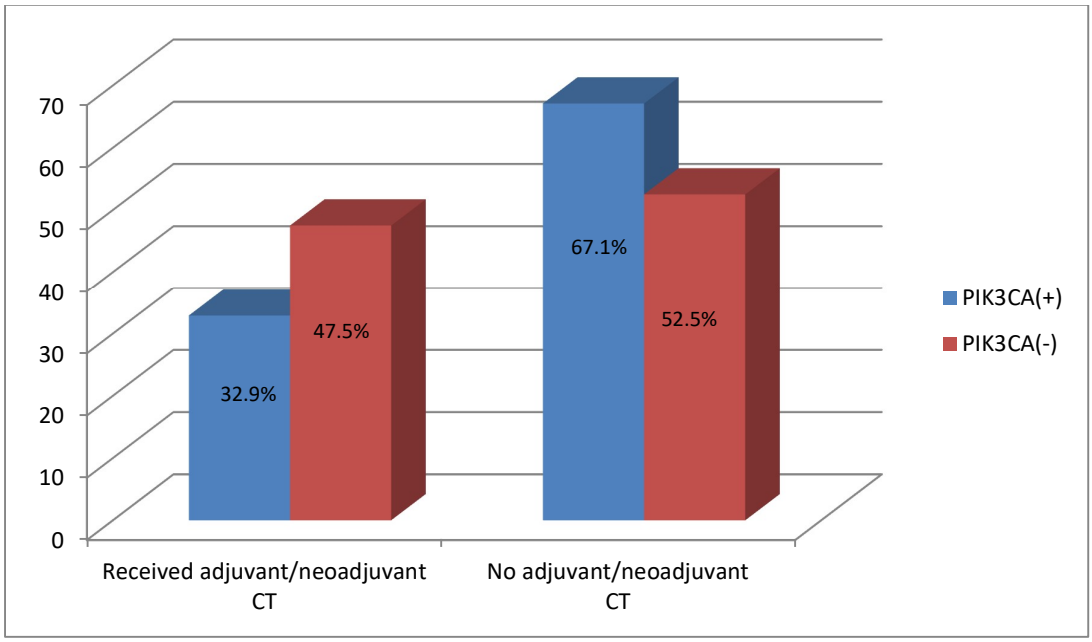
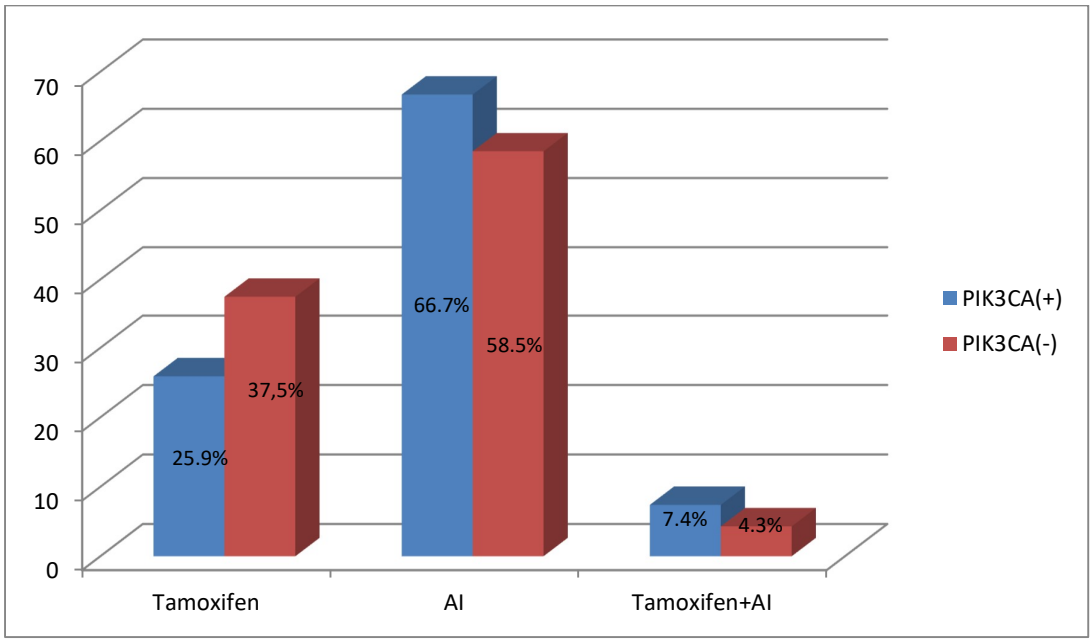


Fig. 23 Distribution of patients according to whether they received adjuvant/neoadjuvant chemotherapy, p=0.034

In the cohort without mutation, most patients received adjuvant/neoadjuvant chemotherapy, while in the cohort without mutation, a higher percentage of patients did not receive chemotherapy, with the results showing statistical significance (p=0.034). (Fig.23)



Фиг.24 Distribution by type of adjuvant endocrine therapy. AI-aromatase inhibitor

Regarding the type of ET received in the adjuvant setting, no statistically significant difference was found between the two groups (p=0.491). (Fig.24)

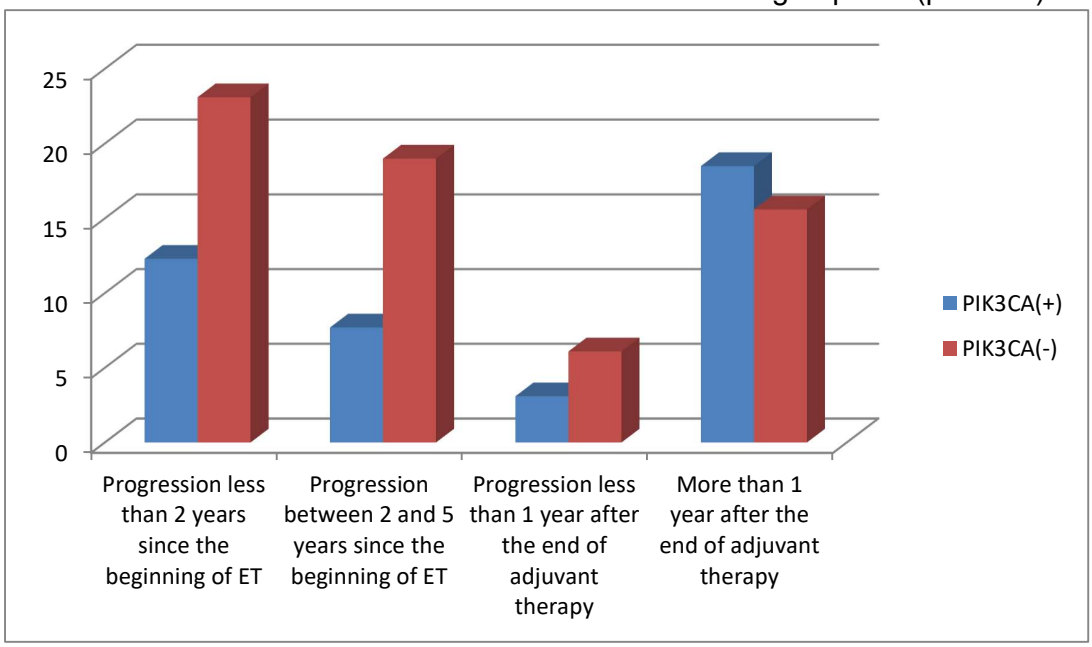


Fig.25 Distribution of patients according to when progression of adjuvant ET occurred.

The patients who were diagnosed at an early stage and who were supposed to receive adjuvant ET were 212, of whom 91 patients refused therapy. The remaining patients were divided into four groups based on when progression occurred. The first group included patients with primary endocrine resistance, i.e. those who progressed before the second year of starting adjuvant ET. The second and third groups included patients with secondary endocrine resistance, with the second group the patients progressed between the second and fifth years of therapy, and the third group patients progressed within the first year after completing adjuvant ET. The fourth group consisted of patients who progressed one year

after finishing adjuvant therapy. The results showed a statistically significant association between the presence of the *PIK3CA* mutation and the development of primary and secondary resistance. (p=0.013). (Fig.25)

4. The relationship between PIK3CA – mutation status and progression-free survival and overall survival.

About 67% of the patients received a CDK4/6 inhibitor in combination with endocrine therapy (AI or fulvestrant) as first-line therapy, while the remaining were treated with tamoxifen, AI, fulvetrant or examestan+everolimus. The results did not show a statistically significant difference between the type of ET used as first-line therapy in two patient cohorts (χ 2 = 2.76, p = 0.55). (Fig. 26)

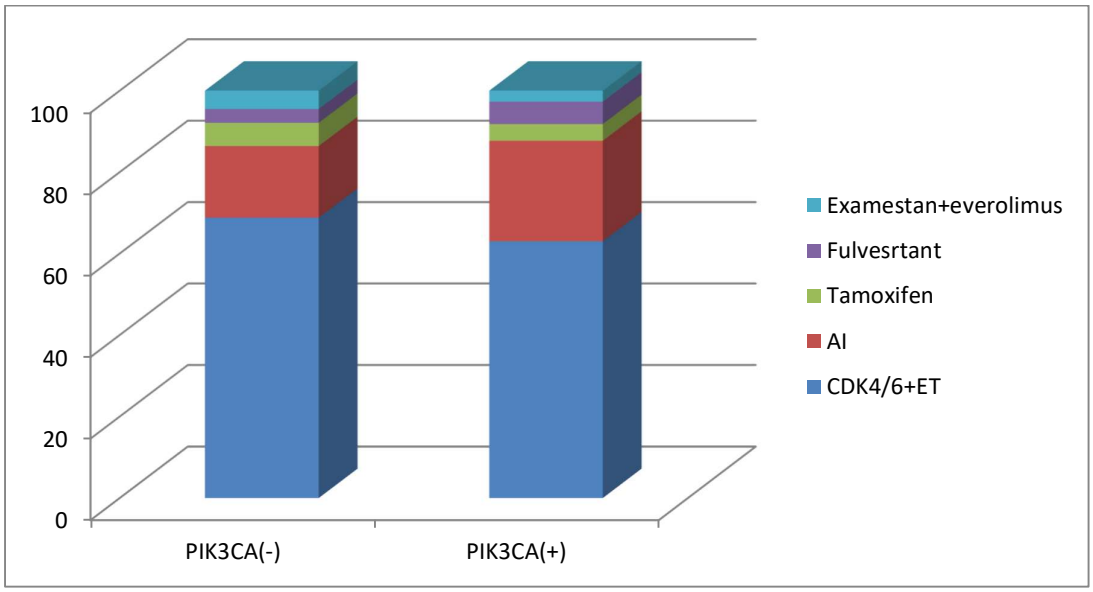
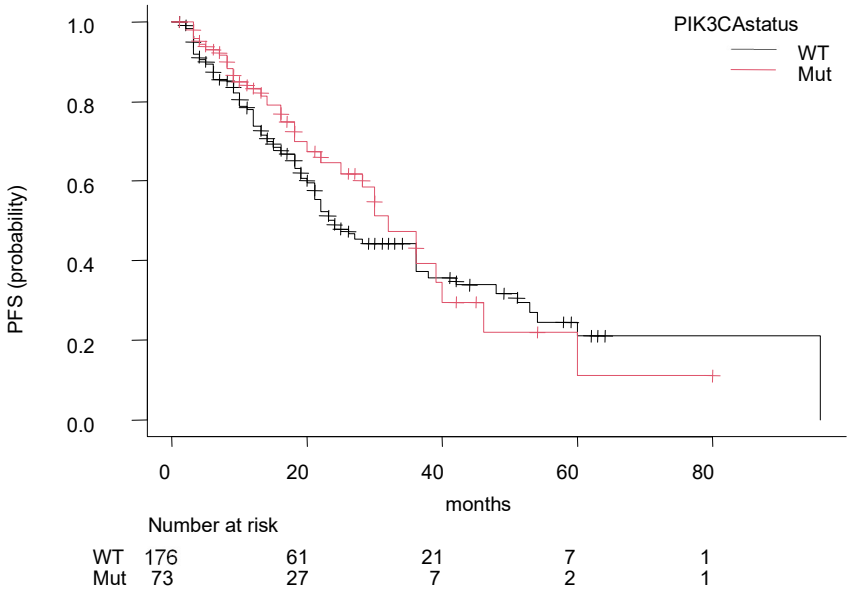


Fig .26 Type of first-line therapy used in the two groups. Et-endocrine therapy; AI-aromatase inhibitor

Regarding progression-free survival (PFS), it was found that there was no statistically significant difference between the two groups. The median PFS in the cohort without a mutation was not longer than that in the mutation group, respectively 32 months (95%, CI: 22–40) in the *PIK3CA* (+) group compared to 24 months in the non-mutation group. (95%, CI: 21–36, p =0.45 ; HR = 0.86 (95%, CI: 0.5–1.3, p= 0.46). (Fig.27)



Фиг.27 Kaplan-Maierestimates of progression free survival (PFS). (A). Patients with *PIK3CA* mutations did not have significantly different median PFS compared to WT patients (32 months [95%, CI: 22–40] vs. 24 months [95%, CI: 21–36], [p = 0.45])

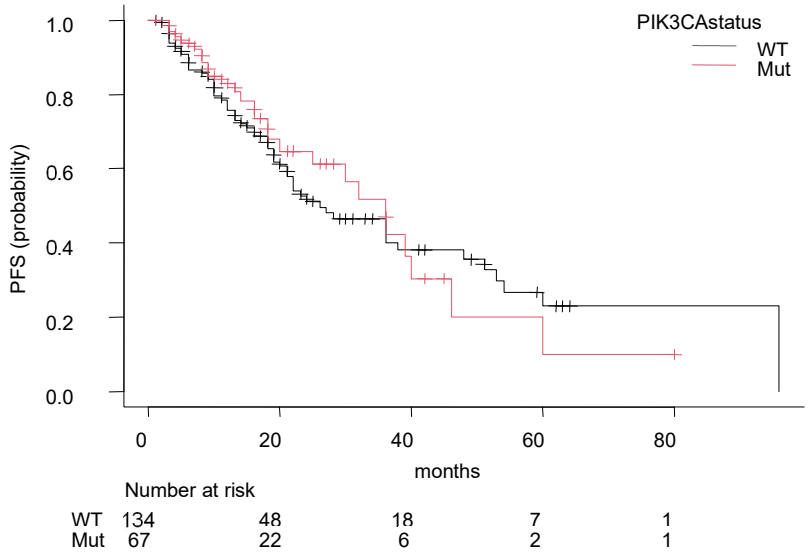


Fig. 28 Propensity matching score analysis (matching data for treatment administered as a first-line endocrine therapy (ET), menopausal status, and locations of metastatic disease), patients with *PIK3CA* mutations did not have significantly different median PFS compared to WT (36 months [95%, CI: 20–40] vs. 26 months [95%, CI: 21–38], (p = 0.69)

The results for PFS were confirmed using propensity matching score analysis, through which the two patient cohorts were balanced in terms of receiving first-line therapy, menopausal status and type of metastases, respectively 36 months [95% CI: 20–40] versus

26 months [95% CI: 21–38], p = 0.69). (Fig.28) Disease progression was observed in 26 patients with primary endocrine resistance, i.e., treated for less than 6 months with first-line ET. Among patients with *PIK3CA* mutation and primary endocrine resistance, six were affected, 50% of whom (n= 3) received a CDK4/6 inhibitor plus ET. Patients without mutation and primary endocrine resistance were twenty, 35% of whom (n=7) received CDK4/6 inhibitor plus ET. No statistically significant difference was observed between patients with primary endocrine resistance and *PIK3CA* mutation status, namely 8.2% and 11.2% (p=0.47).

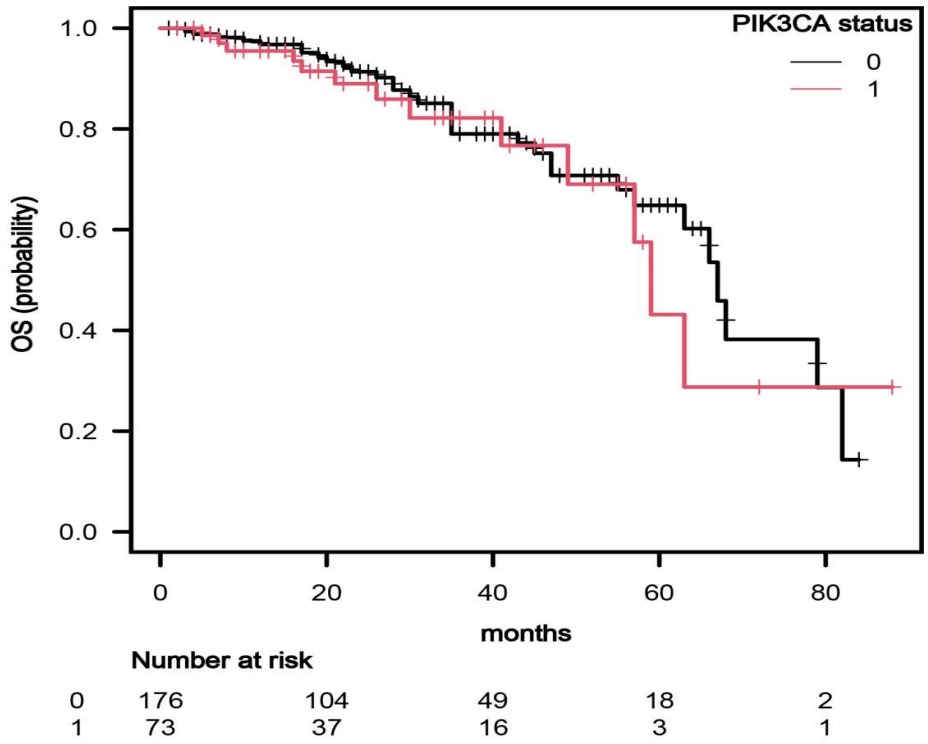


Fig. 29 Kaplan-Maierestimates of overall survival (OS). Patients with *PIK3CA* mutations did not have significantly different median OS compared to WT patients (59 months [95%, CI: 47–69] vs. 61 months [95%, CI: 55–67], [p = .60]).

The Cox regression analysis conducted did not reveal a difference in the risk of death between the two patient cohorts. (Fig. 29)

V. DISCUSSION

The breast cancer (BC) is among the most common oncological diseases by the women worldwide, as well as in Bulgaria, making it a socially significant disease. Despite improved diagnosis and treatment of this socially significant disease, there are still cases in which the disease is diagnosed at a metastatic stage. The goal for these patients is to prolong a life by a good quality of life.

In more than 70% of cases, breast cancer is hormone receptor-positive and negative for human epidermal growth factor receptor 2 (HER2). In 30-40% of patients with HR(+), HER2(-) breast cancer, activating mutations are found in the *PIK3CA* gene, making it one of the most commonly mutated genes in this type of tumor. The PI3K signaling pathway is a key regulator of cell growth, metabolism, proliferation, survival and invasion. Regulation of PI3K is crucial for maintaining genomic stability, chemoresistance, and cell survival, as it is involved in extensive DNA replication and the regulation of cell cycle processes. Several genetic alterations have been identified in various tumorigenesis pathways, including PTEN deletion, AKT1 and *PIK3CA* amplification, and somatic mutations in PIK3CA and AKT1. Most of the point mutations in the *PIK3CA* gene are located in the p110 cluster, particularly around two hotspots - E542/5 in the helix (exon 9) and H1047R near the catalytic domain (exon 20). These mutations lead to amino acid changes (E545K, E542K and H1047R), that increase the activity of the PI3K holoenzyme, resulting in the constitutive activation of AKT. Activating *PIK3CA* mutations are present from the onset ot oncogenesis in BC and are generally neither lost nor acquired during clonal evolution in the later stages of the disease, suggesting that they are driver mutations.

According to the results of the present study, the frequency of the PIK3CA Mutation among Bulgarian patients is 29.2%, which is comparable to the frequency reported in several international studies. Anderson and colleagues, 2020 conducted a systematic review of 572 articles and conference abstracts to determine the prevalence of the *PIK3CA* mutation in HR(+) HER2(-) metastatic breast cancer. Based on the included literature, the average frequency of the *PIK3CA* mutation is 36.4%.

In our study, we did not find a statistically significant difference in median progression-free survival (PFS) on first-line ET ± CDK4/6 inhibitor between patients with confirmed PIK3CA mutation and those without mutation. A similar result was observed for overall survival as well. According to the latest results from studies and mata-analyses, patients in WT cohort have a longer median PFS than patients with *PIK3CA* mutation. The difference between our results and those reported in other studies may be due to the small number of patients included in our study and the higher percentage of patients with endocrine resistance in the non-mutated group.
Similar results to ours were reported after the subgroup analysis of the MONARCH 2 study, where it was found that population with a positive *PIK3CA* mutation and those without a detectable mutation had comparable PFS and OS, suggesting that the presence of the mutation likely did not play a role in the development of therapeutic resistance in patients who received a CDK4/6 inhibitor and ET. Another analysis conducted by Ortega et al., 2020,

found that the presence of a *PIK3CA* mutation was not associated with resistance to CDK4/6 inhibitors in terms of PFS. On other hand, the analysis found that in patients who progressed on first-line therapy before the sixth month, there was a higher frequency of the *PIK3CA* mutation (46.67%), i.e. these are patients with primary endocrine resistance. In our study, 26 patients progressed within six months of starting first-line endocrine therapy, and no statistically significant difference was found regarding primary endocrine resistance between the two patients' cohorts. Our results also showed that the presence of a *PIK3CA* mutation is associated with a more advanced stage at diagnosis, suggesting a more aggressive disease when the mutation is present.

Despite the established association in our study, it cannot be concluded that the presence of *PIK3CA* is unrelated to prognosis and response to first-line endocrine therapy, as this is a retrospective study with a relatively small number of patients. There may also have been bias on patients' selection; also, this is not a randomized study. Regarding the cause of death, for the dead patients, we do not have information on whether it was due to disease progression or unrelated to it.

Despite the limitations mentioned above, our study for the first time examines the frequency of the *PIK3CA* mutation among Bulgarian patients, as well as its association with first-line therapy response and overall survival.

In conclusion our study offers important insights into the topic, however due to limitations such as its retrospective design and the small number of participants, the results need to be confirmed by prospective studies with larger sample size before more definitive conclusions can be made.

VI. CONCLUSION

1. No difference was found in the frequency of the *PIK3CA* mutation in Bulgarian patients with HR(+), HER2(-) mBC compared to its prevalence worldwide.
2. No association was found between mutation status and clinico-pathological characteristics such as age, menopausal status, type of metastases , type of adjuvant ET, and type of semple used for detection of *PIK3CA* mutation.
3. It was found a statistically significant association between the presence of the *PIK3CA* mutation and the diagnosis of patients at a more advanced stage.
4. The results showed that the presence of the *PIK3CA* mutation does not reduce the effectiveness of endocrine therapy administered as first-line treatment.
5. No difference in overall survival (OS) was observed between the group with the *PIK3CA* mutation and the group without the mutation, i.e., the presence of the mutation does not lead to shorter OS.
6. It was found that the majority of Bulgarian patients in the study were treated according to European and international guidelines, i.e., there is no difference between the treatment of patients with HR(+), HER2(-), metastatic breast cancer in Bulgaria compared to the therapy received by patients in leading courtries in the the treatment of this type of tumor worldwide.

VII. CONTRIBUTIONS OF THE DISSERTATION WORK

1. For the first time in Bulgaria , the frequency of the *PIK3CA* mutation is being studied among patients with metastatic hormone-positive, HER2(-) negative breast cancer
2. For the first time in Bulgaria is being study, the relationship between *PIK3CA* мутацията mutation and progression-free survival in relation to the applied first-line endocrine therapy.
3. For the first time in Bulgaria, the correlation between the presence of the *PIK3CA* mutation and overall survival is being analyzed.
4. For the first time, the relationship between the presence of a *PIK3CA* mutation and the development of endocrine resistance to first-line therapy is being studied.

VIII. SCIENTIFIC PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS RELATIED TO THE TOPIC OF THE DISSERTATION

1 Ivanova, A., **Gencheva, R.** : *Abemaciclib – modern treatment for metastatic hormone receptor-positive HER2-negative breast cancer.* MedicPlus .14-18, 09/2021

2 **Radostina Gencheva**, **Радостина Генчева**, assoc. prof. Eleonora Dimitrova *Endocrine resistance in metastatic hormone-positive HER2 negative breast cancer – mechanisms of development*, *MedicPlus, VII, 5-9 ,11/2025*

3 **R. Gencheva**, M. Petrova, P. Kraleva, S. Hadjidekova, M. Radanova, N. Conev, D. Stoyanov, J. Arabadjiev, E. Tazimova, S. Bachurska, M. Eneva, M. Tsvetkova, G. Zhbantov, T. Karanikolova, D. Manov, A. Ivanova, M. Taushanova-Hadjieva, R. Staneva, E. Dimitrova, I. Donev: *Prevalence and prognosis of PIK3CA mutations in Bulgarian patients with metastatic breast cancer receiving endocrine therapy in first-line setting., Cancer Reports. 2024;7:e1966.*

4 **Radostina Gencheva** , Eleonora Dimitrova, Mila Petrova, Sonya Draganova, Rosalina Pechlivanova, Ivan Donev: *PIK3CA mutation in breast cancer as a prognostic factor. MedicPlus 28-30, 03/2025*

5 **Radostina Gencheva**, Savina Hadjidekova, Mila Petrova, Dimo Krustev, Petya Kraleva, Georgi Zhbantov, Nikolay Conev, Jeliazko Arabadjiev, Eliz Tazimova, Svitlana Bachurska, Ivan Galev, Mariyana Eneva, Mariela Tsvetkova, Rada Staneva, Eleonora Dimitrova, Rosen Hadjiev and Ivan Donev, Abstract 2023 ASCO Annual Meeting: *PI3KCA mutation prevalence and outcome among patients with metastatic breast cancer in Bulgaria treated with first-line endocrine therapy.e13005*

6 IX Scientific annual conference of BAMO, 17-19.06.2022г: *PI3KCA mutation prevalence among patients with HR(+) HER2(-) metastatic breast cancer in Bulgaria.* **Radostina Gencheva**, MHAT”Nadezhda”

7 X Scientific annual conference of BAMO, 12-14.05.2023г: *What should we know when treating patients with solid tumors over 75 yars old?*
Georgi Zhbantov, **Radostina Gencheva**, Mohamed Alhalabi, MHAT “Nadezhda”