



Oncology

IN CLINICAL PRACTICE

2021, Vol. 17, Number 1

ISSN 2450-1654
e-ISSN 2450-6478

Ewa Anna Kosakowska, Andrzej Rutkowski, Piotr T. Wysocki, Wojciech Michalski, Anna Cencelewicz-Lesikow, Michał Kunkiel, Agnieszka Jagiełło-Gruszfeld

Evaluation of the efficacy of chemotherapy with capecitabine and oxaliplatin in patients with disseminated colorectal cancer. The impact of primary cancer focus on treatment efficacy

Neslihan Duzkale, Hikmet Taner Teker

The study of genetic and clinicopathological characterisation of Turkish bilateral breast cancer patients

Marcin Kaczor, Wojciech Staśkiewicz, Monika Homa, Magdalena Górecka, Rafał Wójcik, Piotr Potemski

Bevacizumab or standard chemotherapy in previously treated patients with metastatic colorectal cancer — a systematic review

Aleksandra Sobiborowicz, Anna M. Czarnecka, Anna Szumera-Ciećkiewicz, Piotr Rutkowski, Tomasz Świtaj

Diagnosis and treatment of lymphangioleiomyomatosis (LAM) from the PEComa group

Tomasz Sachańbiński, Maciej Miodoński, Barbara Radecka
Invasive breast cancer in ectopic axillary breast tissue — case report



ONCOLOGY IN CLINICAL PRACTICE

Official Journal of the Polish Society of Clinical Oncology

https://journals.viamedica.pl/oncology_in_clinical_practice

Editor-in-Chief

prof. dr hab. n. med. Maciej Krzakowski

Deputy Editors

prof. dr hab. n. med. Andrzej Kawecki
dr hab. med. n. Tomasz Kubiawski, prof. CMKP
prof. dr hab. n. med. Piotr Potemski
prof. dr hab. n. med. Piotr Rutkowski
prof. dr hab. n. med. Piotr Wysocki

Scientific Board

dr Edita Baltruskeviciene (Vilnius, Lithuania)
prof. Tomasz M. Beer (Portland, USA)
prof. Bartosz Chmielowski (Los Angeles, USA)
dr hab. n. med. Anna M. Czarnecka
dr n. med. Rafał Czyżykowski
dr hab. n. med. Joanna Didkowska
prof. dr hab. n. med. Renata Duchnowska
dr Rick Haas (Leiden, The Netherlands)
dr n. med. Beata Jagielska
dr n. med. Jerzy Jarosz
prof. dr hab. n. med. Jacek Jassem
prof. dr hab. n. med. Arkadiusz Jeziorski
dr hab. n. med. Ewa Kalinka

prof. dr hab. n. med. Radziław Kordek
lek. Łukasz Kwinta
dr hab. n. med. Maria Litwiniuk
dr n. med. Aleksandra Łacko
prof. Ruggero De Maria (Rome, Italy)
dr Mario Mandala (Bergamo, Italy)
dr hab. n. med. Radosław Mądry
dr n. med. Janusz Meder
dr hab. n. med. Sergiusz Nawrocki
prof. dr hab. n. med. Włodzimierz Olszewski
dr n. med. Adam Płuzański
prof. dr hab. n. med. Maria Podolak-Dawidziak
dr hab. n. med. Barbara Radecka
prof. dr hab. n. med. Tadeusz Robak
prof. dr hab. n. med. Kazimierz Roszkowski
prof. dr hab. n. med. Ewa Sierko
dr Silvia Stacchiotti (Milan, Italy)
dr Ryszard Szydło (London, UK)
prof. dr hab. n. med. Jerzy Walecki
prof. dr hab. n. med. Jan Walewski
prof. dr hab. n. med. Krzysztof Warzocha
prof. dr hab. n. med. Marek Wojtukiewicz
dr Agnieszka Wozniak (Leuven, Belgium)
prof. Christoph Zielinski (Vienna, Austria)

Managing Editor

Aleksandra Cielecka

Opinions presented in the articles do not necessarily represent the opinions of the Editors

Oncology in Clinical Practice (ISSN 2450–1654) is published six times a year by

VM Media sp. z o.o. VM Group sp.k.
ul. Świętokrzyska 73, 80–180 Gdańsk, Poland
Phone: (+48 58) 320 94 94, fax: (+48 58) 320 94 60
e-mail: redakcja@viamedica.pl,
<http://www.viamedica.pl>, wapp.viamedica.pl



20-0826.010.001

Editorial Address

Klinika Nowotworów Płuca i Klatki Piersiowej
Narodowy Instytut Onkologii im. Marii Skłodowskiej-Curie — Państwowy Instytut Badawczy
ul. Roentgena 5, 02–781 Warszawa, Poland
Phone: (+48 22) 546 21 69
e-mail: sekretariat4@pib-nio.pl

Advertising

For details on media opportunities within this journal please contact the advertising sales department, ul. Świętokrzyska 73, 80–180 Gdańsk, Poland, phone: (+48 58) 320 94 94; e-mail: dsk@viamedica.pl

The Editors accept no responsibility for the advertisement contents.

All rights reserved, including translation into foreign languages. No part of this periodical, either text or illustration, may be used in any form whatsoever. It is particularly forbidden for any part of this material to be copied or translated into a mechanical or electronic language and also to be recorded in whatever form, stored in any kind of retrieval system or transmitted, whether in an electronic or mechanical form or with the aid of photocopying, microfilm, recording, scanning or in any other form, without the prior written permission of the publisher. The rights of the publisher are protected by national copyright laws and by international conventions, and their violation will be punishable by penal sanctions.

Legal note: <http://czasopisma.viamedica.pl/owpk/about/legalNote>

Indexed in Index Copernicus (ICV 2019 = 89.65), Ulrich's Periodicals Directory and CAS.

According to the statement of the Polish Ministry of Science and Higher Education publication in the journal has been awarded with 20 points.

Editorial policies and author guidelines are published on journal website: http://journals.viamedica.pl/oncology_in_clinical_practice



ONCOLOGY IN CLINICAL PRACTICE

Official Journal of the Polish Society of Clinical Oncology

https://journals.viamedica.pl/oncology_in_clinical_practice

2021, Vol. 17, Number 1

ORIGINAL ARTICLES

Evaluation of the efficacy of chemotherapy with capecitabine and oxaliplatin in patients with disseminated colorectal cancer. The impact of primary cancer focus on treatment efficacy

Ewa Anna Kosakowska, Andrzej Rutkowski, Piotr T. Wysocki, Wojciech Michalski, Anna Cencelewicz-Lesikow, Michał Kunkiel, Agnieszka Jagiełło-Gruszczyńska..... 1

The study of genetic and clinicopathological characterisation of Turkish bilateral breast cancer patients

Neslihan Duzkale, Hikmet Taner Teker 6

REVIEW ARTICLES

Bevacizumab or standard chemotherapy in previously treated patients with metastatic colorectal cancer — a systematic review

Marcin Kaczor, Wojciech Staśkiewicz, Monika Homa, Magdalena Górecka, Rafał Wójcik, Piotr Potemski..... 14

Diagnosis and treatment of lymphangioleiomyomatosis (LAM) from the PEComa group

Aleksandra Sobiborowicz, Anna M. Czarnecka, Anna Szumera-Ciećkiewicz, Piotr Rutkowski, Tomasz Świtaj..... 28

CASE REPORT

Invasive breast cancer in ectopic axillary breast tissue — case report

Tomasz Sacharbiński, Maciej Miodoński, Barbara Radecka 42

Professor Krzysztof Krzemieniecki Award for the best case report accepted for publication

Case Report Contest Policies

This policy defines the scope, requirements and regulations regarding **The Krzysztof Krzemieniecki Award** for the best case report published in “Oncology in Clinical Practice” (OCP) Fifth Edition.

1. The aim of the contest is to encourage submission of quality case reports related to oncological practice and to promote them in the scientific deliberations.
2. All respective manuscripts submitted to OCP between June 1st, 2020 and May 31st, 2021 and accepted for publication will qualify.
3. Manuscripts should be prepared in line with Authors’ guidelines and should be submitted only through the manuscript system available at Journal’s website: https://journals.viamedica.pl/oncology_in_clinical_practice
4. All submitted manuscripts will be evaluated during the peer review process and authors will be informed about their qualification for publication in OCP. Accepted papers will be evaluated by the Contest Committee based upon fulfillment of the Contest criteria as well as practical significance, originality, applicability and addressing of current/critical concerns.
5. The first author of the winning paper will be eligible for a prize of gross 1000,00 Euro gross (one thousand euro).
6. Results will be announced during the XXIV National Congress of The Polish Society of Clinical Oncology and subsequently at the Journal website.
7. Winner will be notified via email.
8. Contest Committee may exclude a paper from participation in case of potential conflict of interest or ask submitting author for adequate clarifications.
9. The Sponsor at any stage and in any respect, will not participate in the evaluation of entries and selection of a winning paper.
10. The award amount shall be paid based on the copyright transfer agreement to the paper.
11. These Regulations are the sole and exclusive document defining the principles and conditions for the Contest. In all matters not regulated, decisions are made by The Organizer.

Contest Organizer:

VM Media sp. z o.o. VM Group sp. k., seated at 73 Swietokrzyska Street, 80-180 Gdansk, Poland (Register of Entrepreneurs kept by the District Court for Gdansk, Commercial Division VII of the National Court Register under KRS No 0000266430, VAT Reg. No PL 583-28-39-187).

Patronage  NOVARTIS

Ewa Anna Kosakowska^{1, 2}, Andrzej Rutkowski², Piotr T. Wysocki², Wojciech Michalski³, Anna Cencelewicz-Lesikow², Michał Kunkiel⁴, Agnieszka Jagiełło-Gruszfeld⁴

¹Oncology and Breast Diseases Department, Medical Center of Postgraduate Education, Warsaw, Poland

²Department of Oncological Gastroenterology, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland

³Laboratory of Bioinformatics and Biostatistics, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland

⁴Department of Breast Cancer and Surgery, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland

Evaluation of the efficacy of chemotherapy with capecitabine and oxaliplatin in patients with disseminated colorectal cancer. The impact of primary cancer focus on treatment efficacy

Address for correspondence:

Dr n. med. Agnieszka Jagiełło-Gruszfeld
 Klinika Nowotworów Piersi
 i Chirurgii Rekonstrukcyjnej
 Narodowy Instytut Onkologii
 im. Marii Skłodowskiej-Curie
 — Państwowy Instytut Badawczy
 ul. W.K. Roentgena 5, 02-781 Warszawa
 e-mail: agnieszka.jagiello-gruszfeld@coi.pl

Oncology in Clinical Practice
 2021, Vol. 17, No. 1, 1–5

DOI: 10.5603/OCP.2020.0046

Translation: dr n. med. Dariusz Stencel

Copyright © 2021 Via Medica

ISSN 2450-1654

ABSTRACT

Introduction. Colorectal cancer is an increasingly common cancer, and due to the possibility of using many drugs and combination therapy, it bears the hallmarks of a chronic disease. Improving the quality of life is important.

Material and methods. The following analysis applies to the oxaliplatin and capecitabine (CAPOX) regimen in a group of 305 patients. This chemotherapy was used as part of palliative treatment lines I, II or III.

Results. The work proved the effectiveness of the scheme despite the reduction of drug doses in about 50% of patients, and toxicity grade 3 was only present in 5% (grade 4 complications were not observed). The group of patients in which CAPOX was used as the first treatment line was considered representative, and the effectiveness of the treatment depending on the location of the primary tumour was evaluated.

Conclusion. Differences in overall survival of patients after stratification were observed relative to the location of the primary tumour. Survival was longer in patients with left-sided primary tumour compared to right-sided localisation and was, respectively, 20.4 (95% CI, 17.5–23.4) and 12.1 months (95% CI, 10.5–13.8) ($P = 0.014$).

Key words: metastatic colorectal cancer, oxaliplatin, capecitabine, primary tumour location

Oncol Clin Pract 2021; 17, 1: 1–5

Introduction

Palliative chemotherapy has been used in patients with generalised colorectal cancer (CRC) for many years. At the time of diagnosis, a generalised disease is found in approximately 15–20% of patients.

Even after radical surgery (R0 resection), approximately 50% of patients will develop metastatic lesions, including 30–35% having only liver metastases. In this cohort, 10–25% are eligible for surgical treatment, and 75–90% of patients will be offered palliative chemotherapy. The use of chemotherapy allows objective response to be obtained in 50% of

cases in imaging tests, and extended progression-free survival (PFS) to 10 months and overall survival (OS) to 20–24 months [1].

In about 15–20% of patients, generalised colorectal cancer is an asymptomatic or slow-growing disease; therefore, aggressive treatment is not required [2]. It should be emphasised that the quality of life of patients receiving palliative treatment, apart from the toxicity of treatment, is significantly influenced by the frequency and length of hospitalisation.

Currently, an important argument in choosing a treatment regimen is patients' quality of life, which includes — among others — the frequency and route of drug admini-

Table 1. Characteristics of patients in the observed group

| Treatment line | First-line treatment | Second-line treatment | Third-line treatment | |
|--------------------------|----------------------|-----------------------|-------------------------|-------------------------|
| Number of patients | 222 | 66 | 17 | |
| Gender | Male 183 (60%) | Female 122 (40%) | | |
| Age | Mean 64.4 | Range 32–87 | ≥ 65 years 146 (48%) | < 65 years 159 (52%) |
| Prior adjuvant treatment | YES 139 (46%) | NO 166 (54%) | | |
| WHO performance status | 0–1 278 (91%) | 2 27 (9%) | | |

stration. The generalised stage of colorectal cancer often requires lengthy treatment, and the use of oral medications significantly improves the comfort of such treatment. These regimens include: CAPOX, XELIRI, and capecitabine alone.

The CAPOX regimen includes capecitabine and oxaliplatin. Capecitabine is administered orally at a dose of 1000 mg/m² twice daily for 14 days, and oxaliplatin is administered on the first day of the cycle at a dose of 130 mg/m² as a two-hour intravenous infusion. The cycle is repeated every 21 days.

Nonetheless, the most common therapeutic option proposed for patients with stage IV colorectal cancer is systemic treatment, which improves the quality of life and often extends the survival. The most commonly used anticancer drugs (in monotherapy or multi-drug regimens) for colorectal cancer include fluorouracil, irinotecan, oxaliplatin, capecitabine, bevacizumab, aflibercept, cetuximab, panitumumab, and regorafenib. The main goal is to achieve the greatest effectiveness with the least toxicity of treatment.

A regimen containing a combination of capecitabine and oxaliplatin is used in the first-, second-, or third-line treatment, depending on the genetic characteristics.

Currently, the growing importance of primary tumour location in the biology of colorectal cancer is underlined. The location of the primary tumour on the right side is associated with a worse prognosis. More and more publications are devoted to the impact of tumour location on response to targeted therapy with anti-epidermal growth factor receptor (EGFR) or anti-vascular endothelial growth factor (VEGF) antibodies, while there is little data on the effect of tumour location on the effectiveness of chemotherapy.

The aim of the study was to evaluate the effectiveness of chemotherapy with capecitabine and oxaliplatin in patients with generalised colorectal cancer and to compare treatment results depending on the tumour's original location.

Material and methods

We carried out a retrospective analysis of consecutive patients diagnosed with generalised colorectal can-

cer treated at the Colon Cancer Clinic and the Gastrointestinal Cancer Clinic between March 2008 and April 2011. The inclusion criteria included: histopathological diagnosis of colorectal adenocarcinoma, good general condition (WHO 0–2), locally advanced or metastatic colorectal cancer, the use of chemotherapy according to CAPOX scheme (I, II, or III line), and the presence of a measurable lesion. Table 1 presents the characteristics of 305 patients included in the analysis.

On average, six CAPOX cycles were used in each treatment line. A retrospective analysis of response to CAPOX treatment was performed (including disease control rate [DCR], time to progression [TTP], and overall survival), taking into account dose reductions and treatment toxicity. A retrospective analysis of clinical outcome in patients with metastatic colorectal cancer depending on the location of the primary tumour was also made. This analysis included only the group of patients treated with the CAPOX regimen in the first and second line of treatment.

Results

There were no statistically significant differences in disease control rates (DCR) between the analysed groups. In the entire study group, regardless of the treatment line in which the CAPOX regimen was used, DCR was 75.9%; in individual lines: I — 77.3% (n = 167), II — 72.2% (n = 47), and III — 69.2% (n = 9) (P = 0.604).

Table 2 presents the distribution of response to the treatment according to the RECIST 1.1 criteria.

The median overall survival (OS) in the first-line treatment is 19.3 months (95% CI, 17.06–23.5), in the second-line treatment 14.2 (95% CI, 11.61–17.83), and in the third-line treatment 13.96 (95% CI, 11.78–16.73).

There was no grade 4 haematological or non-haematological toxicity in the study group. Grade 3 leukopaenia and neutropaenia were only observed in patients receiving CAPOX regimen in third-line treatment (5.9% — grade 3 leukopaenia, 2.9% — grade 3 neutropaenia). A statistically significant difference in complications

Table 2. Assessment of response to treatment with the CAPOX regimen in individual treatment lines

| Treatment line | Complete response (%) | Partial response (%) | Stable disease (%) | Progressive disease (%) |
|-----------------------|-----------------------|----------------------|--------------------|-------------------------|
| First-line treatment | 4.2 | 35.2 | 38 | 22.6 |
| Second-line treatment | 4.6 | 24.6 | 43.1 | 27.7 |
| Third-line treatment | 0 | 7.7 | 61.5 | 30.8 |

Table 3. Results of treatment of metastatic colorectal cancer with the CAPOX regimen (capecitabine + oxaliplatin) depending on the primary tumour location

| | Right side | Left side | Total |
|----------------------------------|------------------|------------------|------------------|
| DCR (%) | 68.3 | 76.7 | 74.7 |
| Median PFS (months, 95% CI) | 3.9 (3.4–4.5) | 4.2 (3.9–4.5) | 4.1 (3.9–4.4) |
| Median OS (months, 95% CI) | 12.0 (10.0–14.0) | 18.7 (16.4–21.1) | 16.9 (14.9–18.8) |
| First-line mOS [(months, 95% CI) | 12.1 (10.5–13.8) | 20.4 (17.5–23.4) | 19.3 (15.6–23.1) |
| Second-line mOS (months, 95% CI) | 7.2 (6.2–8.2) | 16.1 (12.0–20.1) | 14.2 (11.3–17.1) |

CI — confidence interval; DCR — disease control rate; OS — overall survival; PFS — progression-free survival

according to the treatment line was found only in case of leukopaenia after CAPOX used in third-line treatment ($P < 0.001$). Grade 3 vomiting occurred in 0.9% of patients in first-line treatment and 1.5% in second-line. Grade 3 hand-foot syndrome was observed in 0.5% of patients in first-line treatment. Grade 3 sensory neuropathy was found in 2.8% of patients in first-line treatment and 3% in second-line treatment.

There was a statistically significant increase in the frequency of oxaliplatin dose reduction in subsequent treatment lines (I — 53.5%, II — 69.7%, III — 82.4%; $P = 0.008$) as well as drug withdrawal (I — 12.5%, II — 16.7%, III — 35.3%; $P = 0.034$). No similar difference was found for capecitabine, for which dose reduction rates were similar in all treatment lines (I — 56%, II — 66%, III — 58.8%).

The clinical benefit obtained in the study did not depend on the chemotherapy line in which CAPOX regimen was used in patients with stage IV colorectal cancer.

We did not observe any relationship between the results of CAPOX treatment and primary tumour location in patients treated in the first line (Tab. 3). The percentage of patients achieving disease control was 68.3% for right-sided and 74.7% for left-sided tumour location ($p = 0.188$).

Similarly, the median progression-free survival (PFS) did not differ and was for right-sided and left-sided location 3.9 (95% CI, 3.4–4.5) and 4.2 months (95% CI, 3.9–4.5; $P = 0.443$), respectively. Median PFS for the whole cohort was 4.1 months (95% CI, 3.9–4.4) and was shorter than in published randomised clinical trials for CAPOX regimen (7.1–10.3 months for CAPOX in first-line treatment; 4.7 months for CAPOX in second-line treatment) [3, 4]. The reason for the differ-

ence between our results and data from clinical trials is uncertain, but it is probably due to patient selection for randomised trials.

Median OS in the study population was 16.9 months (95% CI, 14.9–18.8). This value is similar to the results obtained in randomised clinical trials, in which (depending on the study) it was from 16.0 to 24.6 months, average 17–19 months [3, 4]. In one study with use of CAPOX regimen in second-line treatment the median OS was 11.9 months, compared to 14.2 months (95% CI, 11.3–17.0) in an analogous group in our population [6].

However, we observed a statistically significant difference in overall survival for patients stratified according to the primary tumour location. If the tumour was located on the right side of the colon, the median OS was 12.1 months (95% CI, 10.5–13.8), compared to 20.4 months (95% CI, 17.5–23.4) for the disease with left-sided location ($P = 0.014$). In one retrospective study of patients with metastatic colorectal cancer receiving polychemotherapy without targeted drugs, results similar to ours were achieved: for right and left-sided disease the median OS was 13.0 and 17.8 months, respectively [6].

Based on this data, it is difficult to determine whether this difference in OS is to any extent the result of differences in the effectiveness of CAPOX regimen in these two subgroups. It has been reported in many studies, however, that the difference in overall survival is certainly greatly influenced by the more aggressive course of right-sided colorectal cancers [7, 8]. Poorer prognosis of colorectal cancers located on the right side was also confirmed in the group of patients receiving CAPOX regimen in second-line treatment [9].

Discussion

Currently, there are many data related to the effectiveness and toxicity of chemotherapy, but it is worth emphasising the effectiveness of the CAPOX regimen with a significant dose reduction. No grade 4 toxicity was observed, and grade 3 only in 5% of patients.

Particularly interesting is the importance of primary tumour location in the biology of colorectal cancer, and this observation is discussed below. In this context right-sided tumours (proximal to the splenic flexure) and left-sided tumours (distal to this structure) are distinguished.

The biological explanation for differentiating these locations is, among others, distinct embryogenesis of right and left segments of the large intestine (developing from the middle and posterior intestine, respectively), separate vascularisation (superior and inferior mesenteric artery, respectively), and differences in the intestinal microbiome and alternative carcinogenesis pathways occurring in these sections (right-sided cancers more often develop from serrated adenomas or traditional serrated polyps harbouring *BRAF* mutations and/or microsatellite instability; left-sided cancers typically evolve from classic adenomas with *APC* gene mutations) [8].

The distinction between these locations of colorectal cancers also has great prognostic justification. The results of several studies and meta-analyses indicate that cancers located on the left side have a lower risk of death (relative risk in the large meta-analysis 0.82, 95% CI 0.79–0.84) regardless of the presence of other prognostic factors (e.g. clinical stage, chemotherapy, cancer histology, and *BRAF* mutation) [8]. Attention is also paid to the predictive significance of primary tumour location, which can be of great importance when choosing the method of palliative therapy. In patients with left-sided tumours, unequivocal benefit from using anti-EGFR antibodies (e.g. cetuximab, panitumumab) has been proven; in turn, in patients with a primary tumour located on the right side, the use of anti-VEGF antibodies (e.g. bevacizumab) is preferred [11].

Unfortunately, there are very little data available on the impact of colorectal cancer location on response to chemotherapy, including fluoropyrimidines. Based on experimental data, it appears that fluorouracil may be more active in right-sided cancers due to the higher expression of thymidine phosphorylase and lower expression of gamma-glutamyl hydrolase, which promotes higher folic acid levels in cancer cells and higher fluoropyrimidine cytotoxicity [12]. Thymidine phosphorylase is also required to convert the prodrug capecitabine to the active form, fluorouracil [13]; thus, it appears that a higher level of this enzyme in right-sided tumours [14] may contribute to higher capecitabine activity. However, there is no direct evidence confirming this hypothesis.

The negative prognostic value of right-side location of colorectal cancer persists regardless of the treatment used [6, 7]. This does not mean, however, that patients with right-sided tumours do not benefit from chemotherapy; probably the opposite is true: in stage III cancers, adjuvant treatment with fluorouracil or capecitabine with oxaliplatin has a relatively greater benefit in terms of disease-free survival (DFS) in patients with right-sided cancer [15, 16].

Unfortunately, there are also scarce data on the impact of tumour location on chemotherapy results for stage IV cancers. In one study, Negri et al. did not observe differences in objective response rate (ORR) between originally left- and right-sided cancers during treatment with fluorouracil alone or in combination with mitomycin and interferon, although right-sided location was associated with 1.6-times higher risk of death [17].

In the FIRE-1 study comparing FuFIRI (irinotecan, fluorouracil infusion, leucovorin) and mIROX (irinotecan, oxaliplatin) regimens in the first-line treatment, it was found that using the FuFIRI regimen leads to a higher ORR in patients with primary left-sided tumour (33% and 47% for right-sided and left-sided cancer, respectively); however, such differences were not observed for the mIROX regimen (ORR 40% for both locations) [18]. A tendency towards longer OS was also observed when the FuFIRI scheme was used for left-side primary tumour location and the mIROX scheme for right-sided cancer, but these results did not reach statistical significance [18]. Unfortunately, there are no such studies for chemotherapy regimens currently most commonly used in first- and second-line palliative treatment (FOLOX/CAPOX, FOLFIRI/XELIRI), especially taking into consideration the fact that these regimens are nowadays frequently associated with biological drugs for which the location of the primary tumour is a strong predictive factor (i.e. as described above).

An additional issue is the molecular differences between right- and left-sided cancers, which can affect the response to chemotherapy. Particularly important are the differences in the occurrence of microsatellite instability-high (MSI-H) and *BRAF* gene mutations, which are more frequent in cancers originally located on the right side. In the presence of *BRAF* gene mutation (18.4–22.4% of right-sided cancers and 1.3–7.8% of left-sided cancers), which is a poor prognostic factor, patients do not benefit significantly from chemotherapy with fluoropyrimidine, oxaliplatin, or irinotecan [19–22]. In turn, the presence of MSI-H, which is typical for sporadic *BRAF* mutant cancers (52% of patients with *BRAF* mutation also indicate MSI-H), are found in about 5% of metastatic colorectal cancers, almost exclusively right-sided [7]. Tumours with MSI-H are characterised by markedly reduced sensitivity to fluoropyrimidines,

as seen in preclinical studies [22, 23] and confirmed in a number of clinical studies [24–27]. Similarly, the lack of efficacy of fluoropyrimidines is observed in CpG island methylator phenotype (CIMP) cancers, and this is typical for sporadic MSI-H cancers and in cancers of mucocellular histology, which is a manifestation of MSI-H presence [7, 28]. In summary, primary right-sided cancers show a number of molecular features such as MSI-H, CIMP, and *BRAF* mutations that promote resistance to fluorouracil and capecitabine. Molecular aberrations responsible for reduced effectiveness of fluoropyrimidines are found in the absolute minority of right-sided cancers. It remains an open question to what extent these relationships can be extrapolated to all right-sided colorectal cancers.

Conflict of interest

The authors report no conflicts of interest.

References

- Lidia Sc, Meyerhardt J, Winkfield KI, et al. Winkfield K. Colorectal cancer: symptoms and signs cancer.net. Editorial Board. 2018; 11.
- Seufferlein T, Ahn J, Krndjija D, et al. Tumor biology and cancer therapy - an evolving relationship. *Cell Commun Signal.* 2009; 7: 19, doi: [10.1186/1478-811X-7-19](https://doi.org/10.1186/1478-811X-7-19), indexed in Pubmed: [19678929](https://pubmed.ncbi.nlm.nih.gov/19678929/).
- Arkenau HT, Arnold D, Cassidy J, et al. Efficacy of oxaliplatin plus capecitabine or infusional fluorouracil/leucovorin in patients with metastatic colorectal cancer: a pooled analysis of randomized trials. *J Clin Oncol.* 2008; 26(36): 5910–5917, doi: [10.1200/JCO.2008.16.7759](https://doi.org/10.1200/JCO.2008.16.7759), indexed in Pubmed: [19018087](https://pubmed.ncbi.nlm.nih.gov/19018087/).
- Guo Yu, Xiong BH, Zhang T, et al. XELOX vs. FOLFOX in metastatic colorectal cancer: An updated meta-analysis. *Cancer Invest.* 2016; 34(2): 94–104, doi: [10.3109/07357907.2015.1104689](https://doi.org/10.3109/07357907.2015.1104689), indexed in Pubmed: [26864862](https://pubmed.ncbi.nlm.nih.gov/26864862/).
- Rothenberg ML, Cox JV, Butts C, et al. Capecitabine plus oxaliplatin (XELOX) versus 5-fluorouracil/folinic acid plus oxaliplatin (FOLFOX-4) as second-line therapy in metastatic colorectal cancer: a randomized phase III noninferiority study. *Ann Oncol.* 2008; 19(10): 1720–1726, doi: [10.1093/annonc/mdn370](https://doi.org/10.1093/annonc/mdn370), indexed in Pubmed: [18550577](https://pubmed.ncbi.nlm.nih.gov/18550577/).
- Shida D, Tanabe T, Boku N, et al. Prognostic Value of Primary Tumor Sidedness for Unresectable Stage IV Colorectal Cancer: A Retrospective Study. *Ann Surg Oncol.* 2019; 26(5): 1358–1365, doi: [10.1245/s10434-019-07209-x](https://doi.org/10.1245/s10434-019-07209-x), indexed in Pubmed: [30719633](https://pubmed.ncbi.nlm.nih.gov/30719633/).
- Shen H, Yang J, Huang Q, et al. Different treatment strategies and molecular features between right-sided and left-sided colon cancers. *World J Gastroenterol.* 2015; 21(21): 6470–6478, doi: [10.3748/wjg.v21.i21.6470](https://doi.org/10.3748/wjg.v21.i21.6470), indexed in Pubmed: [26074686](https://pubmed.ncbi.nlm.nih.gov/26074686/).
- Lee MS, Menter DG, Kopetz S. Right versus left colon cancer biology: integrating the consensus molecular subtypes. *J Natl Compr Canc Netw.* 2017; 15(3): 411–419, doi: [10.6004/jnccn.2017.0038](https://doi.org/10.6004/jnccn.2017.0038), indexed in Pubmed: [28275039](https://pubmed.ncbi.nlm.nih.gov/28275039/).
- Petrelli F, Tomasello G, Borgonovo K, et al. Prognostic survival associated with left-sided vs right-sided colon cancer: a systematic review and meta-analysis. *JAMA Oncol.* 2017; 3(2): 211–219, doi: [10.1001/jamaoncol.2016.4227](https://doi.org/10.1001/jamaoncol.2016.4227), indexed in Pubmed: [27787550](https://pubmed.ncbi.nlm.nih.gov/27787550/).
- Loupakis F, Yang D, Yau L, et al. Primary tumor location as a prognostic factor in metastatic colorectal cancer. *J Natl Cancer Inst.* 2015; 107(3), doi: [10.1093/jnci/dju427](https://doi.org/10.1093/jnci/dju427), indexed in Pubmed: [25713148](https://pubmed.ncbi.nlm.nih.gov/25713148/).
- Holch JW, Ricard I, Stintzing S, et al. The relevance of primary tumour location in patients with metastatic colorectal cancer: A meta-analysis of first-line clinical trials. *Eur J Cancer.* 2017; 70: 87–98, doi: [10.1016/j.ejca.2016.10.007](https://doi.org/10.1016/j.ejca.2016.10.007), indexed in Pubmed: [27907852](https://pubmed.ncbi.nlm.nih.gov/27907852/).
- Gallois C, Pernot S, Zaanan A, et al. Colorectal cancer: why does side matter? *Drugs.* 2018; 78(8): 789–798, doi: [10.1007/s40265-018-0921-7](https://doi.org/10.1007/s40265-018-0921-7), indexed in Pubmed: [29790124](https://pubmed.ncbi.nlm.nih.gov/29790124/).
- Meropol NJ, Gold PJ, Diasio RB, et al. Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. *J Clin Oncol.* 2006; 24(25): 4069–4077, doi: [10.1200/JCO.2005.05.2084](https://doi.org/10.1200/JCO.2005.05.2084), indexed in Pubmed: [16943524](https://pubmed.ncbi.nlm.nih.gov/16943524/).
- Sadahiro S, Suzuki T, Tanaka A, et al. Association of right-sided tumors with high thymidine phosphorylase gene expression levels and the response to oral uracil and tegafur/leucovorin chemotherapy among patients with colorectal cancer. *Cancer Chemother Pharmacol.* 2012; 70(2): 285–291, doi: [10.1007/s00280-012-1909-8](https://doi.org/10.1007/s00280-012-1909-8), indexed in Pubmed: [22752215](https://pubmed.ncbi.nlm.nih.gov/22752215/).
- Elsaleh H, Joseph D, Grieu F, et al. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet.* 2000; 355(9217): 1745–1750, doi: [10.1016/S0140-6736\(00\)02261-3](https://doi.org/10.1016/S0140-6736(00)02261-3), indexed in Pubmed: [10832824](https://pubmed.ncbi.nlm.nih.gov/10832824/).
- Peng J, Li C, Wang F, et al. Right- and left-sided stage III colon cancers present different prognostic outcomes of oxaliplatin-based adjuvant chemotherapy after curative resection. *Cancer Manag Res.* 2018; 10: 2095–2103, doi: [10.2147/CMAR.S163520](https://doi.org/10.2147/CMAR.S163520), indexed in Pubmed: [30140160](https://pubmed.ncbi.nlm.nih.gov/30140160/).
- Negri FV, Wotherspoon A, Cunningham D, et al. Mucinous histology predicts for reduced fluorouracil responsiveness and survival in advanced colorectal cancer. *Ann Oncol.* 2005; 16(8): 1305–1310, doi: [10.1093/annonc/mdi244](https://doi.org/10.1093/annonc/mdi244), indexed in Pubmed: [15857840](https://pubmed.ncbi.nlm.nih.gov/15857840/).
- Modest DP, Schulz C, von Weikersthal LF, et al. Outcome of patients with metastatic colorectal cancer depends on the primary tumor site (midgut vs. hindgut): analysis of the FIRE1-trial (FuFIRI or mIROX as first-line treatment). *Anticancer Drugs.* 2014; 25(2): 212–218, doi: [10.1097/CAD.0000000000000041](https://doi.org/10.1097/CAD.0000000000000041), indexed in Pubmed: [24201305](https://pubmed.ncbi.nlm.nih.gov/24201305/).
- Morris V, Overman MJ, Jiang ZQ, et al. Progression-free survival remains poor over sequential lines of systemic therapy in patients with BRAF-mutated colorectal cancer. *Clin Colorectal Cancer.* 2014; 13(3): 164–171, doi: [10.1016/j.clcc.2014.06.001](https://doi.org/10.1016/j.clcc.2014.06.001), indexed in Pubmed: [25069797](https://pubmed.ncbi.nlm.nih.gov/25069797/).
- Murcia O, Juárez M, Rodríguez-Soler M, et al. Colorectal cancer molecular classification using BRAF, KRAS, microsatellite instability and CIMP status: Prognostic implications and response to chemotherapy. *PLoS One.* 2018; 13(9): e0203051, doi: [10.1371/journal.pone.0203051](https://doi.org/10.1371/journal.pone.0203051), indexed in Pubmed: [30188916](https://pubmed.ncbi.nlm.nih.gov/30188916/).
- Goldstein J, Tran B, Ensor J, et al. Multicenter retrospective analysis of metastatic colorectal cancer (CRC) with high-level microsatellite instability (MSI-H). *Ann Oncol.* 2014; 25(5): 1032–1038, doi: [10.1093/annonc/mdu100](https://doi.org/10.1093/annonc/mdu100), indexed in Pubmed: [24585723](https://pubmed.ncbi.nlm.nih.gov/24585723/).
- Carethers JM, Chauhan DP, Fink D, et al. Mismatch repair proficiency and in vitro response to 5-fluorouracil. *Gastroenterology.* 1999; 117(1): 123–131, doi: [10.1016/s0016-5085\(99\)70558-5](https://doi.org/10.1016/s0016-5085(99)70558-5), indexed in Pubmed: [10381918](https://pubmed.ncbi.nlm.nih.gov/10381918/).
- Meyers M, Wagner MW, Hwang HS, Kinsella TJ, Boothman DA. Role of the hMLH1 DNA mismatch repair protein in fluoropyrimidine-mediated cell death and cell cycle responses. *Cancer Research* 2001; 61: 5193–5201.
- Ribic CM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *The New England Journal of Medicine* 2003; 349: 247–257.
- Sargent DJ, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *Journal of Clinical Oncology* 2010; 28: 3219–3226.
- Carethers JM, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004; 126: 394–401.
- Jover R, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. *Gut* 2006; 55: 848–855.
- Jover R, et al. 5-Fluorouracil adjuvant chemotherapy does not increase survival in patients with CpG island methylator phenotype colorectal cancer. *Gastroenterology* 2011; 140: 1174–1181.

Neslihan Duzkale¹ , Hikmet Taner Teker² 

¹Department of Medical Genetics, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey

²Department of Molecular Biology, Middle East Technical University, Ankara, Turkey

The study of genetic and clinicopathological characterisation of Turkish bilateral breast cancer patients

Address for correspondence:

Neslihan Duzkale, MD
T.C. Ankara Diskapi Yildirim Beyazit
Training and Research Hospital
Omer Halisdemir Avenue 06110
Altindag/Ankara, Turkey
Phone: +90 505 7754500
Fax: +90 5962308
e-mail: neslihanduzkale@gmail.com

ABSTRACT

Introduction. Although bilateral breast cancers are a rare condition in the general population, the incidence has increased significantly in *BRCA1* and *BRCA2* gene carrier breast cancer patients. Besides the genetic susceptibility, many risk factors such as age, first breast cancer diagnosis age, lifestyle, and environmental factors may be effective in the development of this type of cancer. This study aimed to determine *BRCA1/2* gene carriage in patients with bilateral breast cancer and to find out the risk factors that may lead to contralateral cancer formation.

Material and methods. From 2016 to 2018, in Turkey, we grouped 31 women diagnosed with bilateral breast cancer synchronously and metachronously. Analysis of *BRCA1* and *BRCA2* genes of these women evaluated for clinical and pathological tumour characteristics was performed using the NGS technique.

Results. No significant difference was found between the metachronous (MBBC) and synchronous (SBBC) groups in terms of clinical and pathological tumour characteristics. MBBC patients' age at first diagnosis of breast cancer was lower than SBBC. Also, there was a statistically significant relationship between chronic diseases and MBBC cancers ($\chi^2 = 11.519$; $p = 0.001$). In our study, disease-related variants were found only in three patients, and two of these variants were identified the first time in the literature.

Conclusion. The risk of bilateral breast cancer of *BRCA1/2* carriers increases when the first breast cancer is diagnosed at a young age and there is a significant family history of cancer. MBBC is associated with chronic diseases, and large-scale research will contribute to clarifying this relationship.

Key words: *BRCA*, bilateral breast cancer, metachronous, synchronous, chronic disease

Oncology in Clinical Practice
2021, Vol. 17, No. 1, 6–13
DOI: 10.5603/OCP.2020.0026
Copyright © 2021 Via Medica
ISSN 2450–1654

Oncol Clin Pract 2021; 17, 1: 6–13

Introduction

Breast cancer is the most common type of cancer in women all over the world and is one of the first causes of deaths due to female sex cancers [1]. Although this type of cancer is mostly seen in the unilateral breast, approximately 2% to 11% of all events are bilaterally detected, and the second most common malignancy in breast cancer patients is located in the contralateral breast [2]. The development of diagnostic, screening, and treatment techniques in cancer and increased survival of cancer patients are thought to lead to the more frequent observation of bilateral breast cancer. However, the causes of invasive or in situ histological

types of lobular breast cancer, gene mutation, early detection of breast cancer, and a history of radiation exposure in previous cancer treatment are thought to increase the risk of BBC development [3, 4]. While the risk of BBC developmental cumulative incidence is 3.4% in 10 years in patients with unilateral breast cancer, this rate increases to 13–40% in women with *BRCA* mutation [5, 6]. BBC patients, according to the time elapsed between the detection of tumors in both breasts (although many authors have not yet reached a consensus), can be grouped as synchronous (SBBC) or meta-synchronous (MBBC) [7–10]. The number of studies investigating the clinical and pathological characteristics of both groups is not sufficient in the literature. In this study, we aimed

Table 1. Age parameters of SBBC and MBBC groups

| Variable | Synchronous (n = 14) | | Metasynchronous (n = 17) | | Statistical analysis* Probability |
|------------------------------|----------------------|---------------------|--------------------------|---------------------|--------------------------------------|
| | $\bar{X} \pm S.S.$ | Median [Min–Max] | $\bar{X} \pm S.S.$ | Median [Min–Max] | |
| Age (years) | 52.93 \pm 8.75 | 54.5 [39.0–67.0] | 54.29 \pm 8.91 | 53.0 [42.0–73.0] | t = -0.428 p = 0.672 |
| Age at first ca dia. (years) | 48.43 \pm 8.83 | 49.5 [36.0–66.0] | 40.88 \pm 9.47 | 40.0 [19.0–61.0] | T = 2.276 p = 0.030 |
| Age at sec ca dia. (years) | 48.64 \pm 8.78 | 49.5 [36.0–66.0] | 51.94 \pm 8.61 | 49.0 [40.0–67.0] | t = -1.053 p = 0.301 |

*Independent Samples t-test (T-table value) statistics were used to compare the measurement values of two independent variables in the normal distribution data

to evaluate the demographic and clinical characteristics, pathological details of tumours, and *BRCA1/2* mutation status of patients we grouped as MBBC and SBBC.

Methods

We performed this study in 31 patients who were diagnosed with bilateral breast cancer and referred to the genetics department of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital between June 2016 and January 2018 in order to clarify the genetic aetiology. All patients who participated in the study were in accordance with the National Comprehensive Cancer Network (NCCN) guidelines for *BRCA1/BRCA2* test standards [11]. Of the patients' demographic characteristics, background and family history, age at first and second cancer diagnosis, tumour-node-metastasis (TNM) staging, oestrogen receptor (ER)/progesterone receptor (PR), C-erbB-2 status, etc. were obtained from the patients themselves, their medical records, and the electronic database of the hospital during genetic counseling. Bilateral breast cancer of patients was grouped into SBBC or MBBC based on the interval between the first and contralateral tumours (≤ 12 and > 12 months, respectively) [12]. All patients included in the study were informed about this study and gave written, informed consent for publication. The independent Ethics Committee of the Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital approved this descriptive case series study (Document No. 2020-02/536).

Genetic materials obtained from peripheral blood samples of patients were tested by next-generation sequencing methods to detect germline variants of *BRCA1* and *BRCA2* genes. In the genetic analysis of the patients, the OncoPrint™ *BRCA* Research Assay commercial kit was used, and this analysis was performed on the Ion S5™ System (Ion Torrent™) platform. In this analysis, all exonic regions and the part up to 20 base pairs of exon-intron boundaries were examined. The sequence results were compared

with the human genome of hg19, and Ion Reporter Software Version 5.4 (Thermo Fisher Scientific) was used for bioinformatics analysis. The in silico analysis for the gene variants was performed using SIFT, PolyPhen2, DANN, PROVEAN, GERP, MPC, Mutation Assessor, Fathmm, and Mutation Taster. In this study, genomic changes were identified according to ACMG criteria [13].

SPSS (IBM SPSS Statistics 24) was used for all statistical calculations. Independent sample t-test (t-table value) and Mann-Whitney U test (Z-table value) statistics were used to compare the measurement values of two independent variables. χ^2 cross tables were used in the study of the relations of the two qualitative variables. A p-value less than or equal to 0.05 was considered statistically significant.

Results

Of the 31 patients who were treated and followed up for bilateral breast cancer between January 2016 and December 2017, 14 (45.16%) presented with SBBC and 17 (54.84%) were diagnosed with MBBC, and all of them underwent *BRCA1/2* genes analyses. The median age of all patients was 53 (range 39–73 years) years, SBBC patients were 49.5 (range 36–66) years old, and MBBC patients were 40 (range 1–61) years old. There was a statistically significant difference between breast cancers in terms of breast cancer diagnosis age (t = 2,276; p = 0.030). The time interval between cancers, breast CA first diagnosis age of metasynchronised patients was statistically significantly lower than synchronised patients (Tab. 1).

In terms of the 31 bilateral breast cancer patients, patient demographics and tumour-related general factors are shown in Table 2. In the assessment of smoking status, women who smoked at least 10 cigarettes a day for 10 years or more were included in the positive group. Chronic diseases were recognised as conditions requiring periodic monitoring and supportive care (hypertension, diabetes mellitus, goiter, familial Mediterranean

Table 2. Baseline patient demographics and clinical details

| Characteristic | n (%) | Characteristic | n (%) | Characteristic | n (%) |
|---|---------------------|---|-----------|---|-----------|
| Level of education | | Age at first labour | | Mass size (left) | |
| Elementary school | 9 (29.0) | Nulliparity | 1 (3.2) | ≤ 2 cm | 9 (49.9) |
| High school | 15 (48.4) | < 20 | 3 (9.7) | 2–4 cm | 6 (33.4) |
| University | 7 (22.6) | 20–30 | 23 (74.2) | Multifocal | 3 (16.7) |
| | | > 30 | 4 (12.9) | | |
| Working condition | | First breast ca laterality | | Mass size (right) | |
| Yes | 7 (22.6) | Left | 11 (35.6) | ≤ 2 cm | 9 (45.0) |
| No | 24 (77.4) | Right | 10 (32.2) | 2–≤ 4 cm | 7 (35.0) |
| | | Bilateral | 10 (32.2) | > 4 cm | 2 (10.0) |
| | | | | Multifocal | 2 (10.0) |
| Residence | | Time between cancers | | Metastasis (at the first diagnosis) | |
| Rural | 7 (22.6) | Simultaneously | 10 (32.2) | None | 22 (71.0) |
| City | 24 (77.4) | Simultaneously – ≤ 1 year | 4 (13.0) | Axillar | 7 (22.6) |
| | | 1 year – ≤ 5 years | 3 (9.6) | Bone | 1 (3.2) |
| Smoking | | 5 years – ≤ 10 years | 3 (9.6) | Lung | 1 (3.2) |
| Yes | 7 (22.6) | More than 10 years | 11 (35.6) | | |
| No | 24 (77.4) | | | | |
| BMI | | The first breast ca diagnosis | | Metastasis (at the contralateral breast diagnosis) | |
| Normal | 9 (29.0) | Palpable mass | 14 (41.1) | None | 28 (90.4) |
| Overweight | 13 (42.0) | Swelling and disfigurement | 7 (20.6) | Lung | 2 (6.5) |
| Obese | 9 (29.0) | Nipple discharge | 2 (5.9) | Lung and bone | 1 (3.1) |
| | | Routine check | 11 (32.4) | | |
| Menarche age | | | | | |
| < 12 | 3 (9.7) | | | | |
| 12–14 | 26 (83.8) | | | | |
| > 14 | 2 (6.5) | | | | |
| Menstrual periods | | The second breast ca diagnosis | | Histopathology (left) | |
| Regular | 28 (90.3) | Palpable mass | 5 (15.6) | Ductal carcinoma in situ | 2 (7.1) |
| Irregular | 3 (9.7) | Swelling and disfigurement | 2 (6.2) | Invasive ductal carcinoma | 18 (64.3) |
| | | Routine check | 25 (78.2) | Invasive lobular carcinoma | 5 (17.8) |
| Relation between diagnosis and menopause | | | | Mixed invasive carcinoma | 1 (3.6) |
| Pre-menopausal | 16 (51.6) | | | Musinoz carcinoma | 1 (3.6) |
| After menopause | 15 (48.4) | | | Metaplastic carcinoma | 1 (3.6) |
| | | | | | |
| Breast feeding duration | | Number of relatives with cancer | | Histopathology (right) | |
| No | 2 (6.5) | 1 | 8 (44.4) | Ductal carcinoma in situ | 1 (3.4) |
| ≤ 1 year | 20 (64.5) | 2 | 5 (27.8) | Lobular carcinoma in situ | 1 (3.4) |
| 1–2 years | 7 (22.5) | 3 | 3 (16.7) | Invasive ductal carcinoma | 20 (69.0) |
| More than 2 years | 2 (6.5) | 4 and more | 2 (11.1) | Invasive lobular carcinoma | 6 (20.8) |
| | | | | Invasive apocrine carcinoma | 1 (3.4) |
| Chronic disease/Surgical history | | Relative with breast and over Ca | | | |
| Yes | 19 (61.3)/17 (54.8) | Breast | 17 (85) | | |
| No | 12 (38.7)/14 (45.2) | Over | 3 (15) | | |

fever, asthma, etc). Surgical history group; appendectomy, cholecystectomy, haemorrhoidectomy, etc. Patients undergoing surgeries were included. In the evaluation of cancer history in relatives, all cancers diagnosed in many organs such as breast, ovary, colon, and brain were

included. Relatives diagnosed with breast and ovarian cancer were grouped separately. Metastasis status was determined in all patients after both the initial diagnosis and one-year follow-up. When calculating BMI (kg/m^2), 25.0–29.9 was considered as overweight, 30 and over as

obese, and 18.5–24.9 as healthy. While patients were mostly diagnosed with the first breast cancer because of a palpable mass complaint, the second breast cancer was diagnosed in many patients during their routine checks. The most common breast cancer histopathological type of both breasts was invasive ductal carcinoma, and the mass size was ≤ 2 cm.

For *BRCA1* and *BRCA2* gene analysis, the accession numbers of these genes were accepted as NM_007294.3 (*BRCA1*) and NM_000059.3 (*BRCA2*), respectively. Genomic changes in *BRCA1* and *BRCA2* were detected in only nine (29%) of 31 patients (Tab. 3). Seven of these gene changes were in the *BRCA2* (77.8%) gene, and two were in the *BRCA1* (22.2%) gene and were heterozygous conditions. Of these genomic changes, two were pathogenic, one was probably pathogenic, and the remaining seven were variants of uncertain significance (VUS).

In these genes, two variants which were not reported in the literature and classified as pathogenic by us were detected. The variants NM_007294.3 (*BRCA1*):c.2131_2132delAA (p.Lys711Valfs*6) in patient P28 and NM_000059.3 (*BRCA2*):c.1773_1776delTTAT (p.Ile591Metfs*22) in patient P13 were formed in the exonic regions of the genes. These variants caused a loss of function in the gene by means of the frameshift mutation mechanism. Various insilico predictive analysis programs support that these variants have a deleterious effect on the gene or gene product. The variant c.8954-5a> G, detected in the *BRCA2* gene of P14, was previously reported as a likely pathogenic variant in the literature [14, 15]. P13 and P14 patients were grouped as MBBC, and P28 patients were grouped as SBBC. These three patients had their first breast cancer diagnosis in their 40s and their cancer was first detected in the right breast. These patients had numerous cancerous relatives. Patients first consulted a doctor for a palpable mass in the right breast. After the analysis, these three patients were diagnosed with hereditary breast and ovarian cancer syndrome (HBOC) associated with *BRCA1* and *BRCA2* and were given genetic counseling. Because *BRCA* disease-related variants were seen in a small number of patients in our sample, it was not possible to compare them statistically with others in this group.

VUS variants were detected in *BRCA1/2* genes of the remaining six patients, two of which were reported in the literature [16–19]. The variants detected in patients P2, P23, P24, and P26 had not been previously reported in the literature. Among the patients with VUS variant, P9 was remarkable because she was diagnosed as Hodgkin's disease when she was 32 years old. In this patient, two separate VUSs were detected: NM_000059.3 (*BRCA2*):c.3310A > C (p.Thr1104Pro) and NM_000059.3 (*BRCA2*):c.3503T > A (p.Met1168Lys).

The patient was 54 years old, was first diagnosed with MBBC in the left breast, and her family cancer history was not significant for HBOC. In patients with other VUSs, breast cancer was diagnosed almost exclusively in the left breast (except P24) and grouped as MBBC. In addition to giving genetic counseling to these patients, it was also planned to reevaluate all VUSs determined according to ACMG once every six months. In 10 (58.8%) patients with MBBC, the first cancer was detected in the left breast. The first application of patients in this group was usually due to a mass complaint addressed in the breast.

As a result of the comparison of demographic data of the patients grouped as SBBC and MBBC, a statistically significant relationship was found in the breast where the cancer was first localised ($\chi^2 = 18.850$; $p = 0.000$). There was also a statistically significant relationship between the time interval of cancers and chronic disease ($\chi^2 = 11.519$; $p = 0.001$) (Tab. 4). There was no significant relationship between the two groups in the other demographic data. In addition, histopathological data of tumors in both breasts were compared but no statistically significant result was obtained in the groups. We performed a statistical analysis of the number of children and the number of relatives with cancer variables by grouping according to the first diagnosis age of the patients as ≤ 40 years and > 40 years. The result was not statistically significant ($p > 0.05$).

Discussion

The NCCN guideline recommends the analysis of *BRCA1* and *BRCA2* genes in individuals with bilateral breast cancer [20]. In detecting multiple primer breast cancer of patients, the diagnostic criteria that Warren and Gates first determined in 1932 were used. These criteria include the following: that each tumour is malignant, it has its own pathological features and its own metastatic pathway and the diagnosis of metastatic or recurrent tumours can be excluded, and tumours occur in different parts or organs and are not continuous with each other [21].

In this study of *BRCA1/2* gene analysis findings, demographic characteristics of 31 patients with bilateral breast cancer were investigated, and disease-causing gene variants were identified in three patients. In this way, the aetiology of the disease became clear in these patients. Of these gene variants, the frameshift ones were first described in our patient in the literature. These patients were diagnosed as HBOC and therefore were given genetic counseling. In addition, genetic counseling was given to six patients in whom the VUS variants were identified. VUS classification means that there is insufficient or conflicting evidence regarding a molecular alteration's role in the disease, and hence a periodic

Table 3. BRCA1 and BRCA2 gene analysis results and details

| Patient ID | Gene | Nucleotide change/ AA change | Exon/ intron | Function | ACMG scoring | Age at first/second diagnosis | First diagnosis location in breast | SBBC/ MBBC | Background | Cancer history on relatives | References |
|------------|-------|---|--------------|-----------------|--------------|-------------------------------|------------------------------------|------------|---|--|--|
| P13 | BRCA2 | c.1773_1776delTTAT (p.Ile591Metfs*22) | Exon 10 | Frameshift | PAT | 57 40/52 | RIGHT | MBBC | Thrombosis, Steatosis hepatitis | 1 ⁰ 2 Lung, 1 Stomach, 1 Larynx 2 ⁰ 2 Lung, 1 Breast 3 ⁰ 2 Lung, 1 Breast, 1 Endometrium, 1 Brain | Novel |
| P14 | BRCA2 | c.8954-5A > G (p.?) | Intron22 | Splice Acceptor | L.PAT | 61 43/61 | RIGHT | MBBC | Ovarian cyst, Diabetes | 1 ⁰ 1 Breast 2 ⁰ 2 Breast 3 ⁰ 1 Endometrium, | De Garibay et al. (2014), Santos et al. (2014) |
| P28 | BRCA1 | c.2131_2132delAA (p.Lys711Valfs*6) | Exon 10 | Frameshift | PAT | 43 40/41 | RIGHT | SBBC | - | 1 ⁰ 1 Ovary 2 ⁰ - 3 ⁰ 2 Breast, 1 Ovary, 1 Leukaemia | Novel |
| P2 | BRCA1 | c.694G > T (p.Asp232Tyr) | Exon 10 | Missense | VUS | 43 38/42 | LEFT | MBBC | Haemorrhoid, Hypertension | 1 ⁰ 1 Breast 2 ⁰ 1 Stomach 3 ⁰ 1 Lung, 1 Breast, 1 Stomach | Novel |
| P7 | BRCA2 | c.9364G > A (p.Ala3122Thr) | Exon 25 | Missense | VUS | 61 45/59 | LEFT | MBBC | Tonsillectomy, Steatosis hepatitis | 1 ⁰ - 2 ⁰ - 3 ⁰ - | Tavtigian et al. (2008), Iazzite et al. (2012) |
| P9 | BRCA2 | c.3310A > C (p.Thr1104Pro)/c.3503T > A (p.Met1168Lys) | Exon 11 | Missense | VUS | 65 54/64 | LEFT | MBBC | Hodgkin's disease (Diagnosis: 32), Hypertension | 1 ⁰ 1 Brain 2 ⁰ - 3 ⁰ - | DE Silva et al. (2011), Karbassi et al. (2016) |
| P23 | BRCA2 | c.1160T > C (p.Val387Ala) | Exon 10 | Missense | VUS | 42 19/40 | LEFT | MBBC | Haemorrhoid, Goitre | 1 ⁰ 1 Melanoma 2 ⁰ 1 Colon 3 ⁰ - | Novel |
| P24 | BRCA2 | c.8474C > T (p.Ala2825Val) | Exon 19 | Missense | VUS | 61 39/39 | SAME | SBBC | Cholecystectomy, Appendectomy, FMF, Glaucoma | 1 ⁰ - 2 ⁰ 3 Breast 3 ⁰ 1 Breast | Novel |
| P26 | BRCA2 | c.670G > A (p.Asp224Asn) | Exon 8 | Missense | VUS | 49 40/47 | LEFT | MBBC | Hypertension, Goitre, Myomectomy | 1 ⁰ - 2 ⁰ 1 Prostate 3 ⁰ 1 Endometrium, 1 Breast, 1 Colon | Novel |

Table 4. Significant parameters of SBBC and MBBC groups

| Variable | SBBC (n = 14) | | MBBC (n = 17) | | Statistical analysis* Probability |
|------------------------|---------------|------|---------------|------|--------------------------------------|
| | n | % | n | % | |
| First Ca | | | | | |
| Right | 3 | 21.4 | 7 | 41.2 | $\chi^2 = 18.850$ $p = 0.000$ |
| Left | 1 | 7.2 | 10 | 58.8 | |
| Simultaneous | 10 | 71.4 | | | |
| Chronic disease | | | | | |
| No | 10 | 71.4 | 2 | 11.8 | $\chi^2 = 11.519$ $p = 0.001$ |
| Yes | 4 | 28.6 | 15 | 88.2 | |

* χ^2 cross tables were used to examine the relationships between the two qualitative variables

re-evaluation of the VUS identified in patients in the genetic test was planned.

Although there are many studies in the literature regarding the increase in breast cancer risk in individuals carrying *BRCA1/2* gene mutations, there are fewer reports that determine this risk in contralateral breast cancer. In BRCA carriers, the risk of developing breast cancer until the age of 70 years is approximately 50–87%. These carriers have a 32–64% risk for the development of contralateral breast cancer. In the literature some authors claim that the overall risk for contralateral MBBC is approximately 0.5%, and this risk may reach up to 3% of women with BRCA1/2 carriers, and even a 10-year risk of 13–40% can be reached [22, 23]. In another study, 10-year contralateral breast cancer risk in BRCA1 carriers was reported to be 24%, and the same risk for BRCA2 carriers was 19% [6]. The unquestionable joint consequence obtained as a result of research in the literature is that BBC risk increases in carrier women with disease-related variants of the *BRCA1/2* genes. Weitzel et al. searched women in detecting the first breast cancer diagnose age. And they determined that the diagnosis age of first cancer for BRCA1 and BRCA2 was on average 38.6 and 43.6 years old, respectively. In their study, they also examined the time interval between the two cancer diagnoses and found an average of 5.1 years for BRCA1 carriers and 5.2 years for BRCA2 carriers [24]. In another study, individuals with the *BRCA1* mutation were shown to have a 1.6-fold risk of contralateral breast cancer compared to those with *BRCA2* mutations [23]. Rogozińska-Szczepka et al. determined that the age at first diagnosis of bilateral cancer with BRCA carriers and BRCA non-carriers was at the age of 42 and 49 years, respectively [25].

The importance of the first diagnosis age in breast cancer was emphasised in a study conducted by Metcalfe et al., who found that ‘women diagnosed with breast cancer under the age of 40 had a 42% risk of developing contralateral breast cancer for 15 years and an annual risk of 2.8%. The same risk decreased to 19% in women who had their first diagnosis after

50 years of age and the annual risk was 1.3%’ [6]. Graeser et al. conducted a similar study in relatives of *BRCA1* mutation carriers and found that ‘those who received first diagnosis with breast cancer younger than 40 years of age had an increased 25-year contralateral breast cancer risk compared to those older than 50 years (63% and 20%, respectively). The annual risk ratios were 2.5% in the young group and 0.8% in the other group’ [23].

In our study, we first examined 31 patients demographically. We then grouped all patients as SBBC and MBBC and compared them for tumour characteristics. Fourteen of the patients (45.16%) were grouped as SBBC, and 17 (54.84%) were grouped as MBBC. The median age was 53 years for all patients, 49.5 years for SBBC patients, and 40 years for MBBC patients, and all values were the same as those in the literature [26]. 32.4% of the patients were diagnosed with first breast cancer and 78.2% with second breast cancer during routine controls. For this reason, the fact that both healthy and breast cancer women are subject to routine checks plays an important role in the early diagnosis and determination of treatment options for this disease. In the literature, it has been illustrated that young patients are vulnerable to MBCC [7].

There was a statistically significant relationship between chronic disease and MBBC in our patient group ($p = 0.001$). The mean age of first and second cancer diagnosis in the MBCC group was 40 and 49 years, respectively. The time interval between the two cancer diagnoses was 11 years (35.6) or more. It was found that 10 (71.4%) of the patients with SBBC had no chronic disease and 15 (88.2%) patients with MBBC had a chronic disease. Among these diseases, goitre, hypertension, diabetes mellitus, migraine, and some inflammatory diseases such as Behcet’s disease and familial Mediterranean fever can be considered. All these diseases require periodic monitoring, medical supportive care, and/or drug therapy. In order to investigate this relationship in more detail, it is important to divide chronic diseases into subgroups in larger

patient groups and to question the relationship between the time of diagnosis of these subgroups and the duration of diagnosis of first and second breast cancer. In fact, chronic diseases diagnosed after the treatment of cancer, which is itself a kind of chronic disease, may be triggered by the long-term side effects of this treatment. Also, a chronic disease in the organism may provide a basis for facilitating the development of contralateral breast cancer due to itself and/or treatment. In the literature, a study showing an increased relationship of MBCC compared to SBBC in chronic diseases has not been published. The results of this study, which was carried out for the first time in a small group of patients in Turkey, should be confirmed in a large number of patient groups, and the underlying cause of this condition should be discovered.

In our patient groups, following the literature, there was a statistically significant difference between breast cancers in terms of breast cancer diagnosis age ($p = 0.030$). An aspect of the time interval between cancers was that the breast cancer first diagnosis age of MBBC patients was statistically lower than that of SBBC patients. A clear difference was not found between the tumour characteristics of both groups clinicopathologically [12].

Conclusion

Women with bilateral breast cancer who have a *BRCA* mutation carrier receive their first breast cancer diagnosis at an early age and have a remarkable family history of cancer. MBBC patients receive their first diagnosis at an earlier age than those with SBBC. For the first time in the literature, this study demonstrated a significant association between MBBC with chronic diseases and SBCC. Increasing the number of patients and conducting larger-scale studies will help clarify the uncertainties in the relationship between chronic diseases and MBBC.

Ethical compliance

The independent Ethics Committee approved this study.

Conflicts of interest



The authors declare to have no conflict of interest.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36

- cancers in 185 countries. *CA Cancer J Clin*. 2018; 68(6): 394–424, doi: [10.3322/caac.21492](https://doi.org/10.3322/caac.21492), indexed in Pubmed: [30207593](https://pubmed.ncbi.nlm.nih.gov/30207593/).
2. Soerjomataram I, Louwman WJ, de Vries E, et al. Primary malignancy after primary female breast cancer in the South of the Netherlands, 1972–2001. *Breast Cancer Res Treat*. 2005; 93(1): 91–95, doi: [10.1007/s10549-005-4016-2](https://doi.org/10.1007/s10549-005-4016-2), indexed in Pubmed: [16184464](https://pubmed.ncbi.nlm.nih.gov/16184464/).
3. Intra M, Rotmensz N, Viale G, et al. Clinicopathologic characteristics of 143 patients with synchronous bilateral invasive breast carcinomas treated in a single institution. *Cancer*. 2004; 101(5): 905–912, doi: [10.1002/cncr.20452](https://doi.org/10.1002/cncr.20452), indexed in Pubmed: [15329896](https://pubmed.ncbi.nlm.nih.gov/15329896/).
4. Cook LS, White E, Schwartz SM, et al. A population-based study of contralateral breast cancer following a first primary breast cancer (Washington, United States). *Cancer Causes Control*. 1996; 7(3): 382–390, doi: [10.1007/BF00052945](https://doi.org/10.1007/BF00052945), indexed in Pubmed: [8734833](https://pubmed.ncbi.nlm.nih.gov/8734833/).
5. Lu W, Schaapveld M, Jansen L, et al. The value of surveillance mammography of the contralateral breast in patients with a history of breast cancer. *Eur J Cancer*. 2009; 45(17): 3000–3007, doi: [10.1016/j.ejca.2009.08.007](https://doi.org/10.1016/j.ejca.2009.08.007), indexed in Pubmed: [19744851](https://pubmed.ncbi.nlm.nih.gov/19744851/).
6. Metcalfe K, Gershman S, Lynch HT, et al. Predictors of contralateral breast cancer in *BRCA1* and *BRCA2* mutation carriers. *Br J Cancer*. 2011; 104(9): 1384–1392, doi: [10.1038/bjc.2011.120](https://doi.org/10.1038/bjc.2011.120), indexed in Pubmed: [21487411](https://pubmed.ncbi.nlm.nih.gov/21487411/).
7. Hartman M, Czene K, Reilly M, et al. Incidence and prognosis of synchronous and metachronous bilateral breast cancer. *J Clin Oncol*. 2007; 25(27): 4210–4216, doi: [10.1200/JCO.2006.10.5056](https://doi.org/10.1200/JCO.2006.10.5056), indexed in Pubmed: [17878475](https://pubmed.ncbi.nlm.nih.gov/17878475/).
8. Hartman M, Czene K, Reilly M, et al. Genetic implications of bilateral breast cancer: a population based cohort study. *Lancet Oncol*. 2005; 6(6): 377–382, doi: [10.1016/S1470-2045\(05\)70174-1](https://doi.org/10.1016/S1470-2045(05)70174-1), indexed in Pubmed: [15925815](https://pubmed.ncbi.nlm.nih.gov/15925815/).
9. Kuo WH, Yen AMF, Lee PH, et al. Incidence and risk factors associated with bilateral breast cancer in area with early age diagnosis but low incidence of primary breast cancer: analysis of 10-year longitudinal cohort in Taiwan. *Breast Cancer Res Treat*. 2006; 99(2): 221–228, doi: [10.1007/s10549-006-9194-z](https://doi.org/10.1007/s10549-006-9194-z), indexed in Pubmed: [16544057](https://pubmed.ncbi.nlm.nih.gov/16544057/).
10. Jobsen JJ, van der Palen J, Ong F, et al. Bilateral breast cancer, synchronous and metachronous; differences and outcome. *Breast Cancer Res Treat*. 2015; 153(2): 277–283, doi: [10.1007/s10549-015-3538-5](https://doi.org/10.1007/s10549-015-3538-5), indexed in Pubmed: [26268697](https://pubmed.ncbi.nlm.nih.gov/26268697/).
11. Beck AC, Yuan H, Liao J, et al. Rate of *BRCA* mutation in patients tested under NCCN genetic testing criteria. *Am J Surg*. 2020; 219(1): 145–149, doi: [10.1016/j.amjsurg.2019.06.012](https://doi.org/10.1016/j.amjsurg.2019.06.012), indexed in Pubmed: [31255259](https://pubmed.ncbi.nlm.nih.gov/31255259/).
12. Ozturk A, Alco G, Sarsenov D, et al. Synchronous and metachronous bilateral breast cancer: A long-term experience. *J BUON*. 2018; 23(6): 1591–1600, indexed in Pubmed: [30610782](https://pubmed.ncbi.nlm.nih.gov/30610782/).
13. Richards S, Aziz N, Bale S, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015; 17(5): 405–424, doi: [10.1038/gim.2015.30](https://doi.org/10.1038/gim.2015.30), indexed in Pubmed: [25741868](https://pubmed.ncbi.nlm.nih.gov/25741868/).
14. de Garibay GR, Aceido A, Garcia-Casado Z, et al. Capillary electrophoresis analysis of conventional splicing assays: IARC analytical and clinical classification of 31 *BRCA2* genetic variants. *Hum Mutat*. 2014; 35(1): 53–57, doi: [10.1002/humu.22456](https://doi.org/10.1002/humu.22456), indexed in Pubmed: [24123850](https://pubmed.ncbi.nlm.nih.gov/24123850/).
15. Santos C, Peixoto A, Rocha P, et al. Pathogenicity evaluation of *BRCA1* and *BRCA2* unclassified variants identified in Portuguese breast/ovarian cancer families. *J Mol Diagn*. 2014; 16(3): 324–334, doi: [10.1016/j.jmoldx.2014.01.005](https://doi.org/10.1016/j.jmoldx.2014.01.005), indexed in Pubmed: [24607278](https://pubmed.ncbi.nlm.nih.gov/24607278/).
16. Tazzite A, Jouhadi H, Nadifi S, et al. *BRCA1* and *BRCA2* germline mutations in Moroccan breast/ovarian cancer families: novel mutations and unclassified variants. *Gynecol Oncol*. 2012; 125(3): 687–692, doi: [10.1016/j.ygyno.2012.03.007](https://doi.org/10.1016/j.ygyno.2012.03.007), indexed in Pubmed: [22425665](https://pubmed.ncbi.nlm.nih.gov/22425665/).
17. Tavtigian SV, Byrnes GB, Goldgar DE, et al. Classification of rare missense substitutions, using risk surfaces, with genetic- and molecular-epidemiology applications. *Hum Mutat*. 2008; 29(11): 1342–1354, doi: [10.1002/humu.20896](https://doi.org/10.1002/humu.20896), indexed in Pubmed: [18951461](https://pubmed.ncbi.nlm.nih.gov/18951461/).
18. De Silva S, Tennekoon KH, Karunanayake EH, et al. Novel sequence variants and common recurrent polymorphisms of *BRCA2* in Sri Lankan breast cancer patients and a family with *BRCA1* mutations. *Exp Ther Med*. 2011; 2(6): 1163–1170, doi: [10.3892/etm.2011.337](https://doi.org/10.3892/etm.2011.337), indexed in Pubmed: [22977638](https://pubmed.ncbi.nlm.nih.gov/22977638/).
19. Karbassi I, Maston GA, Love A, et al. A standardized DNA variant scoring system for pathogenicity assessments in mendelian disorders. *Hum Mutat*. 2016; 37(1): 127–134, doi: [10.1002/humu.22918](https://doi.org/10.1002/humu.22918), indexed in Pubmed: [26467025](https://pubmed.ncbi.nlm.nih.gov/26467025/).
20. Goetz MP, Gradishar WJ, Anderson BO, et al. NCCN Guidelines Insights: Breast Cancer, Version 3.2018. *J Natl Compr Canc Netw*.

- 2019; 17(2): 118–126, doi: [10.6004/jnccn.2019.0009](https://doi.org/10.6004/jnccn.2019.0009), indexed in Pubmed: [30787125](https://pubmed.ncbi.nlm.nih.gov/30787125/).
21. Warren S, Gates O. Multiple primary malignant tumors. *Am J Cancer*. 1932; 16: 1358.
22. Narod SA. Bilateral breast cancers. *Nat Rev Clin Oncol*. 2014; 11(3): 157–166, doi: [10.1038/nrclinonc.2014.3](https://doi.org/10.1038/nrclinonc.2014.3), indexed in Pubmed: [24492834](https://pubmed.ncbi.nlm.nih.gov/24492834/).
23. Graeser MK, Engel C, Rhiem K, et al. Contralateral breast cancer risk in BRCA1 and BRCA2 mutation carriers. *J Clin Oncol*. 2009; 27(35): 5887–5892, doi: [10.1200/JCO.2008.19.9430](https://doi.org/10.1200/JCO.2008.19.9430), indexed in Pubmed: [19858402](https://pubmed.ncbi.nlm.nih.gov/19858402/).
24. Weitzel JN, Robson M, Pasini B, et al. A comparison of bilateral breast cancers in BRCA carriers. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(6): 1534–1538, doi: [10.1158/1055-9965.EPI-05-0070](https://doi.org/10.1158/1055-9965.EPI-05-0070), indexed in Pubmed: [15941968](https://pubmed.ncbi.nlm.nih.gov/15941968/).
25. Rogozińska-Szczepka J, Utracka-Hutka B, Grzybowska E, et al. BRCA1 and BRCA2 mutations as prognostic factors in bilateral breast cancer patients. *Ann Oncol*. 2004; 15(9): 1373–1376, doi: [10.1093/annonc/mdh352](https://doi.org/10.1093/annonc/mdh352).
26. Sim Y, Tan VKM, Sidek NAB, et al. Bilateral breast cancers in an Asian population, and a comparison between synchronous and metachronous tumours. *ANZ J Surg*. 2018; 88(10): 982–987, doi: [10.1111/ans.14773](https://doi.org/10.1111/ans.14773), indexed in Pubmed: [30141242](https://pubmed.ncbi.nlm.nih.gov/30141242/).

Marcin Kaczor^{1, 2} , Wojciech Staśkiewicz², Monika Homa², Magdalena Górecka², Rafał Wójcik², Piotr Potemski³ 

¹Jagiellonian University Medical College, Krakow

²Aestimo, Krakow

³Department of Cancer Chemotherapy Medical University of Lodz, WWCOiT M. Copernicus, Lodz

Bevacizumab or standard chemotherapy in previously treated patients with metastatic colorectal cancer — a systematic review

Address for correspondence:

Dr n. med. Marcin Kaczor
II Katedra Chorób Wewnętrznych
im. prof. Andrzeja Szczeklika
Uniwersytet Jagielloński
Collegium Medicum w Krakowie
ul. Skawińska 8, 31-066 Kraków
e-mail: marcin.kaczor@uj.edu.pl

ABSTRACT

Introduction. The *BRAF V600E* mutation (*BRAFmt*) occurring in the metastatic colorectal cancer (mCRC) patients is associated with poorer prognosis, in comparison to the wild-type variant of the *BRAF* gene (*BRAFwt*). Aim of this work was to assess the clinical efficacy of bevacizumab (BEVA) or standard chemotherapy (ChT) in the 2nd or further lines of treatment in mCRC *BRAFmt* population.

Material and methods. MEDLINE/PubMed, Embase and Cochrane CENTRAL databases were systematically searched. The reference lists of relevant studies were also checked.

Results. 6 eligible trials were identified: MOMA (BEVA ± ChT), allowing for limited overall survival (OS) assessment, WJOG 6210G (BEVA + FOLFIRI), RAISE and 20050181 (FOLFIRI), PICCOLO and Spindler 2013 (irinotecan monotherapy). None of those trials were designed for the treatment evaluation in *BRAFmt* population. Available evidence was restricted to limited analyses in small subgroups (from a few to several dozens of patients), occasionally comprising *RAS* gene mutation (*RASmt*) as well. Based on the identified studies, the comparison of BEVA ± ChT vs. ChT or among different ChTs in *BRAFmt* population was not feasible.

In case of BEVA (MOMA), OS hazard ratio (HR) for *BRAFmt* vs. *BRAFwt* was 1.52 (95% CI: 0.79–2.89) with difference in medians equal to 12.1 months (19.2 vs. 31.3 months, respectively), and *BRAFmt* or *RASmt* patients had median OS lower by 7.9 months and median progression free survival (PFS) by 3.0 months in WJOG 6210G trial. In case of ChT, median PFS was lower in *BRAFmt* by 12–67% (HRs range: 1.01–5.3), and median OS by 34–73% (HRs range: 1.05–5.00).

Conclusions. Due to limited clinical evidence, assessment of further lines of treatment in *BRAFmt* mCRC patients is uncertain, however existing data consistently suggest lower effectiveness of BEVA ± ChT or ChT in *BRAFmt*, than in *BRAFwt* subgroup. Hopefully, combining anti-EGFR therapies with BRAF/MEK inhibitor is expected to improve prognosis of those patients.

Key words: BRAF, colorectal cancer, systematic review, bevacizumab, chemotherapy

Oncology in Clinical Practice
2021, Vol. 17, No. 1, 14–27
DOI: 10.5603/OCP.2020.0011

Translation: dr n. med. Dariusz Stencel
Copyright © 2021 Via Medica
ISSN 2450–1654

Oncol Clin Pract 2021; 17, 1: 14–27

Introduction

Substitution of valine (Val) with glutamic acid (Glu) in codon 600 (*V600E*) of the proto-oncogenic BRAF kinase gene that is part of the mitogen-activated protein

kinase (MAPK or RAS-RAF-MEK-ERK) signalling pathway, is present in 8–12% of metastatic colorectal cancer (mCRC) cases, more often with right-sided primary tumor location [1, 2]. This pathway plays an essential role in the regulation of cell proliferation,

differentiation, survival, and apoptosis, it is also responsible for signal transduction from growth factor receptors, including epidermal growth factor receptor (EGFR) [1–3]. This point mutation leads to constitutive kinase phosphorylation, which drives sustained activation of MAPK signalling pathway. The mechanism of this process has not been fully understood, but it seems that cancers with such a genetic abnormality constitute a distinct phenotypic group [2, 4–6]. The V600E *BRAF* mutation is detected in 40–60% of sporadic cancers with microsatellite instability but almost never in Lynch syndrome (about 1%) and in tumors with *KRAS* and *NRAS* mutations [1, 2, 7]. However, the co-occurrence of *BRAF* aberration and microsatellite instability may be associated with a better prognosis by abolishing the opposing effects of both genetic changes [2, 7, 8]; this mechanism is not fully understood [2]. It is widely accepted that the presence of *BRAF V600E* mutation in patients with colorectal cancer is associated with a poor prognosis at any stage of the disease [1, 2], and the median overall survival may be up to three times lower compared to patients with a wild-type gene variant [9]. *BRAF* mutations other than V600 occur much less frequently and most likely bear no adverse prognostic significance [1, 2].

Most available data in mCRC patients relate to first-line treatment; there are no clear differences in progression-free survival (PFS) when chemotherapy alone is used, however, overall survival is markedly shorter in *BRAF*mt group [2, 10]. Despite the limited scientific evidence, bevacizumab added to FOLFOXIRI chemotherapy is currently the recommended molecularly targeted drug in the first-line treatment of advanced disease [9–14]. However, according to available clinical data [15, 16], a response to anti-EGFR therapy (cetuximab, panitumumab) is unlikely, and the occurrence of the *V600E* mutation is a contraindication to such treatment unless it is combined with anti-*BRAF* therapy [7, 14]. There is scarce data concerning the clinical efficacy of further treatment lines. The aim of this study was to systematically review the clinical trials assessing bevacizumab or irinotecan- or oxaliplatin-based chemotherapy in second and further treatment lines of mCRC with *BRAF* mutation.

Methods

A systematic search of MEDLINE, Embase and Cochrane CENTRAL databases was conducted on August 5, 2019. The search strategy included all types of studies, i.e. secondary and primary, including both randomized and non-randomized clinical trials, as well as non-controlled ones, assessing the use of bevacizumab or chemotherapy containing irinotecan

or oxaliplatin in second or further treatment lines in advanced CRC. Studies assessing clinical efficacy (OS, PFS, objective response rate — ORR) in patients with the *BRAF V600E* gene mutation were included, encompassing comparative assessment between sought interventions in the target population or assessment in relation to patients without the *BRAF* mutation. The defined strategy also allowed to find secondary studies. Detailed information on the search strategy and systematic review is provided in the Supplemental materials (Tab. S1, S2, Fig. S1).

Two-stage publication selection (titles and abstracts analysis followed by full texts analysis) in accordance with the defined PICOS scheme (Tab. S2) as well as the assessment of study quality and risk of bias in the ROB 2.0 [17] and ROBINS-I [18] scales were performed by two independently working researchers (W.S., M.H.) (Tab. S3). Data extraction was carried out in pairs in which one of the persons checked the correctness of the data. Doubts were discussed with the third person (M.K.) until consensus was reached. The above assumptions were pre-determined before the actual review. Presenting the results, the data for *BRAF*mt subgroup were extracted, referring to *BRAF*wt group when possible. In some cases, the necessary calculations were made to present the result for *BRAF*mt vs. *BRAF*wt comparison and based on available data the relative benefit (RB), response rate and a difference in median survival were estimated. The systematic review was carried out in accordance with current Health Technology Assessment (HTA) guidelines of the Agency for Health Technology Assessment and Tariffs (AOTMiT, Agencja Oceny Technologii Medycznych i Taryfikacji) [19].

Results

As a result of the systematic review (Tab. S1), six primary trials (presented in six publications) were found: MOMA [20], WJOG 6210G [21], RAISE [22], 20050181 [23], PICCOLO [24] and Spindler 2013 [25] (Fig. S1). The results of additional analysis of data from the PICCOLO — Seligmann 2016 study were also taken into account [10]. Five of the included studies [20–24] were randomized clinical trials (RCT), but none of them was specifically targeted at the population with *BRAF* mutation — determination of this mutation was not required by inclusion criteria, and the assessment of the significance of *BRAF* mutation was exploratory and included only a subgroup of patients with available material and genotyping results. Furthermore, each study in one arm used intervention not included in the criteria of the presented review — the combination of anti-EGFR or anti-VEGF drug with chemotherapy. In one study [20] only limited assessment of OS was pos-

sible, including the use of BEVA with chemotherapy in the next treatment line after disease progression in the majority of patients. Only the observational study Spindler 2013 [25] was aimed at assessing the impact of *BRAF* mutations. Available results were sufficient only for analysis of clinical efficacy within a small *BRAF*mt subgroups (from several to several dozen patients), sometimes including *RAS*mt [21] and referring them to *BRAF*wt population. The identified studies did not allow for comparative assessment of BEVA with chemotherapy vs. CHT or various CHTs within the *BRAF*mt population. No systematic reviews were found assessing the use of the given intervention in further treatment lines. The characteristics of the included trials are presented in Table S4 and the main results are summarized in Table 1. No meta-analyses of the results were performed due to high clinical heterogeneity.

Bevacizumab (BEVA) + chemotherapy

In the MOMA trial (Cremolini 2019) [20], 232 patients with mCRC were randomized to one of two protocols: 8 cycles of first-line induction therapy with FOLFOXIRI + BEVA, followed by maintenance therapy continued to disease progression — BEVA or BEVA + metronome chemotherapy (capecitabine and cyclophosphamide). Central determination of *BRAF* (exon 15 [V600E] assessment with use of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight MassARRAY system or *RAS* gene mutations was performed in 203 patients, and in 20 (10%) patients mutated *BRAF* status was detected. During a median follow-up of 47.8 months, a total of 210 patients progressed and 152 (72%) received next treatment line, of which 91 (60%) were re-treated with BEVA + FOLFOXIRI, and 31 (20%) — BEVA + FOLFIRI/FOLFOX, and 3 (2%) — BEVA + fluorouracil. In total, BEVA was used in 82% of patients receiving the subsequent treatment line. Therefore, overall survival (OS) analysis also included the use of BEVA in the second treatment line, however, it can be assumed that the observation concerned a maximum of approximately 11 *BRAF*mt patients who had progressed and received BEVA again in the next line. In the *BRAF*mt population, the median OS was 19.2 months and was significantly lower than in *RAS*wt and *BRAF*wt patients ($N = 36$) — 31.3 months (difference of 12.1 months), similarly to *RAS*mt ($N = 150$) — 24.9 months (difference of 6.4 months). In the whole group, the risk of death at a given time point was higher for *BRAF*mt compared to *BRAF*wt and *RAS*wt, but the difference did not reach the statistical significance threshold: HR = 1.52 (95% CI: 0.79–2.89), $P = 0.208$ [20] (Fig. 1.).

The evaluation of bevacizumab in further treatment lines was also carried out in a randomized West Japan

Oncology Group (WJOG 6210G) study (Shitara 2016) [21], which included patients with mCRC or inoperable, locally advanced CRC, with clinically or radiologically confirmed progression during or up to 3 months after the last dose of first-line chemotherapy with fluoropyrimidine, oxaliplatin and bevacizumab. In addition, it was required to exclude *KRAS* gene mutation (*KRAS*wt) in exon 2 (codon 12 or 13) in the central or local evaluation of paraffin-embedded tumor tissue. The study included 121 patients who were randomized to receive BEVA + FOLFIRI or panitumumab + FOLFIRI. Two patients in each group were excluded from further efficacy analysis due to failure to meet inclusion criteria. After progression, 77.8% of patients received another line of treatment, of which 34.1% received bevacizumab. In addition, 109 patients underwent extended genetic profiling covering *KRAS* and *NRAS* gene mutations — exon 2 (codons 12 and 13), exon 3 (codons 59, 61, 117 and 146) and *BRAF* — exon 15 (codon 600) using next-generation sequencing (NGS) of circulating tumor DNA in serum. *BRAF* gene mutation was detected in 5 (4.6%) patients and *RAS* genes mutations in 14 (12.8%) patients. The results are presented in a way that allows comparison of the combined subgroup with *BRAF* or *RAS* mutation with tumors without mutations in the tested genes (wild-type). Among *BRAF*mt or *RAS*mt patients receiving BEVA + FOLFIRI treatment in the second line ($N = 11$), the median OS was 8.2 months (95% CI: 6.0–13.7) and was 7.9 months lower compared to wild type subgroups ($N = 44$) — 16.1 months (95% CI: 12.7–21.1). The median PFS was lower by approximately 3 months in the *BRAF*mt and *RAS*mt groups: 3.7 months (95% CI: 1.8–6.0) vs. 6.7 months (95% CI: 5.4–9.4), respectively. The authors also reported that among patients with measurable disease receiving BEVA + FOLFIRI, the objective response rate in the *BRAF*mt or *RAS*mt subgroup was 18.2% and 2.6% in non-mutated patients, respectively. The available data did not allow further calculations, and when interpreting the results it should also be considered that in the BEVA + FOLFIRI group only 3 patients achieved an objective response [21].

Chemotherapy

The assessment of chemotherapy in further treatment lines in patients with mCRC harboring *BRAF* mutation was based on four clinical trials, two of which enabled the evaluation of FOLFIRI scheme: RAISE [22] and 20050181 [23]; and another two irinotecan monotherapy: PICCOLO [24] and Spindler 2013 [25].

The RAISE study evaluated the efficacy and safety of ramucirumab combined with FOLFIRI compared to placebo + FOLFIRI in patients with progression of mCRC during or within 6 months after the last dose of

Table 1. The main results of studies enabling the assessment of clinical efficacy in patients with BRAF V600E-mutated mCRC

| Study | Sample size | OS | PFS | ORR | RoB |
|--|---|--|---|---|----------|
| Bevacizumab ± chemotherapy | | | | | |
| MOMA [20] | BRAFmt vs. BRAFwt and RASwt ¹ : 20 vs. 36 | Median (-39%*): 19.2 vs. 31.3 months HR = 1.52 (95% CI: 0.79–2.89); P = 0.208 | – | – | Critical |
| WJOG 6210G [21] | BRAFmt or RASmt vs. BRAFwt and RASwt ^{2,3} : 11 vs. 44 | Median (-49%*): 8.2 (95% CI: 6.0–13.7) vs. 16.1 (95% CI: 12.7–21.1) months | Median (-45%*): 3.7 (95% CI: 1.8–6.0) vs. 6.7 (95% CI: 5.4–9.4) months | 18.2% vs. 2.6% ⁴ | Critical |
| FOLFIRI | | | | | |
| RAISE [22] | BRAFmt vs. BRAFwt and RASwt ⁵ : 21 vs. 143 | Median (-73%*): 4.2 vs. 15.5 months | Median (-53%*): 2.7 vs. 5.7 months | – | Critical |
| 20050181 [23] | BRAFmt and RASwt vs. BRAFwt and RASwt ⁶ : 23 vs. 190 | Median (-63%*): 5.7 vs. 15.4 months HR* = 5.00 (95% CI: 3.03–7.69) | Median (-67%*): 1.8 vs. 5.5 months HR* = 3.23 (95% CI: 1.96–5.26) | – | Critical |
| Irinotecan monotherapy | | | | | |
| PICCOLO [24] | BRAFmt and KRASwt vs. BRAFwt and RASwt and PIK3CAwt ⁷ : 31 vs. 163 | HR = 1.56 (95% CI: 1.03; 2.37); P = 0.035 | – | n/N: 6.5%* (2/31) vs. 12.3%* (20/163) RB* = 0.53 (95% CI: 0.13–2.14); P = 0.3688 | Critical |
| PICCOLO — additional analysis [10] | BRAFmt vs. BRAFwt: 40 vs. 419 | Median (-34%*): 6.7 (95% CI: 3.9–18.6) vs. 10.2 (95% CI: 5.4–18.1) months HR ⁸ = 1.21 (95% CI: 0.84–1.76); P = 0.31 | Median (-12%*): 3.5 (95% CI: 2.6–7.3) vs. 4.0 (95% CI: 2.7–8.0) months HR ⁸ = 1.01 (95% CI: 0.69–1.49); P = 0.93 | 5.0% vs. 8.1% OR ⁸ = 0.56 (95% CI: 0.13–2.49); P = 0.45 | Serious |
| Spindler 2013 — prospective cohort [25] | BRAFmt vs. BRAFwt: 8 vs. 89 | HR* = 3.33 (95% CI: 0.96–11.11) | HR* = 3.57 (95% CI: 0.99–12.50) | 0% vs. 14% (NS) | Critical |
| Spindler 2013 — retrospective cohort [25] | BRAFmt vs. BRAFwt: 8 vs. 101 | HR* = 1.05 (95% CI: 0.45–2.50) | HR* = 1.79 (95% CI: 0.70–4.55) | 0% vs. 15% (NS) | Critical |
| Spindler 2013 — multivariate analysis ⁹ [25] | – | HR = 4.3 (95% CI: 1.7–10.6); P = 0.002 | HR = 5.3 (95% CI: 2.1–13.0); P = 0.002 | – | Critical |

OS — overall survival; PFS — progression-free survival; ORR — objective response rate; RoB — risk of bias; HR — hazard ratio; RB — relative benefit; OR — odds ratio; NS — not significant

RoB is the lowest rating among the ROBINS-I scale domains. For almost all included studies, this arises from a critical risk assessment resulting from the presence of interfering factors in the study population (the exception is PICCOLO — additional analysis, where this risk was assessed as serious).

*Estimated based on available data; ¹overall survival (OS) analysis included the use of BEVA in 2nd treatment line (for BRAFmt population it can be assumed that the follow-up ultimately involved a maximum of approximately 11 patients who had progressed and re-received BEVA in the next treatment line); ²in BEVA + FOLFIRI subgroup; ³including three BRAFmt patients, taking into account that in total in BRAFmt or RASmt group they accounted for 26%; ⁴the total number of patients with objective response in the BEVA + FOLFIRI group was 3; ⁵in placebo + FOLFIRI subgroup; ⁶in FOLFIRI subgroup; ⁷in irinotecan monotherapy subgroup; ⁸results adjusted to response to previous treatment, performance status, presence of peritoneal metastases, primary tumor resection, and tumor location; ⁹multivariate analysis taking into account age, performance status and BRAF and KRAS genes status

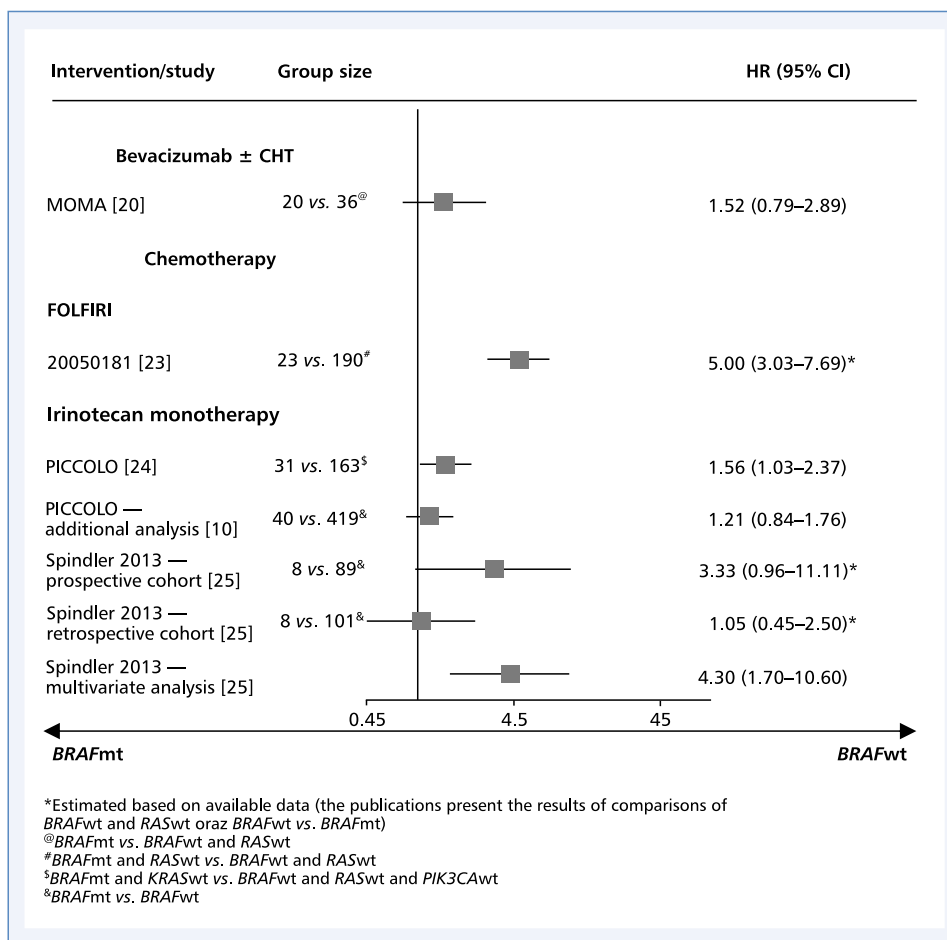


Figure 1. Hazard ratio (HR) for overall survival (OS)

first-line therapy of metastatic disease, including bevacizumab, oxaliplatin and fluoropyrimidine, if they received at least one cycle of therapy [22]. In total, 1,072 patients were included, of which exploratory assessment of the effect of RAS and BRAF mutations in the tumor tissue on the clinical effectiveness of the intervention was possible in 912 patients, and BRAFmt (V600E) was detected in 41 (4.5%) patients. Among patients receiving FOLFIRI chemotherapy alone, the median OS (4.2 months) was 11.3 months lower in BRAFmt patients (N = 21), compared to BRAFwt and RASwt groups (N = 143) — 15.5 months, and 7.3 months compared to RASmt patients (N = 294) — 11.5 months. Similarly, the median PFS was 2.7 months in BRAFmt patients compared to 5.7 months in BRAFwt and RASwt patients and 4.3 months in RASmt patients [22].

The 20050181 trial was another study enabling the evaluation of the FOLFIRI regimen in further treatment lines in patients with mCRC harboring BRAF mutation [23]. A total of 1,186 patients who progressed during or within 6 months after completing the first line FU-containing chemotherapy were randomized

to panitumumab + FOLFIRI or FOLFIRI. Of these, 1,014 (85%) patients had assessed RAS mutations, and then among 421 RASwt patients, 45 (11%) were found to have BRAFmt. A total of 638 (54%) patients had RAS or BRAF mutations. Extended genetic diagnostics of paraffin-embedded tumor tissue in patients with normal exon 2 of the KRAS gene included Sanger sequencing of exon 3 (codons 59/61) and 4 (codons 117/146) of the KRAS gene; exon 2 (codons 12/13), 3 (codons 59/61) and 4 (codons 117/146) of the NRAS gene and exon 15 (codon 600) of the BRAF gene.

The authors of 20050181 study performed an exploratory analysis of clinical efficacy depending on the BRAF status. Among patients treated with FOLFIRI, the median OS was lower by 9.7 months in patients with BRAFmt and RASwt tumors (N = 23) — 5.7 months, compared to BRAFwt and RASwt (N = 190) — 15.4 months: HR = 5.00 (95% CI: 3.03–7.69) (Fig. 1). Similarly, the median PFS in BRAFmt and RASwt group was 1.8 months, e.g. 3.7 months lower than in BRAFwt and RASwt groups — 5.5 months: HR = 3.23 (95% CI: 1.96–5.26) (Fig. 2). In both cases, the observed diffe-

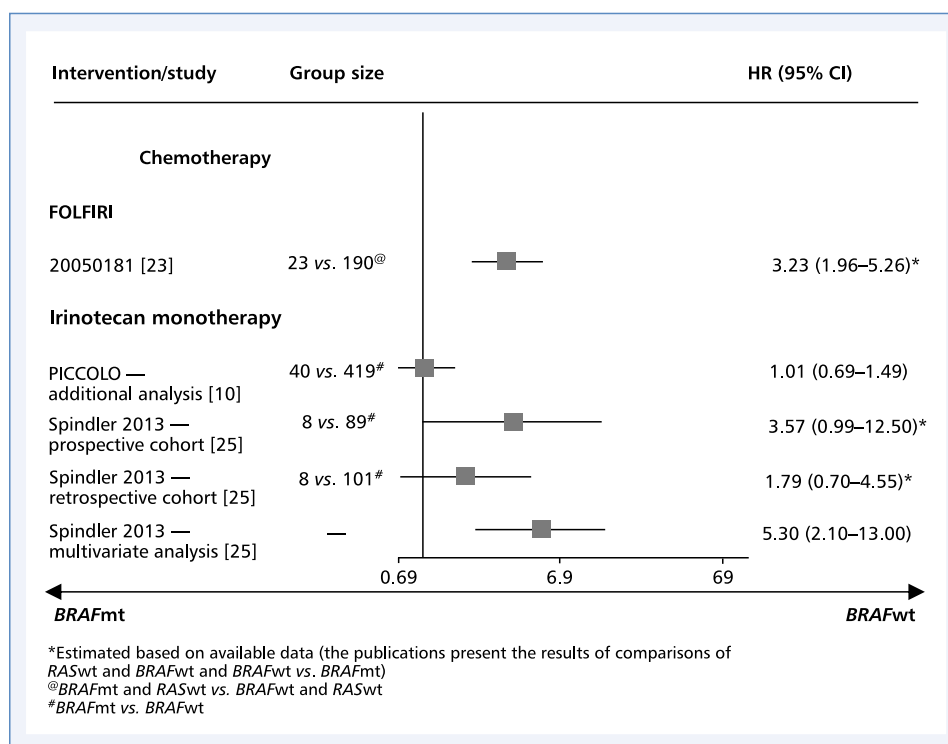


Figure 2. Hazard ratio (HR) for progression-free survival (PFS)

rences depending on the presence of *BRAF* mutations were statistically significant.

Another two trials evaluated irinotecan-based chemotherapy in the further treatment lines of advanced CRC with *BRAF* gene mutation. PICCOLO RCT included patients with inoperable, locally advanced, or metastatic CRC with prior progression during or after fluoropyrimidine-containing chemotherapy. Almost all patients had previously received oxaliplatin. After protocol amending, only patients with wild-type 12, 13 and 16 codons of *KRAS* gene were recruited (no prior anti-EGFR therapy was required). Finally, the analysis included 460 patients randomly assigned to receive panitumumab + irinotecan or irinotecan alone [24]. Further pyrosequencing of available paraffin-embedded tumor tissue was also carried out including codon 146 of *KRAS* gene, codon 12, 13 and 61 of *NRAS* gene, codon 542, 545, 546 and 1047 of *PIK3CA* gene and codon 600 of *BRAF* gene. Mutations in the *BRAF* gene were detected in 68 (14.8%) patients. OS in patients with *BRAF* gene mutation receiving irinotecan alone ($N = 31$) was significantly shorter compared to the group with wild type of all of the genes listed (*BRAF*wt, *RAS*wt and *PIK3CA*wt) ($N = 163$): HR = 1.56 (95% CI: 1.03–2.37), $P = 0.035$. This study also assessed objective response rate (ORR) according to RECIST criteria, which was twice lower in *BRAF*mt patients compared to all-wt: 2 (6.5%) vs. 20 (12.3%); RB = 0.53 (95% CI: 0.13–2.14), however with no statistical significance ($P = 0.3688$) [24].

In addition, Seligmann et al. [10] assessed the effect of *BRAF* mutation on the effectiveness of irinotecan monotherapy based on patients-level data from the PICCOLO study, considering the entire population, regardless of *KRAS* mutation status. The analysis included 459 patients with available results of *BRAF* mutation assessment, among which in 40 patients *V600E* mutation was found. Median OS in *BRAF*mt individuals was 3.5 months lower compared to the *BRAF*wt group: 6.7 vs. 10.2 months, but the difference did not reach statistical significance: HR = 1.21 (95% CI: 0.84–1.76). PFS medians were similar: 3.5 vs. 4.0 months; HR = 1.01 (95% CI: 0.69–1.49), while ORR probability was lower by 44%, but not reaching statistical significance: 5.0% vs. 8.1%, OR = 0.56 (95% CI: 0.13–2.49); $P = 0.45$.

The last analyzed trial was the non-randomized study — Spindler 2013 [25], evaluating the effect of *KRAS* and *BRAF* gene mutations on the outcomes in mCRC patients receiving irinotecan monotherapy in the second line (in prospective and retrospective cohort). The study included 110 patients in the prospective cohort, of which in 97 patients *BRAF* mutation status of tumor tissue was evaluated, with 8 (7%) positive results; and 111 patients in the retrospective cohort, among whom 109 were genotyped and *BRAF*mt was detected in 8 (8%) subjects. Assessment of mutation in 600 codon of *BRAF* gene was performed with the use of Amplification Refractory Mutation System-Quantitative PCR of DNA isolated from paraffin-embedded tumor tissue.

In the prospective cohort, HR for OS in *BRAF*mt (N = 8) vs. *BRAF*wt (N = 89) was 3.33 (95% CI: 0.96–11.11), while in the retrospective cohort — 1.05 (95% CI: 0.45–2.50) in 8 and 101 patients, respectively. Similarly, the risk of progression or death (PFS analysis) was higher in *BRAF*mt patients in both the prospective (HR = 3.57 [95% CI: 0.99–12.50]) and the retrospective cohort (HR = 1.79 [95% CI: 0.70–4.54]), however with no statistical significance. In the multivariate analysis considering age, performance status (PS) and *BRAF* and *KRAS* genes mutational status, the presence of *BRAF* mutations was associated with significantly worse prognosis: HR = 4.3 (95% CI: 1.7–10.6), P = 0.002, for comparison *BRAF*mt vs. *BRAF*wt in OS analysis (Fig. 1) and HR = 5.3 (95% CI: 2.1–13.0), P = 0.0002 in PFS analysis (Fig. 2). No *BRAF*mt patient achieved the objective response compared to 14% of *BRAF*wt patients in the prospective cohort and 15% in the retrospective cohort, but these differences did not reach statistical significance [25].

Risk of bias assessment

Five RCTs were included in the systematic review [20–24], however, the randomization did not refer to the subject of this review: *BRAF* gene mutational status was neither an inclusion criterion nor a stratification factor of randomization, genetic analysis was performed only in part of included patients, and the analysis of *BRAF* mutation impact was exploratory. These studies were not designed to compare interventions that *BRAF*mt patients were randomized to, and one of the trial arms included intervention whose assessment was not the purpose of this review (panitumumab + FOLFIRI [21], ramucirumab + FOLFIRI [22], panitumumab + irinotecan [24]). In one study [20] only limited inference based on OS assessment was possible due to the fact that observation within this endpoint also included BEVA in the subsequent treatment line after disease progression used in the majority of patients. Ultimately, in these RCTs, it was only possible to assess clinical efficacy within one study arm among patients with a known *BRAF* mutation and to refer these results to patients with a wild genotype. Accordingly, it was considered that in the context of presented study it would be appropriate to assess the risk of bias using a scale for non-randomized trials, as it will allow taking into account the baseline differences in demographic and clinical characteristics resulting from the lack of effective randomization. Table S3 presents the result of the risk of bias assessment of all 6 publications included in the systematic review in the ROBINS-I scale. The risk of bias was generally high, and most of the limitations found resulted from the analysis of outcomes only in subgroups distinguished based on *BRAF* gene mutational status, for which many significant

data were not presented in publications yet. Considering the construction of the ROBINS-I scale, such a severe limitation in the interfering factors domain translates into a critically high risk in the overall assessment of the likelihood of endpoints reliability, regardless of the result of the assessment in other domains in the scale. An exception was the additional analysis in the PICCOLO study [10], in which the use of appropriate statistical adjustments allowed to partially eliminate the risk of bias associated with the uneven distribution of prognostic factors between groups — therefore the cumulative risk of bias was assessed as high.

Discussion

According to published reports, the *BRAF* mutation is associated with a significantly reduced survival of colorectal cancer patients receiving chemotherapy, both in the early and advanced stages [26]. While some evidence is available on the efficacy of 1st line treatment in advanced disease, there is limited data regarding further treatment. To our knowledge, this is the first published systematic review of available evidence assessing the efficacy of bevacizumab and chemotherapy in 2nd and further treatment lines of advanced colorectal cancer with the *BRAF V600E* mutation.

Although the presented review included predominantly RCTs, the available results only allowed for assessment of clinical efficacy within particular treatment arms, and the comparison did not relate to different interventions in the *BRAF*wt population (presence of *BRAF* mutation or even a requirement for genetic evaluation of this genetic abnormality were not an inclusion criterion in any of the RCTs), but only a reference of the outcomes observed in subjects receiving the same intervention with the mutation to those with the wild gene. Therefore, the assessment of the impact of *BRAF* mutation on treatment outcomes had an exploratory nature and was only possible in some patients with available material and genetic tests performed. The analysis of the effect of *BRAF* mutation on the effectiveness of irinotecan monotherapy was a goal of only Spindler 2013 observational study [25]. To WJOG 6210G [21] and PICCOLO [24] (after protocol amendment) trials only patients with non-mutated *KRAS* gene were enrolled. Similarly, in study 20050181 [23], only patients with *KRAS*wt underwent extended genetic diagnostics, including *BRAF* gene assessment. In general, control groups in RCTs included patients with wild genotype, according to both *BRAF* and *RAS* mutations (and additionally *PIK3CA* [24]). Only in one study, the extended genetic profiling was carried out using peripheral blood circulating tumor DNA [21], in others, they were performed using paraffin-embedded

tissue specimens. Patients with metastatic CRC were included in most clinical trials, and only two enrolled patients with inoperable, locally advanced tumors [21, 24].

Severely limited data was found to assess the efficacy of bevacizumab (\pm CHT) in 2nd and subsequent treatment lines in patients with advanced CRC harboring *BRAF* mutation. In one study (MOMA) with bevacizumab in the 1st treatment line of metastatic disease only limited OS analysis was possible because this observation also included subsequent treatment lines, and most patients received re-treatment with bevacizumab [20]. However, it is estimated that up to app. 11 patients with the *BRAF* mutation were subjected to such analysis. On the other hand, in another study (WJOG 6210G), the assessment included a total of 11 patients with either *BRAF* or *RAS* mutation and it can be assumed that the former one occurred only in about 3 patients [21]. Nevertheless, in both studies, the median OS was consistently reduced in patients with the *BRAF* mutation, by 39% and 49%, respectively [20, 21], and the risk of death was 1.5 times higher [20]; similarly, the median PFS was reduced by 45% [21]. The data on the objective response rates in the bevacizumab group were insufficient, which makes impossible to draw plausible conclusions.

Studies on the efficacy of chemotherapy in further treatment lines (FOLFIRI or irinotecan monotherapy) also had significant limitations but evaluated *BRAF*mt population was greater and included from 16 to 31 (40, taking into account the alternative analysis of PICCOLO study data [10]) patients in particular studies [22–25], a total of 91 patients (100 including [10]). When using FOLFIRI in one study, the median OS was 73% lower in the *BRAF*mt group and PFS by 53% [22], while in the other by 63% and 67%, respectively [23]. The risk of death at a given time point was several times higher if the mutation was present — five times [23], about 1.5 times [10, 24] and more than four times (multivariate analysis [25]), and the differences were statistically significant. Similarly, the risk of death or disease progression was more than three and four times higher ([23] and [25] — multivariate analysis, respectively), although in an alternative estimation of the PICCOLO study results there was no significant difference in PFS (median 12% lower, HR = 1.01 [10]).

Regarding the objective response rate, available data was markedly limited, in one study the incidence of this endpoint was almost twice lower in *BRAF*mt patients (statistically insignificant difference) [10, 24], while in the other study no patient with *BRAF* mutation ORR was reported [25].

Seligmann et al. [10] assessed the effect of *BRAF* mutation on the results of treatment of advanced CRC with standard chemotherapy using patient-level data from RCTs: COIN [27, 28] and FOCUS [29] (oxalipla-

tin and fluorouracil in the 1st line) and PICCOLO [24] (irinotecan in the 2nd line). The results of this additional analysis regarding the PICCOLO are presented in the main part of this publication. For the 1st line treatment of advanced disease, the authors found that the presence of the *BRAF* mutation is a significant OS prognostic factor (cumulative data for both RCTs: 10.8 vs. 16.4 months [HR = 1.49 (95% CI: 1.23–1.80); P < 0.001]), also after matching with respect to baseline characteristics. However, no clear impact of the mutation on PFS and ORR was observed. Survival after progression was also assessed, defined as the time from progression to death among patients with disease progression; when the date of progression was unknown, the date of the last chemotherapy cycle was taken into account. Patients with the *BRAF* mutation had a shorter survival after progression compared to those with the wild-type gene in both 1st line studies (COIN and FOCUS), the results for both clinical trials: 3.2 vs. 8.6 months; HR = 1.72 (95% CI: 1.35–2.19), P < 0.001 [10]. It is worth noting that significantly fewer *BRAF*mt patients received subsequent treatment line: 33% vs. 51%, P < 0.001; and a significantly higher percentage of *BRAF*mt patients with rapid progression (< 6 months) was observed in both the 1st and 2nd treatment line — 36.5% compared to — 21.9% in non-mutated patients; P < 0.001 [10].

It should be noted that inference based on the collected data is subject to uncertainty, due to the small size of *BRAF*mt population, and on the other hand with methodological limitations of included trials. In addition, the generally high risk of systematic error in the included studies greatly limits conclusions of the analysis. This is mainly due to the nature of the analyzes that were only possible when the included studies were treated as single-arm. It should be noted that the result of the assessment in the other domains of the ROBINS-I scale was better, although this does not change the overall assessment of systematic error risk. The clinical heterogeneity of the trials (especially in terms of interventions used) prevents proper data synthesis and may affect the interpretation and the ability to relate the review results to the target population of metastatic colorectal cancer patients.

Despite the aforementioned numerous limitations, the analysis quite clearly indicates lower effectiveness of evaluated interventions (bevacizumab \pm chemotherapy or chemotherapy) in *BRAF*mt patients. In this group, none of the studied therapies were as effective as in *BRAF*wt population. The advantage of this systematic review is the extended search, which was carried out in 3 databases and also included non-randomized studies to comprehensively assess the effectiveness of the examined intervention. However, gray literature not being indexed in medical databases and ongoing research were not included which could

affect the scope of the evidence described. Only full-text articles in Polish or English were selected, and there were few studies, and some of them did not fully answer the clinical question, which is also a limitation of this analysis.

Combination of BRAF inhibitors with other medications may be more effective than monotherapy, which may lead to resistance by secondary activation of the MAPK pathway — this mechanism may be due to increased EGFR and MEK/ERK pathway signalling activity [1, 2, 30, 31]. Therefore, particular hope is given in the combination of BRAF inhibitors with drugs directed against parallel signalling pathways [1, 6]. There are promising recently published results of phase III BEACON study, in which the combination of cetuximab (anti-EGFR drug), encorafenib (BRAF inhibitor) and binimetinib (MEK inhibitor), as well as cetuximab with encorafenib in the 2nd or 3rd line of treatment significantly increased overall survival of patients with *BRAF V600E* mutation compared to standard therapy [32]. Some hope is also raised by a treatment regimen containing dabrafenib (BRAF inhibitor), panitumumab (anti-EGFR) and trametinib (MEK inhibitor), which has shown promising efficacy in patients with the *BRAF V600E* mutation [33]. Both of these regimens are currently recommended in the US NCCN guidelines for the treatment of patients with mCRC with the current *BRAF* mutation in 2nd and further treatment lines [7].

Clinical comment (P.P.)

When colorectal cancer progresses to metastatic disease, the *BRAF V600E* mutation, which is detected in about 10% of patients, becomes a factor that significantly worsens prognosis. The median survival of patients enrolled in current phase III clinical trials is over 30 months, but if the *BRAF* mutation is present, this value is two or three times lower [36].

It has been demonstrated that patients with *BRAF* mutation undergoing chemotherapy receive systemic treatment of subsequent lines less frequently than other patients, which results from the rather dynamic progression often causing symptoms and deterioration of the general condition preventing further cancer treatment. Until recently, this molecular abnormality has not been routinely studied in patients enrolled in clinical trials, so data on the efficacy of various chemotherapy and biological treatment regimens are based largely on retrospective analyses and are inevitably burdened with selection bias. However, these data quite consistently indicate that, apart from adverse effect on prognosis, the *BRAF V600E* mutation is a determinant of ineffectiveness or very little benefit from the use of anti-EGFR antibodies, especially in monotherapy.

For the reasons given above, it is suggested that in patients with this molecular disorder, 1st line systemic treatment should be as intensive as possible (at least doublet or triplet chemotherapy, i.e. FOLFOXIRI regimen) preferably with the addition of bevacizumab.

The data on the value of subsequent line therapies are extremely scarce, but the efficacy of chemotherapy and anti-angiogenic drugs appears to be low, as confirmed by this systematic review.

After successes in melanoma patients, BRAF tyrosine kinase inhibitors appeared to be the natural choice for next-line therapy, especially in combination with MEK inhibitors, but early phase clinical trials were disappointing [33]. Some optimism was brought only by attempts to use triple therapy additionally containing anti-EGFR antibody. The results of the BEACON phase III study dedicated to previously treated patients with *BRAF V600E* mutation have been recently published. Combination of cetuximab with a BRAF inhibitor encorafenib, as well as triple therapy containing an additional MEK inhibitor binimetinib, have been shown to increase overall survival and time to the quality of life deterioration compared to cetuximab combined with irinotecan-based chemotherapy [32, 37].

Another potentially very effective treatment method may be anti-PD1 immunotherapy or a combination of anti-PD1 and anti-CTLA4 because *BRAF V600E* mutation quite often coexists with microsatellite instability, which is a favorable predictor for this treatment [38]. Available data, however, come from phase II non-controlled studies, and patients with *BRAF V600* mutation were a minority [39].

Conflict of interest

The authors have no conflict of interest to declare.

Founding

The systematic review was financed by Pierre Fabre Médicament Polska Sp. z o.o.

References

1. Potocki PM, Wysocki PJ. BRAF — a new therapeutic target in colorectal cancer. *Oncol Clin Pract* 2018; 14, doi: 10.5603/OCP.2018.0013.
2. Ducreux M, Chamseddine A, Laurent-Puig P, et al. Molecular targeted therapy of BRAF-mutant colorectal cancer. *Ther Adv Med Oncol*. 2019; 11: 1758835919856494, doi: 10.1177/1758835919856494, indexed in Pubmed: 31244912.
3. Zaleśna I, Hartman ML, Czyż M. [BRAF mutation in progression and therapy of melanoma, papillary thyroid carcinoma and colorectal adenocarcinoma]. *Postepy Hig Med Dosw (Online)*. 2016; 70: 471–488, doi: 10.5604/17322693.1201719, indexed in Pubmed: 27180965.
4. Rad R, Cadiñanos J, Rad L, et al. A genetic progression model of Braf(V600E)-induced intestinal tumorigenesis reveals targets for therapeutic intervention. *Cancer Cell*. 2013; 24(1): 15–29, doi: 10.1016/j.ccr.2013.05.014, indexed in Pubmed: 23845441.

5. Clancy C, Burke JP, Kalady MF, et al. BRAF mutation is associated with distinct clinicopathological characteristics in colorectal cancer: a systematic review and meta-analysis. *Colorectal Dis.* 2013; 15(12): e711–e718, doi: [10.1111/colodi.12427](https://doi.org/10.1111/colodi.12427), indexed in Pubmed: [24112392](https://pubmed.ncbi.nlm.nih.gov/24112392/).
6. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer.* 2011; 117(20): 4623–4632, doi: [10.1002/cncr.26086](https://doi.org/10.1002/cncr.26086), indexed in Pubmed: [21456008](https://pubmed.ncbi.nlm.nih.gov/21456008/).
7. Benson AB, Venook AP, Al-Hawary MM, et al. NCCN Clinical Practice Guidelines in Oncology — Colon Cancer. Version 1.2020 — December 19, 2019.
8. Seppälä TT, Böhm JP, Friman M, et al. Combination of microsatellite instability and BRAF mutation status for subtyping colorectal cancer. *Br J Cancer.* 2015; 112(12): 1966–1975, doi: [10.1038/bjc.2015.160](https://doi.org/10.1038/bjc.2015.160), indexed in Pubmed: [25973534](https://pubmed.ncbi.nlm.nih.gov/25973534/).
9. Krakowska M, Potemski P. New treatment options for patients with metastatic colorectal cancer in Poland. *Oncol Clin Pract.* 2017; 13: 156–160, doi: [10.5603/OCP.2017.0014](https://doi.org/10.5603/OCP.2017.0014).
10. Seligmann JF, Fisher D, Smith CG, et al. Investigating the poor outcomes of BRAF-mutant advanced colorectal cancer: analysis from 2530 patients in randomised clinical trials. *Ann Oncol.* 2017; 28(3): 562–568, doi: [10.1093/annonc/mdw645](https://doi.org/10.1093/annonc/mdw645), indexed in Pubmed: [27993800](https://pubmed.ncbi.nlm.nih.gov/27993800/).
11. Masi G, Loupakis F, Salvatore L, et al. Bevacizumab with FOLFOXIRI (irinotecan, oxaliplatin, fluorouracil, and folinate) as first-line treatment for metastatic colorectal cancer: a phase 2 trial. *Lancet Oncol.* 2010; 11(9): 845–852, doi: [10.1016/S1470-2045\(10\)70175-3](https://doi.org/10.1016/S1470-2045(10)70175-3), indexed in Pubmed: [20702138](https://pubmed.ncbi.nlm.nih.gov/20702138/).
12. Loupakis F, Cremolini C, Salvatore L, et al. FOLFOXIRI plus bevacizumab as first-line treatment in BRAF mutant metastatic colorectal cancer. *Eur J Cancer.* 2014; 50(1): 57–63, doi: [10.1016/j.ejca.2013.08.024](https://doi.org/10.1016/j.ejca.2013.08.024), indexed in Pubmed: [24138831](https://pubmed.ncbi.nlm.nih.gov/24138831/).
13. Cremolini C, Loupakis F, Antoniotti C, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol.* 2015; 16(13): 1306–1315, doi: [10.1016/S1470-2045\(15\)00122-9](https://doi.org/10.1016/S1470-2045(15)00122-9), indexed in Pubmed: [26338525](https://pubmed.ncbi.nlm.nih.gov/26338525/).
14. Obwieszczenie Ministra Zdrowia z dnia 20 grudnia 2019 r. w sprawie wykazu refundowanych leków, środków spożywczych specjalnego przeznaczenia żywieniowego oraz wyrobów medycznych na 1 stycznia 2020 r.
15. Pietrantonio F, Petrelli F, Coiu A, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. *Eur J Cancer.* 2015; 51(5): 587–594, doi: [10.1016/j.ejca.2015.01.054](https://doi.org/10.1016/j.ejca.2015.01.054), indexed in Pubmed: [25673558](https://pubmed.ncbi.nlm.nih.gov/25673558/).
16. Rowland A, Dias MM, Wiese MD, et al. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. *Br J Cancer.* 2015; 112(12): 1888–1894, doi: [10.1038/bjc.2015.173](https://doi.org/10.1038/bjc.2015.173), indexed in Pubmed: [25989278](https://pubmed.ncbi.nlm.nih.gov/25989278/).
17. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ.* 2019; 366: i4898, doi: [10.1136/bmj.i4898](https://doi.org/10.1136/bmj.i4898), indexed in Pubmed: [31462531](https://pubmed.ncbi.nlm.nih.gov/31462531/).
18. Sterne JAC, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ.* 2016; 355: i4919, doi: [10.1136/bmj.i4919](https://doi.org/10.1136/bmj.i4919), indexed in Pubmed: [27733354](https://pubmed.ncbi.nlm.nih.gov/27733354/).
19. Wytyczne oceny technologii medycznych (HTA, ang. health technology assessment), wersja 3.0. Agencja Oceny Technologii Medycznych i Taryfikacji, Warszawa, sierpień 2016.
20. Cremolini C, Antoniotti C, Rossini D, et al. GONO Foundation Investigators. Phase II randomised study of maintenance treatment with bevacizumab or bevacizumab plus metronomic chemotherapy after first-line induction with FOLFOXIRI plus Bevacizumab for metastatic colorectal cancer patients: the MOMA trial. *Eur J Cancer.* 2019; 109: 175–182, doi: [10.1016/j.ejca.2018.12.028](https://doi.org/10.1016/j.ejca.2018.12.028), indexed in Pubmed: [30735919](https://pubmed.ncbi.nlm.nih.gov/30735919/).
21. Shitara K, Yonesaka K, Denda T, et al. Randomized study of FOLFIRI plus either panitumumab or bevacizumab for wild-type KRAS colorectal cancer-WJOG 6210G. *Cancer Sci.* 2016; 107(12): 1843–1850, doi: [10.1111/cas.13098](https://doi.org/10.1111/cas.13098), indexed in Pubmed: [27712015](https://pubmed.ncbi.nlm.nih.gov/27712015/).
22. Yoshino T, Portnoy DC, Obermannová R, et al. Biomarker analysis beyond angiogenesis: RAS/RAF mutation status, tumour sidedness, and second-line ramucicromab efficacy in patients with metastatic colorectal carcinoma from RAISE—a global phase III study. *Ann Oncol.* 2019; 30(1): 124–131, doi: [10.1093/annonc/mdy461](https://doi.org/10.1093/annonc/mdy461), indexed in Pubmed: [30339194](https://pubmed.ncbi.nlm.nih.gov/30339194/).
23. Peeters M, Oliner KS, Price TJ, et al. Analysis of KRAS/NRAS mutations in a phase III study of panitumumab with FOLFIRI compared with FOLFIRI alone as second-line treatment for metastatic colorectal cancer. *Clin Cancer Res.* 2015; 21(24): 5469–5479, doi: [10.1158/1078-0432.CCR-15-0526](https://doi.org/10.1158/1078-0432.CCR-15-0526), indexed in Pubmed: [26341920](https://pubmed.ncbi.nlm.nih.gov/26341920/).
24. Seymour MT, Brown SR, Middleton G, et al. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. *Lancet Oncol.* 2013; 14(8): 749–759, doi: [10.1016/S1470-2045\(13\)70163-3](https://doi.org/10.1016/S1470-2045(13)70163-3), indexed in Pubmed: [23725851](https://pubmed.ncbi.nlm.nih.gov/23725851/).
25. Spindler KG, Appelt AL, Pallisgaard N, et al. KRAS-mutated plasma DNA as predictor of outcome from irinotecan monotherapy in metastatic colorectal cancer. *Br J Cancer.* 2013; 109(12): 3067–3072, doi: [10.1038/bjc.2013.633](https://doi.org/10.1038/bjc.2013.633), indexed in Pubmed: [24263065](https://pubmed.ncbi.nlm.nih.gov/24263065/).
26. Sanz-Garcia E, Argiles G, Elez E, et al. BRAF mutant colorectal cancer: prognosis, treatment, and new perspectives. *Ann Oncol.* 2017; 28(11): 2648–2657, doi: [10.1093/annonc/mdx401](https://doi.org/10.1093/annonc/mdx401), indexed in Pubmed: [29045527](https://pubmed.ncbi.nlm.nih.gov/29045527/).
27. Maughan TS, Adams RA, Smith CG, et al. MRC COIN Trial Investigators. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet.* 2011; 377(9783): 2103–2114, doi: [10.1016/S0140-6736\(11\)60613-2](https://doi.org/10.1016/S0140-6736(11)60613-2), indexed in Pubmed: [21641636](https://pubmed.ncbi.nlm.nih.gov/21641636/).
28. Adams RA, Meade AM, Seymour MT, et al. MRC COIN Trial Investigators. Intermittent versus continuous oxaliplatin and fluoropyrimidine combination chemotherapy for first-line treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet Oncol.* 2011; 12(7): 642–653, doi: [10.1016/S1470-2045\(11\)70102-4](https://doi.org/10.1016/S1470-2045(11)70102-4), indexed in Pubmed: [21641867](https://pubmed.ncbi.nlm.nih.gov/21641867/).
29. Seymour MT, Maughan TS, Ledermann JA, et al. FOCUS Trial Investigators, National Cancer Research Institute Colorectal Clinical Studies Group. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. *Lancet.* 2007; 370(9582): 143–152, doi: [10.1016/S0140-6736\(07\)61087-3](https://doi.org/10.1016/S0140-6736(07)61087-3), indexed in Pubmed: [17630037](https://pubmed.ncbi.nlm.nih.gov/17630037/).
30. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature.* 2012; 483(7387): 100–103, doi: [10.1038/nature10868](https://doi.org/10.1038/nature10868), indexed in Pubmed: [22281684](https://pubmed.ncbi.nlm.nih.gov/22281684/).
31. Corcoran RB, Ebi H, Turke AB, et al. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov.* 2012; 2(3): 227–235, doi: [10.1158/2159-8290.CD-11-0341](https://doi.org/10.1158/2159-8290.CD-11-0341), indexed in Pubmed: [22448344](https://pubmed.ncbi.nlm.nih.gov/22448344/).
32. Kopetz S, Grothey A, Yaeger R, et al. Encorafenib, binimetinib, and cetuximab in v600e-mutated colorectal cancer. *N Engl J Med.* 2019; 381(17): 1632–1643, doi: [10.1056/NEJMoa1908075](https://doi.org/10.1056/NEJMoa1908075), indexed in Pubmed: [31566309](https://pubmed.ncbi.nlm.nih.gov/31566309/).
33. Corcoran RB, André T, Atreya CE, et al. Combined BRAF, EGFR, and MEK inhibition in patients with -mutant colorectal cancer. *Cancer Discov.* 2018; 8(4): 428–443, doi: [10.1158/2159-8290.CD-17-1226](https://doi.org/10.1158/2159-8290.CD-17-1226), indexed in Pubmed: [29431699](https://pubmed.ncbi.nlm.nih.gov/29431699/).
34. Tabernero J, Takayuki Y, Cohn AL, et al. RAISE Study Investigators. Ramucicromab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol.* 2015; 16(5): 499–508, doi: [10.1016/S1470-2045\(15\)70127-0](https://doi.org/10.1016/S1470-2045(15)70127-0), indexed in Pubmed: [25877855](https://pubmed.ncbi.nlm.nih.gov/25877855/).
35. Peeters M, Price TJ, Cervantes A, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol.* 2010; 28(31): 4706–4713, doi: [10.1200/JCO.2009.27.6055](https://doi.org/10.1200/JCO.2009.27.6055), indexed in Pubmed: [20921462](https://pubmed.ncbi.nlm.nih.gov/20921462/).
36. Ursem C, Atreya CE, Van Loon K. Emerging treatment options for -mutant colorectal cancer. *Gastrointest Cancer.* 2018; 8: 13–23, doi: [10.2147/GICTT.S125940](https://doi.org/10.2147/GICTT.S125940), indexed in Pubmed: [29628780](https://pubmed.ncbi.nlm.nih.gov/29628780/).
37. Kopetz S, Grothey A, Cutsem EV, et al. Encorafenib plus cetuximab with or without binimetinib for BRAF V600E-mutant metastatic colorectal cancer: Quality-of-life results from a randomized, three-arm, phase III study versus the choice of either irinotecan or FOLFIRI plus cetuximab (BEACON CRC). *J Clin Oncol.* 2020; 38(4_suppl): 8–8, doi: [10.1200/jco.2020.38.4_suppl.8](https://doi.org/10.1200/jco.2020.38.4_suppl.8).
38. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol.* 2017; 18(9): 1182–1191, doi: [10.1016/S1470-2045\(17\)30422-9](https://doi.org/10.1016/S1470-2045(17)30422-9), indexed in Pubmed: [28734759](https://pubmed.ncbi.nlm.nih.gov/28734759/).
39. Morse MA, Hochster H, Benson AL. Perspectives on treatment of metastatic colorectal cancer with immune checkpoint inhibitor therapy. *Oncologist.* 2020; 25(1): 33–45, doi: [10.1634/theoncologist.2019-0176](https://doi.org/10.1634/theoncologist.2019-0176), indexed in Pubmed: [31383813](https://pubmed.ncbi.nlm.nih.gov/31383813/).

Appendix

Table S1. Search strategy

| # | Query |
|-----------------|---|
| PubMed | |
| #2 | advanced OR metastatic |
| #3 | colon cancer OR colorectal cancer |
| #4 | (#2 AND #3) |
| #7 | randomized controlled trial[pt] |
| #8 | random allocation[mh] |
| #9 | random*[tiab] |
| #10 | controlled[tiab] |
| #11 | (#7 OR #8 OR #9 OR #10) |
| #13 | BRAF |
| #16 | (bevacizumab OR FOLFOXIRI OR FOLFIRI OR FOLFOX OR oxaliplatin OR irinotecan) |
| #17 | (#4 AND #16) |
| #18 | (#17 AND #11) |
| #20 | (#13 AND #17) |
| #22 | (#18 OR #20) |
| Cochrane | |
| #1 | advanced OR metastatic in Trials |
| #2 | [mh „colorectal neoplasms“] OR „colon cancer“ in Trials |
| #3 | #1 AND #2 in Trials |
| #4 | bevacizumab OR FOLFOXIRI OR FOLFIRI OR FOLFOX OR oxaliplatin OR irinotecan in Trials |
| #5 | #3 AND #4 in Trials |
| #6 | BRAF in Trials |
| #7 | #6 AND #5 in Trials |
| #8 | #7 OR #5 in Trials |
| Embase | |
| #1 | (advanced:de OR metastatic:de) AND [embase]/lim |
| #2 | („colon cancer“:de OR „colorectal cancer“/exp) AND [embase]/lim |
| #3 | („bevacizumab“:de OR folfoxiri:de OR „folfiri“:de OR „folfox“:de OR „oxaliplatin“:de OR „irinotecan“:de) AND [embase]/lim |
| #4 | #1 AND #2 |
| #5 | #3 AND #4 |
| #6 | [randomized controlled trial]/lim AND [embase]/lim |
| #7 | random*:ab,ti AND [embase]/lim |
| #8 | controlled:ab,ti AND [embase]/lim |
| #9 | randomization:de AND [embase]/lim |
| #10 | #6 OR #7 OR #8 OR #9 |
| #11 | #5 AND #10 |
| #12 | braf AND [embase]/lim |
| #13 | #5 AND #12 |
| #14 | #11 OR #13 |

Table S2. PICOS scheme

| Parameter | Inclusion criteria |
|--------------|--|
| Population | Adults with advanced CRC and assessed <i>BRAF</i> (V600E) status, progression after first-line treatment of advanced disease |
| Intervention | Bevacizumab (\pm CHT) in 2 nd or further treatment line due to advanced disease Oxaliplatin- or irinotecan-based chemotherapy regimens in 2 nd or further treatment line due to advanced disease |
| Comparison | As above or none |
| Outcomes | Overall survival (OS) Progression-free survival (PFS) Objective response rate (ORR) Studies enabling comparative assessment of sought interventions in the <i>BRAF</i> mt population or relating their effectiveness to <i>BRAF</i> wt patients were included |
| Study design | Randomized, controlled clinical trials, controlled or non-controlled non-randomized studies, published in full-text in English or Polish Systematic reviews published in English or Polish |

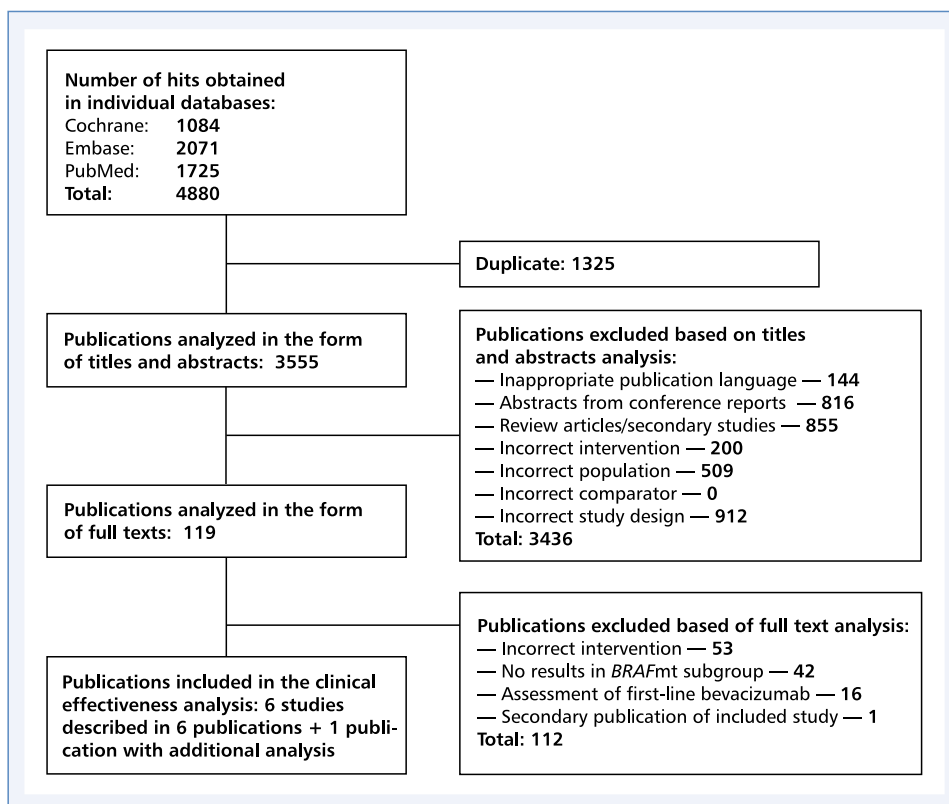


Figure S1. Search results. List of excluded studies at the stage of full texts analysis along with the reasons for exclusion is available on request

Table S3. Assessment of risk of bias in the included studies using the ROBINS-I tool

| Study name | Domain | Disturbing factors | Patients selection | Intervention classification | Deviations from planned interventions | Missing data | Outcomes | Selection of described results | Total rating |
|--|--------|--------------------|--------------------|-----------------------------|---------------------------------------|--------------|----------|--------------------------------|--------------|
| MOMA [20] | OS | Critical | Low | Low | Low | Moderate | Low | Low | Critical |
| WJOG 6210G [21] | OS | Critical | Low | Low | Low | Moderate | Low | Low | Critical |
| | PFS | | | | | | Serious | | |
| RAISE [22] | OS | Critical | Low | Low | Low | Moderate | Low | Low | Critical |
| | PFS | | | | | | Moderate | | |
| 20050181 [23] | OS | Critical | Low | Low | Low | Serious | Low | Low | Critical |
| | PFS | | | | | | Serious | | |
| PICCOLO [24] | OS | Critical | Low | Low | Low | Moderate | Low | Low | Critical |
| | PFS | | | | | | Serious | | |
| | ORR | | | | | | Serious | | |
| PICCOLO — additional analysis [10] | OS | Serious | Low | Low | Low | Moderate | Low | Low | Serious |
| | PFS | | | | | | Serious | | |
| | ORR | | | | | | Serious | | |
| Spindler 2013 — prospective cohort, multivariate analysis [25] | OS | Critical | Low | Low | Low | Moderate | Low | Low | Critical |
| | PFS | | | | | | Serious | | |
| Spindler 2013 — retrospective cohort [25] | OS | Critical | Low | Low | Low | Low | Low | Low | Critical |
| | PFS | | | | | | Serious | | |

The risk of bias on the ROBINS-I scale can be assessed as (in order from lowest to highest): low, moderate, serious and critical, and in the absence of relevant information: unspecified. The total risk error rating is not higher than the lowest among the results in individual domains.

Table S4. Characteristics of included clinical trials

| Study name | Study type and location | Intervention | Comparator | Population ^a | Total number/ /number analyzed for mutation/ /BRAFmt number | Observation period, endpoints | Funding |
|-----------------|---|--|---|---|--|---|---|
| MOMA [20] | RCT, non-blinded 16 cancer centers in Italy | After 8 cycles of FOLFOXIRI induction therapy (irinotecan 165 mg/m ² iv + next oxaliplatin 85 mg/m ² iv + levofolinic acid 200 mg/m ² + next fluorouracil 3200 mg/m ² in continuous 48 h infusion) + BEVA (5 mg/kg iv) (q2w) randomized to BEVA (7.5 mg/kg iv) or BEVA (7.5 mg/kg iv) + metronomic chemotherapy (capecitabine [500 mg 3 × daily] and cyclophosphamide [50 mg daily]) | – | mCRC | 232/206/20 | Median 47.8 months I: PFS II: OS, objective response rate ^b , resection rate, -La Roche safety | GONO Foundation, the ARCO Foundation and F. Hoffmann-La Roche |
| WJOG 6210G [21] | RCT, non-blinded, multicenter (Japan) | Panitumumab (6 mg/kg) + FOLFIRI (doses not described) (q2w) | BEVA (5 mg/kg) + FOLFIRI (doses not described) (q2w) | mCRC or locally advanced inoperable CRC, progression after treatment with fluoropyrimidine, oxaliplatin and bevacizumab, KRASwt in exon 2 | 117/109/5 | 15.4 months vs. 13.4 months I: OS II: PFS, objective response rate ^b , safety, biomarkers analysis | West Japan Oncology Group (WJOG) |
| RAISE* [22] | RCT, double-blinded (blinded): patients, sites personnel involved, study sponsor), multicenter, international | Ramucirumab (8 mg/kg in 60 min) + next FOLFIRI (irinotecan 180 mg/m ² in 90 min iv + next or simultaneously folinic acid 400 mg/m ² in 120 min iv + next fluorouracil 400 mg/m ² in 2–4 min bolus iv + next fluorouracil 2400 mg/m ² in continuous 48 h infusion) (q2w) | Placebo (in 60 min) + next FOLFIRI (irinotecan 180 mg/m ² in 90 min iv + next or simultaneously folinic acid 400 mg/m ² in 120 min iv + next fluorouracil 400 mg/m ² in 2–4 min bolus iv + next fluorouracil 2400 mg/m ² in continuous 48 h infusion) (q2w) | mCRC, progression after treatment with fluoropyrimidine, oxaliplatin and bevacizumab | 1072/912/41 | – I: OS II: PFS, objective response rate ^b , disease control, safety, PROs (PROs questionnaires: EORTC QLQ-C30 version 3.0, EQ-5D) | Eli Lilly and Company |



Table S4 cont. Characteristics of included clinical trials

| Study name | Study type and location | Intervention | Comparator | Population ^a | Total number/number analyzed for mutation/ BRAF number | Observation period, endpoints | Funding |
|--------------------|---|--|---|--|--|---|---|
| 20050181* [23] | RCT, non-blinded multicenter, international | FOLFIRI (irinotecan 180 mg/m ² iv + folinic acid 400 mg/m ² iv or levofolinate 200 mg/m ² iv + fluorouracil 400 mg/m ² bolus iv + fluorouracil 2400 mg/m ² as a continuous infusion over 2 days) | Panitumumab (6.0 mg/kg in 60 min + next in 30 min in subsequent infusions) + next FOLFIRI (irinotecan 180 mg/m ² iv + folinic acid 400 mg/m ² iv or levofolinate 200 mg/m ² iv + fluorouracil 400 mg/m ² bolus iv + fluorouracil 2400 mg/m ² as a continuous infusion over 2 days) | mCRC, progression after fluoropyrimidine-based CTH | 1186/421 ^c /45 | Median 48 wks. I: OS, PFS II: objective response rate ^b and duration, safety, PROs | Amgen Inc |
| PICCOLO [24] | RCT, non-blinded 60 sites in the UK | Panitumumab (9 mg/kg iv) + irinotecan (350 mg/m ² ; if age > 70 years or performance status 2 — 300 mg/m ²) (q3w) | Irinotecan (350 mg/m ² ; if age > 70 years or performance status 2 — 300 mg/m ²) (q3w) | mCRC or locally advanced inoperable CRC, progression after fluoropyrimidine-based CTH, no previous anti-EGFR therapy | 460/bd./68 (in all KRAS _{c.12-13,61} wt) Additional analysis — irinotecan [10]: 511/459/40 | Median of treatment cycles: 4 (range: 0-28) I: OS II: PFS, objective response rate ^b , PROs, safety (questionnaire: EORTC QLQ-C30) | Cancer Research UK, Amgen Inc. |
| Spindler 2013 [25] | Non-RCT, prospective-retrospective, single center (Denmark) | Irinotecan (both cohorts: 350 mg/m ²) (q3w) | — | mCRC, 2 nd line treatment | Prospective cohort — 110/97/8 Retrospective cohort — 111/109/8 | Prospective cohort: median of treatment cycles 4 (1-15); Retrospective cohort: median of treatment cycles 6 (2-15) OS, PFS, objective response rate, safety | Tryg Fonden and the Research Council Hospital Lillebaelt |

RCT — randomized controlled trial; BEVA — bevacizumab; q2w/q3w — dose administered every 2 and 3 weeks, respectively; PROs — patient-reported outcomes, CTH — chemotherapy
*Detailed data on the research methodology come from the main publications that were not included at the stage of research selection due to the lack of results related to the analysis — RAISE [34], 20050181 [35]

^bDetailed population data for the subgroups analyzed was not available

^cRASwt patients

Aleksandra Sobiborowicz^{1,2}, Anna M. Czarnecka^{1,3}, Anna Szumera-Ciećkiewicz^{4,5},
Piotr Rutkowski¹, Tomasz Świtaj¹

¹Department of Soft Tissue/Bone, Sarcoma and Melanoma, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland

²Medical Faculty, Medical University of Warsaw, Warsaw, Poland

³Department of Experimental Pharmacology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

⁴Pathology Laboratory, Department of Pathology and Laboratory Diagnostics, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland

⁵Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Diagnosis and treatment of lymphangiomyomatosis (LAM) from the PEComa group

Address for correspondence:

Dr n. med. Tomasz Świtaj
Klinika Nowotworów Tkanek Miękkich,
Kości i Czerniaków Narodowy Instytut
Onkologii im. Marii Skłodowskiej-Curie —
Państwowy Instytut Badawczy w Warszawie
ul. Roentgena 5, 02-781 Warszawa
Tel.: 22 546 20 31; faks: 22 643 93 75
e-mail: tomasz.switaj@pib-nio.pl

Oncology in Clinical Practice
2021, Vol. 17, No. 1, 28–41
DOI: 10.5603/OCP.2020.0034
Translation: prof. Ewa Bartnik
Copyright © 2021 Via Medica
ISSN 2450-1654

ABSTRACT

Lymphangiomyomatosis (LAM) is a rare, proliferative lung disease, leading to progressive damage of their structure and is a member of the PEComa neoplasm family (perivascular epithelioid cell tumors). In the patients, solid-cystic masses described as lymphangiomyoma or extrapulmonary LAM (E-LAM) can occur. E-LAM foci have been described in the mediastinum, supraclavicular lymph nodes, the liver, walls of the small and large intestine, the pancreas, mesentery. E-LAM masses can attain very large sizes — tumors 15–22 cm long have been described. On the basis of positive results of clinical trials sirolimus, a drug from the group of mTOR kinase inhibitors, was registered by the Food and Drug Administration (FDA) in May 2015 as the first and currently only drug for systemic LAM therapy. Sirolimus use is recommended in patients with LAM, accompanied by rapidly progressing deterioration of respiratory system function or FEV₁ ≤ 70% predicted value and in patients with pleural lymph exudate before applying invasive methods of treatment.

Key words: lymphangiomyomatosis, sirolimus

Oncol Clin Pract 2021; 17, 1: 28–41

Introduction

Lymphangiomyomatosis (LAM) is a rare, proliferative lung disease leading to progressive damage to their structure with the formation of numerous small cysts and lymph accumulation in the pleural cavity [1]. At its basis is the multifocal proliferation of smooth muscle cells and perivascular epithelioid cells, (PECs); in LAM they are also designated LAM cells in the lung interstitium and is therefore included in the family of PEComa type neoplasms (perivascular epithelioid cell tumors) [2]. The PEComa group also includes angiomyolipoma (AML), clear-cell sugar tumor (CCST) — pulmonary and extrapulmonary forms (primary extrapulmonary sugar tumor, PEST), clear-cell myomelanocytic tumor (CCMMT) and primary cutaneous PEComa, CCCMT — cutaneous clear cell myomelanocytic tumor and PEComa NOS (not otherwise specified) [3]. Pulmonary LAM (P-LAM) occurs in two clinical forms: associated

with tuberous sclerosis [Bourneville-Pringle disease, tuberous sclerosis complex (TSC)] — a genetic syndrome caused by inactivating germline mutations in the *TSC1* and *TSC2* genes, characterized by the occurrence of many tumors of the hamartoma type, perturbations of the nervous system, including epileptic seizures, autism and intellectual disability of various degrees [4], and as a sporadic form — sporadic LAM (S-LAM), in women with no predisposing factors [5]. In addition to pulmonary manifestation of LAM, in these patients, numerous irregularities are observed in the lymph system outside the chest such as perturbations of the patency of the thoracic duct, lymph exudate in the retro-orbital space and pelvis, or lymphadenopathy [5]. Moreover, in rare cases, there is a proliferation of pathological lymph vessels infiltrated by LAM cells, leading to an obstruction of their lumen and accumulation of lymph resulting in the formation of solid-cystic masses described as lymphangiomyoma or extrapulmonary LAM (E-LAM) [6].

Frequency of occurrence

The frequency of occurrence of lung LAM differs depending on the clinical type — pulmonary LAM is found in 30–50% women and approximately 10% men with tuberous sclerosis [7, 8], whereas the sporadic form occurs in one woman out of 200–400 thousand [5, 7]. Lymphangioleiomyomatosis concerns almost exclusively women of reproductive age — the median age at diagnosis is in the range of 35–41 years [9–12]. Pleural LAM is rarely diagnosed in postmenopausal patients — of note here is the often occurring information about the use of hormonal replacement therapy [12, 13]. Single cases have been described of the occurrence of the disease in children and men without predisposing genetic factors [14–16]. Extrapulmonary perturbations within the lymphatic system are found often in patients with the pulmonary LAM form — perturbations in the patency of the thoracic duct are found in over 70%, lymph exudate in the retroperitoneal space is observed in 30% of the cases [17]. Lymphangioleiomyoma is less common — in 10–21% of patients with pulmonary S-LAM [12, 17–19] and is often accompanied by a swelling of lymph nodes within the abdominal cavity, ascites and a broadening of the thoracic duct [18]. It seems that the frequency of occurrence of lymphangioleiomyoma in patients with the pulmonary form of LAM increases with the severity of its course [18], but they are not more common in patients with tuberous sclerosis — in a retrospective analysis from the Mayo Clinic, among 403 patients with TSC, E-LAM occurred in only 3 [20]. In the analysis of autopsy material or material obtained from gynecological surgery in 10 female patients with the pulmonary form of LAM, the presence of small LAM foci in the uterus, adnexa or broad ligament of the uterus was observed in as many as 90% , and in 80% — the occupation of the lymph nodes in the retroperitoneal space [21]. It is not clear whether the presence of E-LAM foci predisposes to the development of the pulmonary form of LAM. In a large retrospective analysis of material obtained from 1732 patients without a history of LAM (median age 56 years), who underwent gynecological surgery with lymphadenectomy, extrapulmonary LAM foci were found in 8 patients [22]. In one of them a pulmonary form of LAM was diagnosed, 7 years after the gynecological surgery whereas the remaining women did not have a LAM relapse in any form (median observation 26 months) [22]. In a similar analysis among 19 patients in whom asymptomatic extrapulmonary LAM had been detected occupying the uterus and pelvic and paraaortal lymph nodes during an average 33 month observation none developed the pulmonary form [23]. Besides the description of cases concerning women with LAM foci in paraaortal and pelvic lymph nodes, in whom pulmonary LAM did not occur nor tuberous sclerosis [24, 25], descriptions are also available in which extrapulmonary

LAM was the first symptom of the development of the pulmonary LAM form, which if it occurs is most commonly diagnosed within about 2 years from the diagnosis of the extrapulmonary form [6, 26–31].

Anatomy and clinical picture

Pulmonary LAM leads to progressive destruction of the lung parenchyma and its replacement by numerous small cysts, causing a progressive respiratory deficiency [7]. In the course of LAM exercise tolerance is progressively limited as well as everyday physical activity to a degree greater than in for example chronic obstructive pulmonary disease [32]. Among the most common early symptoms of the disease are: dyspnea, persistent coughing and hemoptysis [7, 11, 12]. Dyspnea at rest and the need for oxygen therapy appears in most patients within 10 years from the diagnosis [33]. The course of the disease is complicated by recurring pneumothorax, occurring in about 5% of the patients per year from the moment of diagnosis; additionally, in about one-half of the cases they are the first symptom of developing LAM [3, 12, 34]. Moreover, because of the perturbation of lymphatic vessel patency, there are lymph exudates in the pleural cavity causing an intensified dyspnea and chest pain [35]. LAM is also a rare cause of occurrence of pulmonary hypertension (about 7.6% patients LAM) [9].

Lymphangioleiomyoma is most commonly localized in the retroperitoneal space and the uterus and in surrounding lymph nodes [6, 24, 27]. Moreover, E-LAM foci developing in the mediastinum [36], supraclavicular lymph nodes [37, 38], liver [39], walls of the small and large intestine [40, 41], pancreas [26, 42], mesentery [43] have been described. E-LAM masses can reach very large sizes — cases of tumors with the greatest length 15–22 cm have been described [18, 44–46] — and can extend along lymph vessels — coming from the retroperitoneal space to the chest [19], and further even along the neck along with the sternocleidomastoid muscle [47].

The symptoms of lymphangioleiomyoma presence occur in about 55–60% patients [6, 18] and the most commonly are: pain in the vicinity of the tumor [26, 29, 46] and bleeding from the reproductive system in the case of LAM localized in the uterus [24, 48, 49]. Symptoms associated with the pressure of the tumor mass on neighboring organs are also frequently observed: dyspepsia, bloating and symptoms of obstruction of the alimentary tract [46], hydronephrosis or edema and paresthesia of the lower extremities [46, 50]. Lymphangioleiomyoma localized in the mediastinum may cause Horner syndrome, lymph exudates to the pleural cavity and heart rhythm perturbations [6, 18]. If LAM cells infiltrate the ureters, chyluria occurs [51], whereas the occupation of Vater's papilla may lead to cholestasis [45]. In the case of large lymphangioleiomyomas, ascites

linked to a large lymph volume may be observed [6] or bleeding from the tumor to the abdominal cavity [52]. Symptoms due to pressure on neighboring organs such as: bloating, pain in the vicinity of the tumor, pollakiuria, edema and paresthesia of the lower extremities or constipation are more intense during the day [18]. This is associated with an increase in tumor volume during the day due to greater flow of lymph through the abdominal cavity and the pelvis after meals and during daily activity, and an increase in hydrostatic pressure in the erect position [18, 27]. The increase in volume during the day is 140% on average [18]. Changes in lymphangioliomyoma volume during the menstrual cycle have also been described [53].

Diagnosis

According to the recommendations of the European Pulmonological Society, the gold standard in LAM diagnosis is a lung biopsy and high-resolution computer tomography of the chest [7]. Characteristics additionally favoring this diagnosis are: angiomyolipoma currently/in the patient's history, lymph exudates within the chest and abdominal cavity, tuberous sclerosis, the presence of lymphangioliomyoma or microscopically detected occupation of lymph nodes by LAM cells [7]. In the recommendations of the American Thoracic Society/Japanese Respiratory Society from 2017, the following are also included among these properties: plasma concentration of vascular epithelial growth factor D (VEGF-D) ≥ 800 pg/mL and the presence of LAM cells in a cytological examination of the lymph exudates in the pleural cavity [54]. Because LAM is exceedingly rare in males, the final diagnosis should be made in each case on the basis of a typical result of a lung biopsy [7]. A transbronchial biopsy has been shown to be a relatively safe method in the group of LAM patients [55].

High-resolution computer tomography is the examination of choice both in the diagnostic process as well as to observe disease progression [7]. Among typical radiological changes observed in all patients are numerous, small round cysts in the lung (2–5 mm diameter), equally distributed within the lung parenchyma [11]. Because of the frequent co-occurrence of LAM and renal angiomyolipoma computer tomography with an abdominal cavity and pelvic contrast is recommended, and in the case of contraindications for contrast use — a magnetic resonance analysis [7].

Lymphangioliomyoma in computer tomography most commonly takes the form of well-defined, solid-cystic lesions, with walls of different thickness and numerous septa [26, 27, 46]. Less commonly a solid or only cyst-like character is observed [27]. The lesion is generally delimited but a few lymphangioliomyomas with an in-

filtrating type of growth are observed [18]. Solid tumors have a density similar to the liver (59–71 HU), cyst-like lesions a lower one (3–25 HU), corresponding to lymph accumulation [18]. The cyst walls may become more pronounced after contrast [17]. Lymphangioliomyoma under magnetic resonance shows differentiated values of signal both in T1 and in T2 exposure [38, 44, 49, 50]. In PET a small uptake of glucose is observed [26]. In ultrasound E-LAM can give the appearance of an isoechoic, hypo- and hyperechoic lesion, not permitting its differentiation from ovarian neoplasms or lymphomas [27]. However, changes in the structure during the day are characteristic: solid changes in the morning hours, can in the evening have a solid-cystic character and change their echogenicity [27]. Moreover, lymphangioliomyomas have been found to increase their volume during the day which differentiates them from other neoplastic lesions localized in the retroperitoneal space and the pelvis [27].

LAM is also associated with an increased risk of meningioma development, therefore in the case of symptoms from the central nervous system magnetic resonance of the head is justified [7]. Patients with LAM should also be under the care of a genetic counselling facility — TSC is characterized by a broad range of phenotypes and poorly clinically expressed characteristics of this syndrome may lead to incorrect classification of the case as sporadic [7].

Morphology

The typical appearance for LAM encompasses the occurrence of numerous cysts in the parenchymal tissue of the lungs and multifocal proliferation of immature smooth myocytes and perivascular epithelioid cells (PECs), known in the case of lymphangioliomyoma as LAM cells [7]. Foci of proliferation for LAM cells and myocytes are most commonly located around lymph vessels, interlobar septae and the pleura [3]. Within LAM cysts smaller fusiform cells are localized more centrally, whereas epithelioid cells with abundant cytoplasm are localized mainly at the periphery [56]. Cytological atypia and division patterns are generally not present [57].

On the basis of the intensity of two morphological changes typical for LAM: the occurrence of cysts and intensification of LAM cell proliferation within lung biopsy material, Matsui et al. (2001), elaborated a division into III histologic severity grades (LHS, histologic severity of LAM) [34]. The percentage of lung tissue occupied by the described lesions viewed under a small magnification was qualified as follows: LHS1 < 25%; LHS-2: 25–50% and LHS-3 > 50% [34]. The authors also showed a strong correlation of the grade of LHS severity with 10-year overall survival, which was: 100%,

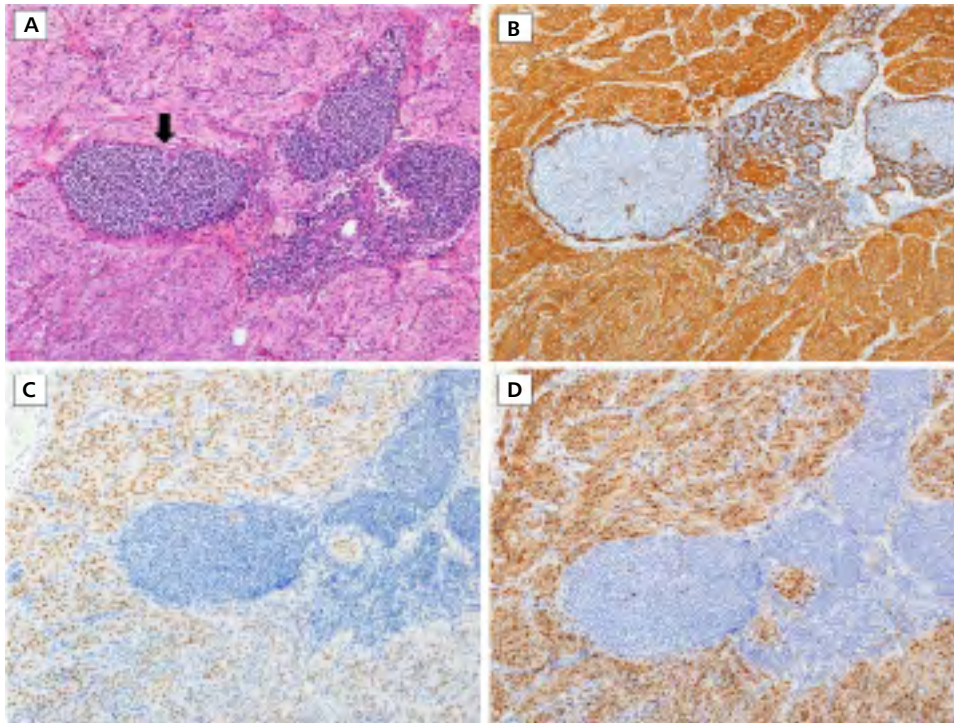


Figure 1A–D. Retroperitoneal LAM with infiltration of lymph node — in the order of staining HE, SMA, HMB-45, progesterone receptors [100×]

74.4% and 52.4%, respectively in w LHS-1, LHS-2 and LHS-3 [34]. LHS is currently an acknowledged prognostic factor in pulmonary LAM [58].

Lymphangioliomyoma is generally described as lesions well delimited from surrounding tissues [21, 26], with the presence of a fibrous capsule [57]. Infiltration of LAM cells beyond the capsule is rare [6]. LAM foci in the uterus are an exception, they are frequently poorly separated from unaffected smooth uterine muscle coat [21, 24]. Differential diagnosis of E-LAM within the uterus encompasses myoma, leiomyosarcoma, endometrial stromal sarcoma or paraganglioma [48]. Lymphangioliomyomas similarly as the pulmonary form is composed of fusiform and epithelioid LAM cells with round and oval nuclei with an abundant slightly acidophilic cytoplasm [6, 24, 50]. These cells form nests — resembling the so-called „zellballen” described in pheochromocytomas [26], separated by slot-like vessels with thin walls lined with endothelial cells [50], which may undergo hyalinization [21, 24, 26]. Typically necrosis and blood extravasation to the tumor are not observed [6, 21, 26, 46]. In most cases the mitotic activity is low [6, 21], in some cases, up to 4 figures for 10 visual fields were observed [24, 37]. A fairly common finding are small clusters of reactive lymphocytes, resembling lymph nodules [6].

LAM cells are characterized by the simultaneous expression of melanocyte (HMB-45, Melan A, MART1)

and smooth muscle (SMA, desmin, actin) markers (Fig. 1) [59, 60]. The expression of HMB-45 is observed in all cases, and the percentage of cells showing the expression of this marker is variable — it is in general 20–40% and concerns predominantly epithelioid cells [11, 56, 60, 61]. In almost all cases the expression of the estrogen (ER) and progesterone (PR) receptor also occurs — mainly within fusiform cells [56], and in general PR expression is stronger [62]. Other markers useful in the differential diagnosis are β -catenin and E-cadherin and cathepsin K, whose expression has been observed in all analyzed cases [56, 63, 64]. The co-occurrence of the expression of lymph vessel endothelium markers is also characteristic: podoplanin (D2-40), PROXI, VEGFR-3 and LYVE1, which occurs both in cells lining lymph vessels and in the LAM cells themselves [56]. Among less typical markers is the epithelial growth factor receptor (EGFR), whose expression was observed in about one-half of cases [56]. Kobayashi et al. (2018) have also demonstrated the cytoplasmic expression of EGFR/ErbB-1 and HER4/ErbB-4 in LAM cells [65]. Maisel et al. (2018) described the strong expression of the programmed death-ligand 1 (PD-L1) in preparations from lung biopsies from 6 patients with LAM, which was greater in preparations from pulmonary nodules than in tissue from healthy lungs [66]. They also found the presence of T lymphocytes showing the expression of programmed death-ligand 1 (PD-1) receptor, infiltrating

LAM foci [66]. In the case of lymphangioliomyoma, LAM cells show the expression of a similar marker profile: HMB45 — particularly in epithelial cells [6], Melan A, calponin, SMA, desmin, nuclear expression of ER and PR [6, 24, 46, 50]; moreover expression of β -catenin [35], E-cadherin [67], MiiTF [25] and in the lymph vessel endothelium: CD34, podoplanin, CD31, and VEGFr-3 [21, 24, 26, 43].

Genetics

Similarly, as other proliferative diseases from the PEComa family, lymphangioliomyoma has a higher frequency of occurrence in patients with tuberous sclerosis — characterized by the presence of germline inactivating mutations and loss of heterozygosity (LOH) in the 16p13.3 region, in the *TSC2* locus, encoding the tuberin protein and in the 9q34 region, locus *TSC1*, encoding the hamartin protein [8, 68]. Tuberin has an inhibiting action on the signalling protein Rheb — a homolog of the Ras protein (Ras homolog enriched in the brain — Rheb), which in turn is a known activator of the mTOR serine-threonine kinase [69]. Hamartin forms a complex with tuberin, stabilizing it and protecting it from degradation in proteosomes [70]. Excessive activation of the Rheb protein due to the loss of function of one of these two proteins leads to activation of the mTORC1 pathway, increased synthesis of proliferation stimulating proteins and angiogenesis resulting in the presence of numerous tumors of the PEComa type in patients with tuberous sclerosis [69]. The pulmonary form of LAM is found in approximately 30–50% women and in approximately 10% men with tuberous sclerosis, these are more commonly patients with mutations within *TSC2* [8, 71]. Similarly, somatic inactivating mutations and loss of heterozygosity in the *TSC1* and *TSC2* genes are observed in LAM cells obtained from patients with the sporadic LAM form though as this form is rare there are no analyses of large groups of patients [72]. Badri et al. (2013) analyzed material obtained from 10 patients with sporadic LAM, showing that in as many as 8 of them LAM cells showed perturbations within the *TSC2* locus, moreover, in 4 of them complete loss of tuberin occurred because of loss of heterozygosity and a mutation in the second allele (3 cases) and two inactivating mutations in both alleles simultaneously in one case [73]. In the paper by Fujita et al. (2015), inactivating somatic *TSC1/TSC2* mutations were detected in LAM cells in 6 out of 9 patients with LAM [74]. Among other genetic changes observed in patients with lymphangioliomyoma are: germline mutations within *BARD1*, *BLM* and *BRCA2* [14] and *EGFR* amplification [56], however, their role in LAM pathogenesis has not been fully analyzed.

Evaluation of the stage of the pulmonary form of LAM and factors affecting the severity of its progression

Among analyses of the functioning of the respiratory system whose results correlate with the irregularities observed in radiological and histopathological analyses and which change with disease progression are: analysis of the coefficient of lung transfer for carbon monoxide (TLco) and a spirometric analysis — especially the measurement of the forced expiratory volume in 1 second (FEV₁) [58]. In the first evaluation of the degree of progression of the disease TLco and spirometric analysis with a bronchodilatory test are recommended [7]. Moreover, in patients from whom a lung biopsy was obtained a histological evaluation of the progression in the LHS scale, which was described earlier, is performed [58]. FEV₁ and TLco measurements should be repeated every 3–6 months in order to evaluate the progression of the disease and the response to treatment and in the case of stable results, the control analyses can be reduced to annual ones [7]. In patients in the initial stage of the disease in general deviations are not observed in gasometric blood analysis, it is used as an indication of indications for oxygen therapy and lung transplantation in patients with advanced disease [7]. An exercise test and a 6-minute marching test (6MWT) find application in the evaluation of the effect of the disease on general performance and response to treatment [58]. The pulmonary LAM form is characterized by a milder course in patients with tuberous sclerosis, in comparison to the sporadic form [7, 58]. Moreover, patients in whom the first LAM symptoms are hemoptysis and dyspnea have a worse disease than those in whom LAM was diagnosed because of pneumothorax, which may be associated with the delay in diagnosis in the first group [58]. A tendency of LAM to progress more slowly in post-menopausal women has been indicated [75]; in the analysis by Gupta et al. (2019) the change in FEV₁ in pre-menopausal women was on the average –118 mL/year, whereas in postmenopausal women it was –74 mL/year (p = 0.003) [76]. A recently described prognostic factor in lymphangioliomyoma is the plasma level of vascular endothelial growth factor D (VEGF-D), which is much higher in patients than in the healthy population, especially in patients with tuberous sclerosis [77]. A high level of VEGF-D (over 800 pg/mL) was correlated with a more rapid rate of deterioration of FEV₁ values [78] and the presence of lymph exudates to the pleural cavity and the number of pulmonary cysts [77]. Other markers which have been associated with the severity of the course of pulmonary LAM are: the concentration of extracellular matrix metalloproteinases (MMP) in urine [58], the plasma concentration of vitamin D binding protein (VTDB) [79, 80], VEGF-3 receptor and chemokine CCL21 [81]

or the expression of the receptor of the human epithelial growth factor 3 (HER3) w LAM cells [61].

Pulmonary LAM treatment

Respiratory rehabilitation encompassing aerobic exercises and exercises for strengthening the breathing muscles exerts a proven, favorable effect on the evaluation of life quality, the 6MWT result, the feeling of dyspnea and the level of everyday activity of LAM patients [82]. By analogy to the procedure in chronic obstructive pulmonary disease, it is recommended that LAM patients be covered by prophylactic vaccinations against pneumonia diplococcus and influenza and because of the frequent occurrence of osteoporosis, patients with LAM should undergo periodic densitometric analyses [7]. LAM is associated with an increased risk of pneumothorax, which occurs in about one-half of patients at the moment of diagnosis and in over 60% of patients in the course of the disease, with a tendency to frequent recurrences [7]. Factors additionally increasing the risk of pneumothorax are pregnancy and flying [7, 83]. Pleurodesis is effective in preventing recurring pneumothorax; the analysis by Gonano et al. (2018) of a group of 145 patients indicated that pleurodesis prevented its occurrence with a probability of 82%, 68% and 59% after one, 5 and 10 years, respectively after the procedure in comparison with 55%, 46% and 39% among patients who did not undergo the procedure ($p = 0.026$) [83]. In the case of abundant lymph exudates to the pleural cavity a diet low in fat is recommended, and if clinical symptoms occur (dyspnea, coughing, chest pain) — traditionally draining of the pleural cavity through thoracentesis is applied [7], however, in the light of new evidence before starting treatment by invasive methods an attempt to control the symptoms by systemic treatment with sirolimus is recommended [5, 35, 84]. In advanced stages of LAM, in patients considered to be class III–IV on the NYHA scale with hypoxemia at rest, lung transplantation can be applied [7]. Average time from diagnosis to the transplantation varies in papers from various centers in the range of 4–5 years [85, 86]. In a large retrospective analysis of data concerning the course of the disease in 138 patients with LAM who had undergone a lung transplantation 1-year, 5-year and 10-year overall survival after the procedure were: 94%, 73% and 56%, respectively, and the causes of death were most commonly: bronchiolitis obliterans and chronic transplant rejection [87]. Such parameters as the presence of lung hypertension and the 6MWT distance before the procedure, the patient's age, time of organ ischemia during the procedure or transplantation of one or both lungs did not affect overall survival after the procedure [87]. In the analysis by Reynaud-Gaubert et al.

(2008), summing up the results of lung transplantation of several French transplantation centers 1-year, 5-year and 10-year overall survival after the procedure were 79.6%, 74.4% and 64.7%, respectively [85]. Baldi et al. (2017) obtained similar results in an analysis encompassing 11 patients with LAM, the probability of surviving one, three and five years after lung transplantation was 90%, 90% and 75%, respectively [86]. There are rare descriptions of patients with a LAM recurrence in the transplanted lung [85, 88].

In recent years a breakthrough has occurred in systemic lymphangioleiomyomatosis treatment, which for many years was resistant to anti-estrogen therapy (removal of ovaries, use of medroxyprogesterone or selective estrogen receptor modulators) whose efficacy had finally not been proven [89]. No efficacy was demonstrated for treating LAM using doxycycline [5] or an aromatase inhibitor — letrozole [90]. On the basis of positive results of clinical trials, sirolimus, a drug from the group of mTOR kinase inhibitors was registered by the Food and Drug Administration (FDA) in May 2015 as the first and currently the only drug for systemic LAM treatment [5]. According to the guidelines of the American Thoracic Society/Japanese Respiratory Society, sirolimus is indicated in patients with LAM, accompanied by a rapidly deteriorating function of the respiratory system or $FEV_1 \leq 70\%$ wn. and in patients with symptomatic pleural lymph exudates, before using invasive treatment methods [5]. Clinical trials concerning the use of mTOR inhibitors (sirolimus, everolimus) in treating lymphangioleiomyomatosis are summarized in Table 1 — without taking retrospective analyses into consideration.

A randomized, multicenter, placebo-controlled, double-blind phase III clinical trial MILES (Multicenter International Lymphangioleiomyomatosis Efficacy And Safety Of Sirolimus), is so far the largest clinical trial concerning the use of sirolimus in LAM [91]. 89 patients were randomly divided into 43 receiving placebo and 46 sirolimus p.o. at an initial dose of 2 mg/d, and then established to maintain the serum concentration of the drug at a level of 5 to 15 ng/mL [91]; this dosage was accepted as a standard in successive trials [84, 92, 93]. After 12 months of treatment in the group receiving sirolimus FEV_1 stabilization was observed ($+1 \pm 2$ mL/month) with worsening FEV_1 at 12 ± 2 mL/month in the placebo group ($p < 0.001$). In the treatment group improvement was also observed in forced vital capacity (FVC), exercise tolerance, evaluation of the quality of life and a decrease in the serum concentration of VEGF-D in comparison with the placebo group. After finishing taking sirolimus, FEV_1 decreased at the same rate in both groups, which suggests that sirolimus therapy does not stop the progression of the disease when therapy is terminated, but also does not speed up this process [94].

Table 1. Clinical trials concerning the use of mTOR inhibitors mTOR (S-sirolimus, E-everolimus) in LAM

| Author, year | Trial type | N | Age- years | TSC % | Drug | Drug concentration/dose | Length | FEV ₁ changes | Other effects |
|-----------------------------------|--|--------------|------------|-------|------|-------------------------|--|--|---|
| Bissler et al. 2008 * [105] | Single center, open | 25 (18 LAM) | - | 66.7 | S | 10-15 ng/mL | 12 months + 12 months observation | At the end of treatment: +118 ± 330 mL vs. wp At the end of observation: + 62 ± 411 mL vs. wp | ↑ FVC, ↓AML volume |
| Dabora et al. 2011 * [106] | Multicenter, open | 36 (21 LAM) | 34 | 100 | S | 9-15 ng/mL | 52 weeks | No changes vs. wp | ↓AML volume, ↓VEGF-D, ↓number of skin changes |
| Davies et al. 2011 * [107] | Multicenter, open | 16 | - | 33 | S | 3-10 ng/mL | 24 months | -76 ± 52 mL/r | ↓AML volume, ↓FVC, ↓DLco |
| McCormack et al. 2011 * [91] | Multicenter, randomized, double blind, placebo control | 89 | 45.4 | 9 | S | 5-15 ng/mL | 12 months + 12 months observation | Sirolimus: +1 ± 2 mL/month placebo: -12 ± 2 mL/month | ↑FVC, ↑QoL, ↓VEGF-D, ↓FRC |
| Neurohr et al. 2011 [93] | Single center, open/observational | 10 | 42.4 | 20 | S | 5-10 ng/mL | 12.1 ± 2.81 months | After 6 months: + 345 ± 58 mL | ↑ FVC, ↑DLco, ↑6MWD |
| Taveira-DaSilva et al. 2011 [84] | Single center, open/observational | 19 | 41 | 0 | S | 5-15 ng/mL | 2.5 years | Before treatment: -2.8% ± 0.8% wn/r sirolimus: + 1.8% ± 0.5% wn/time of observation | ↑FVC, ↑ΔDLco, ↓chyllothorax |
| Bissler et al. 2013* [108] | Multicenter, randomized, double-blind, placebo-control | 118 (29 LAM) | 31 | 96 | E | 10 mg/d | E: average 38 weeks placebo: average 34 weeks | Everolimus: -1% wp placebo: -4% wp | ↓AML volume, ↓ number of skin changes |
| Yao et al. 2014 [109] | Single center, open | 38 | 40.9 | 13 | S | 8.0 ± 2.5 ng/mL | Average 40.8 months | Before treatment: -2.3 ± 0.1% wn/r sirolimus: +1 ± 0.3% wn/r | ↑DLco |
| Goldberg et al. 2015* [110] | Multicenter, open | 24 | 42.5 | 5 | E | 10 mg/d | 26 weeks | + 114 mL (95% CI: 11-217) | ↑ 6MWD, ↓VEGF-D |
| Takada et al. 2016* [92] | Multicenter, open | 63 | 41.5 | 3.2 | S | 5-15 ng/mL | 24 months | FEV ₁ ↓ FEV ₁ ↑ in the group with chyllothorax in history | - |
| Bee et al. 2018* [111] | Prospective, cohort | 47 | 43.6 | 19 | S | 1-2 mg/d | Average 35.8 months | In the whole group: +11 mL/r (od +254 do -148 mL/r) In a group of 21 patients in whom Δ FEV ₁ before the trial was known: -150 mL/r before vs. +35 mL/r during | - |
| Taveira-DaSilva et al. 2018 [112] | Single center, open | 25 | 40.6 | 8 | S | 5-15 ng/mL | 54 ± 19.2 months | Before treatment: -7.4% ± 1.4% wn/r sirolimus: -0.3% ± 0.5% wn/r | ↓ AML diameter, ↓VEGF-D, ↓DLco, ↓chyllothorax |

BP — blood pressure; DLCO — lung diffusion capacity for carbon dioxide; DN — adverse effects; DDO — lower respiratory tract; DO — respiratory tract; GDO — upper respiratory tract; FEV₁ — first second forced vital capacity; FVC — forced vital capacity; OO — peripheral edema; N — number of trial participants; TC — total cholesterol; Qo — the quality of life; TG — triglycerides; WBC — white blood count; ZUM — infection of the urinary tract; wn — predicted value; wp — initial value; *trial included in meta-analysis by Gao et al. (2018) [98]

In the MILES trial, VEGF-D serum concentration was indicated as a negative prognostic factor but at the same time as a positive predictive factor for response to sirolimus treatment [95]. A higher VEGF-D level at the beginning of the trial was associated with a better response to treatment in the group receiving sirolimus (improvement in FEV₁ and FVC values), but at the same time a more rapid decrease in the value of these parameters in the placebo group [95]. In the last performed analyses a positive effect of sirolimus on burdensome LAM complications such as lymph exudates to the pleural cavity and recurring pneumothorax was also observed. In the trial by Zhou et al. (2018) in 5 analyzed patients with recurring pneumothorax in spite of pleurodesis, taking sirolimus in doses ensuring the maintenance of the drug concentration in serum at a level 3–10 ng/mL, no recurrences of pneumothorax were observed during the whole time of treatment [96]. However, interruption of therapy or a decrease of the blood concentration of the drug below 3 ng/mL resulted in recurrence of pneumothorax in 2 patients during 2 and 3 year-long observation [96]. In an observational trial by Taveira-DaSilva et al. (2011) planned to evaluate the benefit of using sirolimus in patients with a severe course of LAM and abundant lymph exudates in the pleural cavity in all 12 patients a complete or almost complete reduction of the volume of the accumulating liquid took place which allowed draining of the pleural cavity to be stopped in 2 of them [84]. In two retrospective analyses the effectiveness of sirolimus at a lower dose (target drug concentration in serum below 5 ng/mL) in comparison with a standard dose (drug concentration in serum 5–15 ng/mL on the basis of the MILES trial), was evaluated, yielding contradictory results [10, 97]. Ando et al. (2013) showed an improvement in the function of the respiratory system and withdrawal of lymph exudates in patients treated with low doses, to a degree comparable with the results of trials using the higher dose [10]. However, Yoon et al. (2018) showed lower effectiveness of lower doses of sirolimus, whose use at the same time did not lead to a decreased frequency of undesirable adverse effects [97]. In a meta-analysis by Gao et al. (2018), encompassing 7 clinical trials concerning the use of sirolimus in LAM, a significant improvement of FEV₁ and FVC was confirmed in treated patients – the weighted average of differences was: 0.15 l (95% CI: 0.08–0.22, $p < 0.01$, $I_2 = 0\%$) for FEV₁ and 0.22 l (95% CI: 0.11–0.32, $p < 0.01$, $I_2 = 0\%$) for FVC [98]. However, no improvement of the 6-minute walk test nor the diffusion capacity of the lungs for carbon dioxide was observed. The accumulated frequency of occurrence of adverse events was: 50% for stomatitis, 40% for hyperlipidemia, 23% for headaches, 20% for bone marrow suppression and 19% for diarrhea [98]. Among frequently mentioned adverse effects of sirolimus are also respiratory tract

infections, but sirolimus was not shown to increase their frequency with respect to the population of patients with LAM not using systemic therapy [99].

Sirolimus is currently the only drug whose use in LAM is recommended in the guidelines of the American Thoracic Society/Japanese Respiratory Society, in the light of the lack of convincing evidence for the effectiveness of other substances [5]. Preclinical and clinical trials are also being conducted on autophagy inhibitors [100], statins [101], hydroxychloroquine [102], a synthetic flavonoid — Proxison — an antioxidant normalizing mitochondrial metabolism, which showed synergy with sirolimus in inhibiting LAM cell growth *in vitro* [103], drugs targeting signal pathways connected with the receptor for vascular endothelial growth factor — VEGFR [104] or PD-1 inhibitors, prolonging survival in mouse models of LAM [66].

Treatment and prognosis in E-LAM

Lymphangioleiomyomas, even though they attain large sizes and are often non-resectable [29, 45], in general, have a mild clinical course. In a retrospective analysis by Matsui et al. (2001) among 17 patients with E-LAM, only one died because of an aggressive course of a simultaneously occurring pulmonary LAM form, all the others were alive at the moment of publishing the results with an average observation time of 5.5 years [6]. Radical resection allows long-term control of the disease [16, 24, 39, 40, 42, 50], also when laparoscopic techniques are used [43, 113]. There are reports in the literature about local recurrence after E-LAM resection [37, 114], which can, however, be treated with good effects by a repeated resection [115]. Long survival times were also observed in the case of non-resectable disease; the case of an 11-year old girl has been described in whom a non-radically excised mesenterial lymphangioleiomyoma did not undergo progression in spite of lack of treatment for 10 years [116] and a 47-year old woman with uterine E-LAM and metastases to the lungs and ovary who remained in good overall status for 12 years of observation [117]. Because of the lack of unified methods of lymphangioleiomyoma treatment and their relatively mild clinical course, screening of patients with the pulmonary form of LAM for E-LAM is not recommended, if they do not have any clinical symptoms [7]. If cumbersome clinical symptoms occur such as pain in tumor progression, constipation or edema of the lower extremities, attempts to treat are undertaken similar to the pulmonary form of LAM — by hormone therapy [12] and mTOR inhibitors [118] and radiotherapy for the area of the occupied lymph nodes [119]. Radzikowska et al. (2016) performed a retrospective analysis of the effectiveness of sirolimus in 14 patients with pulmonary LAM (including one with TSC) and lymphangioleiomyoma of the retroperitoneal tract [118]. During 10 months of therapy with sirolimus (at

Table 2. E-LAM cases treated with mTOR inhibitors — sirolimus and everolimus available in the literature

| Author | Age | TSC | Pulmonary LAM | Localization | Max. tumor diameter | Symptoms from tumor | Drug; Dose | Best response | Time of observation | Progression |
|--------------------------------|-----|-----|---------------|---|---------------------|---|---|----------------|---------------------|-------------|
| Chen et al. 2009 [88] | 23 | no | yes | PZ | – | – | Sirolimus; 1 mg/d | – | 36 months | No |
| Possekel et al. 2009 [41] | 23 | no | no | Numerous in liver, mesentery, wall of small and large intestine | 10 cm | A feeling of incomplete elimination, stomach pain | Sirolimus; 1.5 Mg/d | PR | 3 months | No |
| Rosenberg et al. 2013 [121] | 41 | no | yes | PZ | 16.5 cm | Stomach pain, early feeling of satiety | Sirolimus; – | PR | 9 months | No |
| Freitas et al. 2015 [122] | 26 | no | yes | PZ, abdominal lymph nodes | – | – | Sirolimus; 2 mg/d | PR | 12 months | No |
| | 37 | no | later | PZ | 18 cm | Pain in the vicinity of tumor | Sirolimus; 2 mg/d | PR | 6 months | No |
| Hecimovic et al. 2015 [123] | 44 | no | yes | PZ | 7cm | – | Sirolimus; 2–3 mg/d | PR | 10 months | No |
| | 33 | no | later | PZ, pelvis | 10 cm | – | Sirolimus; 1 mg/d | PR (almost CR) | 9 months | No |
| | 34 | no | later | PZ | 8 cm | Stomach pain, increase in stomach diameter, ascites, hemoptysis | Sirolimus; 2 mg/d | PR | 3 months | No |
| Cabeza et al. 2016 [124] | 53 | no | yes | Numerous in PZ | 6.8 cm | Stomach pain | Sirolimus; 2 mg/d | PR (almost CR) | 12 months | No |
| Harrari et al. 2016 [125] | 39 | no | no | Numerous in PZ | – | – | Sirolimus; 2 Mg/d | PR | 18 months | No |
| Ito et al. 2016 [126] | 39 | no | yes | PZ, pelvis | 6.2 cm; | Edema of lower extremity | Sirolimus; 1 mg/d for 2 weeks then 2 mg/d | PR (almost CR) | 18 months | No |
| Wahid et al. 2016 [127] | 37 | yes | no | pelvis | 15.3 cm | Back ache, fainting | Everolimus; 10mg/d | PR | 12 months | No |
| | 45 | no | no | PZ | 8.5 cm | Feeling of satiety, pain in vicinity of tumor | Everolimus; 10mg/d | CR | 18 months | No |
| Lecuelle et al. 2019 [128] | 35 | no | yes | PZ | 8.5 cm | – | Sirolimus; 5mg/d | CR | 30 months | No |
| Ussavarungsi et al. 2019 [129] | 26 | no | yes | PZ | 26 cm | – | Sirolimus; 1 mg/d | SD | 14 months | No |

CR — complete response; d — day; max. — maximal; PD— disease progression; PR— partial response; PZ — retroperitoneal space

Table 3. E-LAM cases with the use of hormone therapy available in the literature

| Author | Age | TSC | Pulmonary LAM | Localization | Max. tumor diameter | Symptoms from tumor | Drug; Dose | Best response | Time of observation | Progression | Drug; Dose |
|-----------------------------|-----|-----|---------------|--|------------------------|---|--|--|---------------------|-------------------|--------------|
| Klein et al. 1992 [130] | 36 | No | Yes | Mediastinum lymph nodes, pz and pelvis | Numerous — max 5 cm | None | Oophorectomy + ifn- α 2b (for 3 months) + t tamoxifen | 3 \times 10 ⁶ j. 3 \times / week + 20 mg/d | CR | 18 months | No |
| de Groot et al. 2008 [45] | 23 | No | Later | Pz | 16 Cm | Pain in the vicinity of tumor | Goserelin + tamoxifen Goserelin + medroxyprogesterone + thalidomide Goserelin + ifn- α 2b | 3.6 mg/month + 40 mg/d 3.6 mg/month + 500 mg/d + 50 mg/d 3.6 mg/m + 3 \times 10 ⁶ j. 3 \times /week | PD PD SD | - - 9 years | - - No |
| Yamashita et al. 2011 [131] | 30 | No | No | Pelvis | - | Recurring stomach pains during menstruation | Leuprorelin | 1.88 mg/ month | SD | 6 months | No |
| Szpurek et al. 2014 [117] | 47 | No | No | Uterus, metastases to ovary and lungs | - | Brak | Tamoxifen | - | SD | 5 months | Yes |
| Basnet et al. 2015 [132] | 24 | No | No | Pz, pelvis, mesentery | Numerous, approx. 3 Cm | Pain in vicinity of tumor | Medroxyprogesterone | 150 mg i.m.; 2 doses given with a 3 month interval | PR | 6 months | Yes |
| Yoshizawa et al. 2019 [133] | 36 | Yes | No | Uterus | - | Stomach pain | Leuprorelin | - | PD | - | - |

CR — complete response; d — day; IFN- α 2b — interferon α 2b; j. — unit; max. — maximum; PD — disease progression; PR — partial response; PZ — retroperitoneal space

a dose of 1–5 mg/d, in order to attain blood concentration of the drug at a level of 5–15 ng/mL), besides improvement of respiratory tract ailments, a significant decrease was observed in the volume of the lesions in comparison to the initial value ($1603.85 \pm 2437.56 \text{ cm}^3$ vs. $198.01 \pm 315.43 \text{ cm}^3$; $p = 0.00026$), and total withdrawal of lymph from the retroperitoneal space and pleura in 13 of them [118]. In the analysis by Mohammadih et al. (2013), encompassing 5 patients with lymphangioliomyoma of the abdominal cavity, the effectiveness of everolimus (at a dose 1–1.5 mg/d; in two divided doses) was evaluated [120]. After 6 months of therapy in 4 out of 5 patients a partial or complete response was observed and withdrawal of the ascites [120]. The descriptions in the literature of cases treated with mTOR inhibitors and hormone therapy in E-LAM are presented in Table 2 and Table 3, respectively. It is worth stressing that among the cases presented in Table 2 (mTOR inhibitors), disease progression did not occur in any of the cases during the administration of a drug from this group.

Summary

Lymphangioliomyomatosis is a member of the PEComa (perivascular epithelioid cell tumors) family [2]. In patients with the pulmonary form of LAM, LAM foci have been described in the uterus, adnexa or broad ligament of the uterus and there is the frequent occupation of lymph nodes in the extraperitoneal space [21]. Lymphangioliomyoma in computer tomography, in general, has the form of a well-separated solid-cystic lesion, with walls of different thickness and numerous septae [26, 27, 46]. Solid or cystic character is less common [27]. The lesion is generally well separated, but a few lymphangioliomyomas with infiltrating growth are also observed [18]. Sirolimus is currently the only drug whose use in LAM is recommended by international recommendations [5]. The randomized, multicenter, placebo-controlled, double-blind clinical phase III MILES trial (Multicenter International Lymphangioliomyomatosis Efficacy And Safety Of Sirolimus), is so far the largest clinical trial concerning the use of sirolimus in LAM [91]. Preclinical and clinical trials also encompass autophagy inhibitors [100], statins [101], hydroxychloroquine [102], anti-VEGFR drugs [104] and PD-1 inhibitors [66].

Conflict of interest

The authors report no conflicts of interest.

References

1. Klimczak A, Pękuł M, Wiater K, et al. PEComa — grupa rzadkich nowotworów pochodzenia mezenchymalnego. NOWOTWORY Journal of Oncology. 2011; 61(1): 52–56.

2. Martignoni G, Pea M, Reghellin D, et al. Molecular pathology of lymphangioliomyomatosis and other perivascular epithelioid cell tumors. Arch Pathol Lab Med. 2010; 134(1): 33–40, doi: [10.1043/2008-0542-RAR1.1](https://doi.org/10.1043/2008-0542-RAR1.1), indexed in Pubmed: [20073603](https://pubmed.ncbi.nlm.nih.gov/20073603/).
3. Thway K, Fisher C. PEComa: morphology and genetics of a complex tumor family. Ann Diagn Pathol. 2015; 19(5): 359–368, doi: [10.1016/j.anndiagpath.2015.06.003](https://doi.org/10.1016/j.anndiagpath.2015.06.003), indexed in Pubmed: [26144278](https://pubmed.ncbi.nlm.nih.gov/26144278/).
4. Słowińska M, Józwiak S, Peron A, et al. Early diagnosis of tuberous sclerosis complex: a race against time. How to make the diagnosis before seizures? Orphanet J Rare Dis. 2018; 13(1): 25, doi: [10.1186/s13023-018-0764-z](https://doi.org/10.1186/s13023-018-0764-z), indexed in Pubmed: [29378663](https://pubmed.ncbi.nlm.nih.gov/29378663/).
5. McCormack F, Gupta N, Finlay G, et al. Official American Thoracic Society/Japanese Respiratory Society Clinical Practice Guidelines: Lymphangioliomyomatosis Diagnosis and Management. American Journal of Respiratory and Critical Care Medicine. 2016; 194(6): 748–761, doi: [10.1164/rccm.201607-1384st](https://doi.org/10.1164/rccm.201607-1384st).
6. Matsui K, Tatsuguchi A, Valencia J, et al. Extrapulmonary lymphangioliomyomatosis (LAM): clinicopathologic features in 22 cases. Hum Pathol. 2000; 31(10): 1242–1248, doi: [10.1053/hupa.2000.18500](https://doi.org/10.1053/hupa.2000.18500), indexed in Pubmed: [11070117](https://pubmed.ncbi.nlm.nih.gov/11070117/).
7. Johnson SR, Cordier JF, Lazor R, et al. Review Panel of the ERS LAM Task Force. European Respiratory Society guidelines for the diagnosis and management of lymphangioliomyomatosis. Eur Respir J. 2010; 35(1): 14–26, doi: [10.1183/09031936.00076209](https://doi.org/10.1183/09031936.00076209), indexed in Pubmed: [20044458](https://pubmed.ncbi.nlm.nih.gov/20044458/).
8. Muzykewicz DA, Sharma A, Muse V, et al. TSC1 and TSC2 mutations in patients with lymphangioliomyomatosis and tuberous sclerosis complex. J Med Genet. 2009; 46(7): 465–468, doi: [10.1136/jmg.2008.065342](https://doi.org/10.1136/jmg.2008.065342), indexed in Pubmed: [19419980](https://pubmed.ncbi.nlm.nih.gov/19419980/).
9. Freitas CSG, Baldi BG, Jardim C, et al. Pulmonary hypertension in lymphangioliomyomatosis: prevalence, severity and the role of carbon monoxide diffusion capacity as a screening method. Orphanet J Rare Dis. 2017; 12(1): 74, doi: [10.1186/s13023-017-0626-0](https://doi.org/10.1186/s13023-017-0626-0), indexed in Pubmed: [28427470](https://pubmed.ncbi.nlm.nih.gov/28427470/).
10. Ando K, Kurihara M, Kataoka H, et al. Efficacy and safety of low-dose sirolimus for treatment of lymphangioliomyomatosis. Respir Investig. 2013; 51(3): 175–183, doi: [10.1016/j.resinv.2013.03.002](https://doi.org/10.1016/j.resinv.2013.03.002), indexed in Pubmed: [23978644](https://pubmed.ncbi.nlm.nih.gov/23978644/).
11. Chu SC, Horiba K, Usuki J, et al. Comprehensive evaluation of 35 patients with lymphangioliomyomatosis. Chest. 1999; 115(4): 1041–1052, doi: [10.1378/chest.115.4.1041](https://doi.org/10.1378/chest.115.4.1041), indexed in Pubmed: [10208206](https://pubmed.ncbi.nlm.nih.gov/10208206/).
12. Johnson SR, Tattersfield AE. Clinical experience of lymphangioliomyomatosis in the UK. Thorax. 2000; 55(12): 1052–1057, doi: [10.1136/thorax.55.12.1052](https://doi.org/10.1136/thorax.55.12.1052), indexed in Pubmed: [11083892](https://pubmed.ncbi.nlm.nih.gov/11083892/).
13. Kebria M, Black D, Borelli C, et al. Primary retroperitoneal lymphangioliomyomatosis in a postmenopausal woman: a case report and review of the literature. Int J Gynecol Cancer. 2007; 17(2): 528–532, doi: [10.1111/j.1525-1438.2007.00785.x](https://doi.org/10.1111/j.1525-1438.2007.00785.x), indexed in Pubmed: [17362325](https://pubmed.ncbi.nlm.nih.gov/17362325/).
14. Liu Yi, Guo Z, Zhao C, et al. Lymphangioliomyomatosis: a case report and review of diagnosis and treatment. Onco Targets Ther. 2018; 11: 5339–5347, doi: [10.2147/OTTS.161360](https://doi.org/10.2147/OTTS.161360), indexed in Pubmed: [30214240](https://pubmed.ncbi.nlm.nih.gov/30214240/).
15. Takia L, Jat KR, Mandal A, et al. Lymphangioliomyomatosis (LAM) presenting as recurrent pneumothorax in an infant with tuberous sclerosis: treated successfully with sirolimus. BMJ Case Rep. 2018; 2018, doi: [10.1136/bcr-2018-226244](https://doi.org/10.1136/bcr-2018-226244), indexed in Pubmed: [30413450](https://pubmed.ncbi.nlm.nih.gov/30413450/).
16. Fiore MG, Sanguedolce F, Lolli I, et al. Abdominal lymphangioliomyomatosis in a man with Klinefelter syndrome: the first reported case. Ann Diagn Pathol. 2005; 9(2): 96–100, doi: [10.1016/j.anndiagpath.2004.12.007](https://doi.org/10.1016/j.anndiagpath.2004.12.007), indexed in Pubmed: [15806517](https://pubmed.ncbi.nlm.nih.gov/15806517/).
17. Zhang C, Chen X, Wen T, et al. Computed tomography lymphangiography findings in 27 cases of lymphangioliomyomatosis. Acta Radiol. 2017; 58(11): 1342–1348, doi: [10.1177/0284185116688381](https://doi.org/10.1177/0284185116688381), indexed in Pubmed: [28132530](https://pubmed.ncbi.nlm.nih.gov/28132530/).
18. Avila NA, Bechtle J, Dwyer AJ, et al. Lymphangioliomyomatosis: CT of diurnal variation of lymphangioliomyomas. Radiology. 2001; 221(2): 415–421, doi: [10.1148/radiol.2212001448](https://doi.org/10.1148/radiol.2212001448), indexed in Pubmed: [11687685](https://pubmed.ncbi.nlm.nih.gov/11687685/).
19. Avila NA, Kelly JA, Chu SC, et al. Lymphangioliomyomatosis: abdominopelvic CT and US findings. Radiology. 2000; 216(1): 147–153, doi: [10.1148/radiology.216.1.r00j42147](https://doi.org/10.1148/radiology.216.1.r00j42147), indexed in Pubmed: [10887241](https://pubmed.ncbi.nlm.nih.gov/10887241/).
20. Torres VE, Björnsson J, King BF, et al. Extrapulmonary lymphangioliomyomatosis and lymphangiomas cysts in tuberous sclerosis complex. Mayo Clin Proc. 1995; 70(7): 641–648, doi: [10.4065/70.7.641](https://doi.org/10.4065/70.7.641), indexed in Pubmed: [7791386](https://pubmed.ncbi.nlm.nih.gov/7791386/).
21. Hayashi T, Kumasaka T, Mitani K, et al. Prevalence of uterine and adnexal involvement in pulmonary lymphangioliomyomatosis: a clinicopathologic study of 10 patients. Am J Surg Pathol. 2011; 35(12): 1776–1785, doi: [10.1097/PAS.0b013e318235edbd](https://doi.org/10.1097/PAS.0b013e318235edbd), indexed in Pubmed: [22020043](https://pubmed.ncbi.nlm.nih.gov/22020043/).

22. Kuno I, Yoshida H, Shimizu H, et al. Incidental lymphangioleiomyomatosis in the lymph nodes of gynecologic surgical specimens. *Eur J Obstet Gynecol Reprod Biol.* 2018; 231: 93–97, doi: [10.1016/j.ejogrb.2018.10.027](https://doi.org/10.1016/j.ejogrb.2018.10.027), indexed in Pubmed: [30336310](https://pubmed.ncbi.nlm.nih.gov/30336310/).
23. Schoolmeester JK, Park KJ. Incidental nodal lymphangioleiomyomatosis is not a harbinger of pulmonary lymphangioleiomyomatosis: a study of 19 cases with evaluation of diagnostic immunohistochemistry. *Am J Surg Pathol.* 2015; 39(10): 1404–1410, doi: [10.1097/PAS.0000000000000470](https://doi.org/10.1097/PAS.0000000000000470), indexed in Pubmed: [26135558](https://pubmed.ncbi.nlm.nih.gov/26135558/).
24. Ando H, Ogawa M, Watanabe Y, et al. Lymphangioleiomyoma of the uterus and pelvic lymph nodes: a report of 3 cases, including the potentially earliest manifestation of extrapulmonary lymphangioleiomyomatosis. *Int J Gynecol Pathol.* 2020; 39(3): 227–232, doi: [10.1097/PGP.0000000000000589](https://doi.org/10.1097/PGP.0000000000000589), indexed in Pubmed: [30789500](https://pubmed.ncbi.nlm.nih.gov/30789500/).
25. Rabban JT, Firetag B, Sangoi AR, et al. Incidental pelvic and para-aortic lymph node lymphangioleiomyomatosis detected during surgical staging of pelvic cancer in women without symptomatic pulmonary lymphangioleiomyomatosis or tuberous sclerosis complex. *Am J Surg Pathol.* 2015; 39(8): 1015–1025, doi: [10.1097/PAS.0000000000000416](https://doi.org/10.1097/PAS.0000000000000416), indexed in Pubmed: [25786086](https://pubmed.ncbi.nlm.nih.gov/25786086/).
26. Abdelkader A, Lam CA, Shahrir KS, et al. Retroperitoneal lymphangioleiomyoma with lymph node involvement: A pathologic-radiologic correlation of a rare form of myomelanocytic tumor. *Ann Diagn Pathol.* 2017; 27: 69–73, doi: [10.1016/j.anndiagpath.2017.01.008](https://doi.org/10.1016/j.anndiagpath.2017.01.008), indexed in Pubmed: [28325364](https://pubmed.ncbi.nlm.nih.gov/28325364/).
27. Avila NA, Dwyer AJ, Murphy-Johnson DV, et al. Sonography of lymphangioleiomyoma in lymphangioleiomyomatosis: demonstration of diurnal variation in lesion size. *AJR Am J Roentgenol.* 2005; 184(2): 459–464, doi: [10.2214/ajr.184.2.01840459](https://doi.org/10.2214/ajr.184.2.01840459), indexed in Pubmed: [15671364](https://pubmed.ncbi.nlm.nih.gov/15671364/).
28. Ernst JC, Sohaey R, Cary JM. Pelvic lymphangioleiomyomatosis. Atypical precursor to pulmonary disease. *Chest.* 1994; 106(4): 1267–1269, doi: [10.1378/chest.106.4.1267](https://doi.org/10.1378/chest.106.4.1267), indexed in Pubmed: [7924509](https://pubmed.ncbi.nlm.nih.gov/7924509/).
29. Kim HS, Park MI, Suh KS. Lymphangioleiomyomatosis arising in the pelvic cavity: a case report. *J Korean Med Sci.* 2005; 20(5): 904–907, doi: [10.3346/jkms.2005.20.5.904](https://doi.org/10.3346/jkms.2005.20.5.904), indexed in Pubmed: [16224174](https://pubmed.ncbi.nlm.nih.gov/16224174/).
30. Lam B, Ooi GC, Wong MP, et al. Extrapulmonary presentation of asymptomatic pulmonary lymphangioleiomyomatosis. *Respirology.* 2003; 8(4): 544–547, doi: [10.1046/j.1440-1843.2003.00498.x](https://doi.org/10.1046/j.1440-1843.2003.00498.x), indexed in Pubmed: [14708559](https://pubmed.ncbi.nlm.nih.gov/14708559/).
31. Lu HC, Wang J, Tsang YM, et al. Lymphangioleiomyomatosis initially presenting with abdominal pain: a case report. *Clin Imaging.* 2003; 27(3): 166–170, doi: [10.1016/s0899-7071\(01\)00349-7](https://doi.org/10.1016/s0899-7071(01)00349-7), indexed in Pubmed: [12727053](https://pubmed.ncbi.nlm.nih.gov/12727053/).
32. Bahmer T, Watz H, Waschki B, et al. Reduced physical activity in lymphangioleiomyomatosis compared with COPD and healthy controls: disease-specific impact and clinical correlates. *Thorax.* 2016; 71(7): 662–663, doi: [10.1136/thoraxjnl-2015-207852](https://doi.org/10.1136/thoraxjnl-2015-207852), indexed in Pubmed: [26968971](https://pubmed.ncbi.nlm.nih.gov/26968971/).
33. Johnson SR, Whale CI, Hubbard RB, et al. Survival and disease progression in UK patients with lymphangioleiomyomatosis. *Thorax.* 2004; 59(9): 800–803, doi: [10.1136/thx.2004.023283](https://doi.org/10.1136/thx.2004.023283), indexed in Pubmed: [15333859](https://pubmed.ncbi.nlm.nih.gov/15333859/).
34. Matsui K, Beasley MB, Nelson WK, et al. Prognostic significance of pulmonary lymphangioleiomyomatosis histologic score. *Am J Surg Pathol.* 2001; 25(4): 479–484, doi: [10.1097/0000478-200104000-00007](https://doi.org/10.1097/0000478-200104000-00007), indexed in Pubmed: [11257622](https://pubmed.ncbi.nlm.nih.gov/11257622/).
35. Lama A, Ferreiro L, Golpe A, et al. Characteristics of patients with lymphangioleiomyomatosis and pleural effusion: a systematic review. *Respiration.* 2016; 91(3): 256–264, doi: [10.1159/00044264](https://doi.org/10.1159/00044264), indexed in Pubmed: [26938102](https://pubmed.ncbi.nlm.nih.gov/26938102/).
36. Pontes M, Barbosa C, Coelho ML, et al. Probable initial pulmonary lymphangioleiomyomatosis and mediastinal lymphangioleiomyoma. *Rev Port Pneumol.* 2014; 20(2): 101–106, doi: [10.1016/j.rppneu.2013.06.007](https://doi.org/10.1016/j.rppneu.2013.06.007), indexed in Pubmed: [24268918](https://pubmed.ncbi.nlm.nih.gov/24268918/).
37. Derweduwén AM, Verbeken E, Stas M, et al. Extrapulmonary lymphangioleiomyomatosis: a wolf in sheep's clothing. *Thorax.* 2013; 68(1): 111–113, doi: [10.1136/thoraxjnl-2012-201973](https://doi.org/10.1136/thoraxjnl-2012-201973), indexed in Pubmed: [22863757](https://pubmed.ncbi.nlm.nih.gov/22863757/).
38. Kamitani T, Yabuuchi H, Soeda H, et al. A case of lymphangioleiomyomatosis affecting the supraclavicular lymph nodes. *J Comput Assist Tomogr.* 2006; 30(2): 279–282, doi: [10.1097/00004728-200603000-00022](https://doi.org/10.1097/00004728-200603000-00022), indexed in Pubmed: [16628048](https://pubmed.ncbi.nlm.nih.gov/16628048/).
39. Fu W, Li Y, Li H, et al. Solitary extrapulmonary lymphangioleiomyomatosis of the liver: A case report and literature review. *Exp Ther Med.* 2016; 12(3): 1499–1502, doi: [10.3892/etm.2016.3502](https://doi.org/10.3892/etm.2016.3502), indexed in Pubmed: [27588070](https://pubmed.ncbi.nlm.nih.gov/27588070/).
40. Goh SG, Ho JM, Chuah KL, et al. Leiomyomatosis-like lymphangioleiomyomatosis of the colon in a female with tuberous sclerosis. *Mod Pathol.* 2001; 14(11): 1141–1146, doi: [10.1038/modpathol.3880449](https://doi.org/10.1038/modpathol.3880449), indexed in Pubmed: [11706076](https://pubmed.ncbi.nlm.nih.gov/11706076/).
41. Possekel AK, Katenkamp D, Brambs HJ, et al. Lymphangioleiomyomatosis: solitary abdominal manifestation (2009: 9b). *Eur Radiol.* 2009; 19(12): 3015–3018, doi: [10.1007/s00330-008-1275-4](https://doi.org/10.1007/s00330-008-1275-4), indexed in Pubmed: [19921526](https://pubmed.ncbi.nlm.nih.gov/19921526/).
42. Singh M, Saroha V, Wadhwa R, et al. Solitary lymphangioleiomyoma of pancreas mimicking pancreatic pseudocyst — a case report. *J Gastrointest Cancer.* 2012; 43(2): 336–339, doi: [10.1007/s12029-010-9183-7](https://doi.org/10.1007/s12029-010-9183-7), indexed in Pubmed: [20602180](https://pubmed.ncbi.nlm.nih.gov/20602180/).
43. Jun Y, Jang S, Han H, et al. Extrapulmonary lymphangioleiomyoma presenting as a mesenteric cystic mass. *Basic and Applied Pathology.* 2011; 4(2): 63–65, doi: [10.1111/j.1755-9294.2011.01097.x](https://doi.org/10.1111/j.1755-9294.2011.01097.x).
44. Ascher KB, Glassberg Csete MK. Lymphangioleiomyomatosis (LAM) of the Uterus: Risk of Pulmonary LAM? D32 ILD: CASE REPORTS II. American Thoracic Society International Conference Abstracts: American Thoracic Society. 2018: A6507–A.
45. de Groot JWB, Vecht J, van den Bergen HA, et al. Extrapulmonary lymphangioleiomyomatosis: an unusual cause of biliary tract obstruction. *Neth J Med.* 2008; 66(1): 31–34, indexed in Pubmed: [18219066](https://pubmed.ncbi.nlm.nih.gov/18219066/).
46. Atallah D, Checrallah A, Rouzier R, et al. Retroperitoneal lymphangioleiomyoma mimicking ovarian tumor emerging after tamoxifen therapy. *Obstet Gynecol.* 2006; 108(3 Pt 2): 762–764, doi: [10.1097/01.AOG.0000192548.92503.ab](https://doi.org/10.1097/01.AOG.0000192548.92503.ab), indexed in Pubmed: [17018494](https://pubmed.ncbi.nlm.nih.gov/17018494/).
47. Favoreel N, Van Meerbeeck SF, Gosselin R. Extrapulmonary manifestation of lymphangioleiomyomatosis. *JBR-BTR.* 2015; 98(1): 53, doi: [10.5334/jbr-btr.759](https://doi.org/10.5334/jbr-btr.759), indexed in Pubmed: [26223072](https://pubmed.ncbi.nlm.nih.gov/26223072/).
48. Grant L, Chipwete S, Soo Hoo S, et al. Extrapulmonary uterine lymphangioleiomyomatosis (LAM) and dysfunctional uterine bleeding: the first presentation of LAM in a tuberous sclerosis complex patient. *BMJ Case Rep.* 2019; 12(2), doi: [10.1136/bcr-2018-226358](https://doi.org/10.1136/bcr-2018-226358), indexed in Pubmed: [30804158](https://pubmed.ncbi.nlm.nih.gov/30804158/).
49. Han JM, Lee KH, Kim SJ, et al. A case of lymphangioleiomyomatosis originated in the pelvic cavity. *J Gynecol Oncol.* 2008; 19(3): 195–198, doi: [10.3802/jgo.2008.19.3.195](https://doi.org/10.3802/jgo.2008.19.3.195), indexed in Pubmed: [19471572](https://pubmed.ncbi.nlm.nih.gov/19471572/).
50. Jaiswal VR, Baird J, Fleming J, et al. Localized retroperitoneal lymphangioleiomyomatosis mimicking malignancy. A case report and review of the literature. *Arch Pathol Lab Med.* 2003; 127(7): 879–882, indexed in Pubmed: [12823048](https://pubmed.ncbi.nlm.nih.gov/12823048/).
51. Gray S, Carrington C, Cornog J. Lymphangioleiomyomatosis: Report of a case with ureteral involvement and chyluria. *Cancer.* 1975; 35(2): 490–498.
52. Longacre TA, Hendrickson MR, Kapp DS, et al. Lymphangioleiomyomatosis of the uterus simulating high-stage endometrial stromal sarcoma. *Gynecol Oncol.* 1996; 63(3): 404–410, doi: [10.1006/gyno.1996.0344](https://doi.org/10.1006/gyno.1996.0344), indexed in Pubmed: [8946880](https://pubmed.ncbi.nlm.nih.gov/8946880/).
53. Sandrini A, Silverstone E, Yates DH. Menstrual cycle variation of retroperitoneal lymphangioleiomyomas in lymphangioleiomyomatosis. *Intern Med J.* 2011; 41(12): 832–835, doi: [10.1111/j.1445-5994.2011.02593.x](https://doi.org/10.1111/j.1445-5994.2011.02593.x), indexed in Pubmed: [22151950](https://pubmed.ncbi.nlm.nih.gov/22151950/).
54. Gupta N, Finlay GA, Kotloff RM, et al. ATS Assembly on Clinical Problems. Lymphangioleiomyomatosis Diagnosis and Management: High-Resolution Chest Computed Tomography, Transbronchial Lung Biopsy, and Pleural Disease Management. An Official American Thoracic Society/Japanese Respiratory Society Clinical Practice Guideline. *Am J Respir Crit Care Med.* 2017; 196(10): 1337–1348, doi: [10.1164/rccm.201709-1965ST](https://doi.org/10.1164/rccm.201709-1965ST), indexed in Pubmed: [29140122](https://pubmed.ncbi.nlm.nih.gov/29140122/).
55. Koba T, Arai T, Kitaichi M, et al. Efficacy and safety of transbronchial lung biopsy for the diagnosis of lymphangioleiomyomatosis: A report of 24 consecutive patients. *Respirology.* 2018; 23(3): 331–338, doi: [10.1111/resp.13190](https://doi.org/10.1111/resp.13190), indexed in Pubmed: [28960664](https://pubmed.ncbi.nlm.nih.gov/28960664/).
56. Grzegorek I, Lenze D, Chabowski M, et al. Immunohistochemical evaluation of pulmonary lymphangioleiomyomatosis. *Anticancer Res.* 2015; 35(6): 3353–3360, indexed in Pubmed: [26026095](https://pubmed.ncbi.nlm.nih.gov/26026095/).
57. Matsui K, Tatsuguchi A, Valencia J, et al. Lymphangioleiomyomatosis (LAM): a review of clinical and morphological features. *J Nippon Med Sch.* 2000; 67(5): 311–329, doi: [10.1272/jnms.67.311](https://doi.org/10.1272/jnms.67.311), indexed in Pubmed: [11031360](https://pubmed.ncbi.nlm.nih.gov/11031360/).
58. Taveira-DaSilva AM, Pacheco-Rodriguez G, Moss J. The natural history of lymphangioleiomyomatosis: markers of severity, rate of progression and prognosis. *Lymphat Res Biol.* 2010; 8(1): 9–19, doi: [10.1089/lrb.2009.0024](https://doi.org/10.1089/lrb.2009.0024), indexed in Pubmed: [20235883](https://pubmed.ncbi.nlm.nih.gov/20235883/).
59. Zhe X, Schuger L. Combined smooth muscle and melanocytic differentiation in lymphangioleiomyomatosis. *J Histochem Cytochem.* 2004; 52(12): 1537–1542, doi: [10.1369/jhc.4A6438.2004](https://doi.org/10.1369/jhc.4A6438.2004), indexed in Pubmed: [15557209](https://pubmed.ncbi.nlm.nih.gov/15557209/).
60. Zhang X, Travis WD. Pulmonary lymphangioleiomyomatosis. *Arch Pathol Lab Med.* 2010; 134(12): 1823–1828, doi: [10.1043/2009-0576-RS.1](https://doi.org/10.1043/2009-0576-RS.1), indexed in Pubmed: [21128782](https://pubmed.ncbi.nlm.nih.gov/21128782/).

61. Davis JM, Hyjek E, Husain AN, et al. Lymphatic endothelial differentiation in pulmonary lymphangioleiomyomatosis cells. *J Histochem Cytochem*. 2013; 61(8): 580–590, doi: [10.1369/0022155413489311](https://doi.org/10.1369/0022155413489311), indexed in Pubmed: [23609227](https://pubmed.ncbi.nlm.nih.gov/23609227/).
62. Gao L, Yue MM, Davis J, et al. In pulmonary lymphangioleiomyomatosis expression of progesterone receptor is frequently higher than that of estrogen receptor. *Virchows Arch*. 2014; 464(4): 495–503, doi: [10.1007/s00428-014-1559-9](https://doi.org/10.1007/s00428-014-1559-9), indexed in Pubmed: [24570392](https://pubmed.ncbi.nlm.nih.gov/24570392/).
63. Flavin R, Cook J, Fiorentino M, et al. β -Catenin Is a Useful Adjunct Immunohistochemical Marker for the Diagnosis of Pulmonary Lymphangioleiomyomatosis. *American Journal of Clinical Pathology*. 2011; 135(5): 776–782, doi: [10.1309/ajcp9ex1zhmra](https://doi.org/10.1309/ajcp9ex1zhmra).
64. Dongre A, Clements D, Fisher AJ, et al. Cathepsin k in lymphangioleiomyomatosis: LAM cell-fibroblast interactions enhance protease activity by extracellular acidification. *Am J Pathol*. 2017; 187(8): 1750–1762, doi: [10.1016/j.ajpath.2017.04.014](https://doi.org/10.1016/j.ajpath.2017.04.014), indexed in Pubmed: [28623674](https://pubmed.ncbi.nlm.nih.gov/28623674/).
65. Kobayashi K, Miki Y, Saito R, et al. Roles of human epidermal growth factor receptor family in pulmonary lymphangioleiomyomatosis. *Hum Pathol*. 2018; 81: 121–130, doi: [10.1016/j.humpath.2018.07.002](https://doi.org/10.1016/j.humpath.2018.07.002), indexed in Pubmed: [30030119](https://pubmed.ncbi.nlm.nih.gov/30030119/).
66. Maisel K, Merrilees MJ, Atochina-Vasserman EN, et al. Immune checkpoint ligand PD-L1 is upregulated in pulmonary lymphangioleiomyomatosis. *Am J Respir Cell Mol Biol*. 2018; 59(6): 723–732, doi: [10.1165/rcmb.2018-0123OC](https://doi.org/10.1165/rcmb.2018-0123OC), indexed in Pubmed: [30095976](https://pubmed.ncbi.nlm.nih.gov/30095976/).
67. Uehara K, Kawakami F, Hirose T, et al. Clinicopathological analysis of clinically occult extrapulmonary lymphangioleiomyomatosis in intra-pelvic and para-aortic lymph nodes associated with pelvic malignant tumors: A study of nine patients. *Pathol Int*. 2019; 69(1): 29–36, doi: [10.1111/pin.12749](https://doi.org/10.1111/pin.12749), indexed in Pubmed: [30688413](https://pubmed.ncbi.nlm.nih.gov/30688413/).
68. Smolarek TA, Wessner LL, McCormack FX, et al. Evidence that lymphangiomyomatosis is caused by TSC2 mutations: chromosome 16p13 loss of heterozygosity in angiomyolipomas and lymph nodes from women with lymphangiomyomatosis. *Am J Hum Genet*. 1998; 62(4): 810–815, doi: [10.1086/301804](https://doi.org/10.1086/301804), indexed in Pubmed: [9529362](https://pubmed.ncbi.nlm.nih.gov/9529362/).
69. Kristof AS, You Z, Han YS, et al. mTOR signaling in lymphangioleiomyomatosis. *Lymphat Res Biol*. 2010; 8(1): 33–42, doi: [10.1089/lrb.2009.0019](https://doi.org/10.1089/lrb.2009.0019), indexed in Pubmed: [20235885](https://pubmed.ncbi.nlm.nih.gov/20235885/).
70. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J*. 2008; 412(2): 179–190, doi: [10.1042/BJ20080281](https://doi.org/10.1042/BJ20080281), indexed in Pubmed: [18466115](https://pubmed.ncbi.nlm.nih.gov/18466115/).
71. Strizheva GD, Carsillo T, Kruger WD, et al. The spectrum of mutations in TSC1 and TSC2 in women with tuberous sclerosis and lymphangiomyomatosis. *Am J Respir Crit Care Med*. 2001; 163(1): 253–258, doi: [10.1164/ajrccm.163.1.2005004](https://doi.org/10.1164/ajrccm.163.1.2005004), indexed in Pubmed: [11208653](https://pubmed.ncbi.nlm.nih.gov/11208653/).
72. Murphy SJ, Terra SB, Harris FR, et al. Genomic rearrangements in sporadic lymphangioleiomyomatosis: an evolving genetic story. *Mod Pathol*. 2017; 30(9): 1223–1233, doi: [10.1038/modpathol.2017.52](https://doi.org/10.1038/modpathol.2017.52), indexed in Pubmed: [28643793](https://pubmed.ncbi.nlm.nih.gov/28643793/).
73. Badri K, Gao L, Hyjek E, et al. Exonic mutations of TSC2/TSC1 are common but not seen in all sporadic pulmonary lymphangioleiomyomatosis. *American Journal of Respiratory and Critical Care Medicine*. 2013; 187(6): 663–665, doi: [10.1164/ajrccm.187.6.663](https://doi.org/10.1164/ajrccm.187.6.663).
74. Fujita A, Ando K, Kobayashi E, et al. Detection of low-prevalence somatic TSC2 mutations in sporadic pulmonary lymphangioleiomyomatosis tissues by deep sequencing. *Hum Genet*. 2016; 135(1): 61–68, doi: [10.1007/s00439-015-1611-0](https://doi.org/10.1007/s00439-015-1611-0), indexed in Pubmed: [26563443](https://pubmed.ncbi.nlm.nih.gov/26563443/).
75. Johnson SR, Tattersfield AE. Decline in lung function in lymphangioleiomyomatosis: relation to menopause and progesterone treatment. *Am J Respir Crit Care Med*. 1999; 160(2): 628–633, doi: [10.1164/ajrccm.160.2.9901027](https://doi.org/10.1164/ajrccm.160.2.9901027), indexed in Pubmed: [10430739](https://pubmed.ncbi.nlm.nih.gov/10430739/).
76. Gupta N, Lee HS, Ryu JH, et al. NHLBI LAM Registry Group. The NHLBI LAM registry: prognostic physiologic and radiologic biomarkers emerge from a 15-year prospective longitudinal analysis. *Chest*. 2019; 155(2): 288–296, doi: [10.1016/j.chest.2018.06.016](https://doi.org/10.1016/j.chest.2018.06.016), indexed in Pubmed: [29940164](https://pubmed.ncbi.nlm.nih.gov/29940164/).
77. Amaral AF, de Oliveira MR, Dias OM, et al. Tuberous Sclerosis, Lymphangioleiomyomatosis and Angiomyolipoma Study Group, Universidade de Sao Paulo, Brazil. Concentration of Serum Vascular Endothelial Growth Factor (VEGF-D) and Its Correlation with Functional and Clinical Parameters in Patients with Lymphangioleiomyomatosis from a Brazilian Reference Center. *Lung*. 2019; 197(2): 139–146, doi: [10.1007/s00408-018-00191-3](https://doi.org/10.1007/s00408-018-00191-3), indexed in Pubmed: [30623243](https://pubmed.ncbi.nlm.nih.gov/30623243/).
78. Radzikowska E, Jaguś P, Sobiecka M, et al. Correlation of serum vascular endothelial growth factor-D concentration with clinical presentation and course of lymphangioleiomyomatosis. *Respir Med*. 2015; 109(11): 1469–1475, doi: [10.1016/j.rmed.2015.09.005](https://doi.org/10.1016/j.rmed.2015.09.005), indexed in Pubmed: [26386638](https://pubmed.ncbi.nlm.nih.gov/26386638/).
79. Miller S, Coveney C, Johnson J, et al. The vitamin D binding protein axis modifies disease severity in lymphangioleiomyomatosis. *Eur Respir J*. 2018; 52(5), doi: [10.1183/13993003.00951-2018](https://doi.org/10.1183/13993003.00951-2018), indexed in Pubmed: [30093573](https://pubmed.ncbi.nlm.nih.gov/30093573/).
80. Liu HJ, Henske EP. Vitamin D binding protein: a new biomarker of disease severity in lymphangioleiomyomatosis. *Eur Respir J*. 2018; 52(5), doi: [10.1183/13993003.01886-2018](https://doi.org/10.1183/13993003.01886-2018), indexed in Pubmed: [30385604](https://pubmed.ncbi.nlm.nih.gov/30385604/).
81. Lamattina AM, Taveira-Dasilva A, Goldberg HJ, et al. Circulating Biomarkers From the Phase 1 Trial of Sirolimus and Autophagy Inhibition for Patients With Lymphangioleiomyomatosis. *Chest*. 2018; 154(5): 1070–1082, doi: [10.1016/j.chest.2018.08.1029](https://doi.org/10.1016/j.chest.2018.08.1029), indexed in Pubmed: [30144422](https://pubmed.ncbi.nlm.nih.gov/30144422/).
82. Araujo MS, Baldi BG, Freitas CSG, et al. Pulmonary rehabilitation in lymphangioleiomyomatosis: a controlled clinical trial. *Eur Respir J*. 2016; 47(5): 1452–1460, doi: [10.1183/13993003.01683-2015](https://doi.org/10.1183/13993003.01683-2015), indexed in Pubmed: [26917604](https://pubmed.ncbi.nlm.nih.gov/26917604/).
83. Gonano C, Pasquier J, Daccord C, et al. Air travel and incidence of pneumothorax in lymphangioleiomyomatosis. *Orphanet J Rare Dis*. 2018; 13(1): 222, doi: [10.1186/s13023-018-0964-6](https://doi.org/10.1186/s13023-018-0964-6), indexed in Pubmed: [30545392](https://pubmed.ncbi.nlm.nih.gov/30545392/).
84. Taveira-DaSilva AM, Hathaway O, Stylianou M, et al. Changes in lung function and chylous effusions in patients with lymphangioleiomyomatosis treated with sirolimus. *Ann Intern Med*. 2011; 154(12): 797–805, doi: [10.7326/0003-4819-154-12-201106210-00007](https://doi.org/10.7326/0003-4819-154-12-201106210-00007), indexed in Pubmed: [21690594](https://pubmed.ncbi.nlm.nih.gov/21690594/).
85. Reynaud-Gaubert M, Mornex JF, Mal H, et al. Lung transplantation for lymphangioleiomyomatosis: the French experience. *Transplantation*. 2008; 86(4): 515–520, doi: [10.1097/TP0b013e31817c15df](https://doi.org/10.1097/TP0b013e31817c15df), indexed in Pubmed: [18724219](https://pubmed.ncbi.nlm.nih.gov/18724219/).
86. Baldi BG, Samano MN, Campos SV, et al. Experience of lung transplantation in patients with lymphangioleiomyomatosis at a Brazilian reference centre. *Lung*. 2017; 195(6): 699–705, doi: [10.1007/s00408-017-0045-y](https://doi.org/10.1007/s00408-017-0045-y), indexed in Pubmed: [28823029](https://pubmed.ncbi.nlm.nih.gov/28823029/).
87. Khawar MU, Yazdani D, Zhu Z, et al. Clinical outcomes and survival following lung transplantation in patients with lymphangioleiomyomatosis. *J Heart Lung Transplant*. 2019; 38(9): 949–955, doi: [10.1016/j.healun.2019.06.015](https://doi.org/10.1016/j.healun.2019.06.015), indexed in Pubmed: [31303421](https://pubmed.ncbi.nlm.nih.gov/31303421/).
88. Chen F, Omasa M, Kondo N, et al. Sirolimus treatment for recurrent lymphangioleiomyomatosis after lung transplantation. *Ann Thorac Surg*. 2009; 87(1): e6–e7, doi: [10.1016/j.athoracsur.2008.07.107](https://doi.org/10.1016/j.athoracsur.2008.07.107), indexed in Pubmed: [19101258](https://pubmed.ncbi.nlm.nih.gov/19101258/).
89. Sobiecka M. Limfangioleiomyomatoza. *Postępy Nauk Medycznych*. 2011; 4: 295–302.
90. Lu C, Lee HS, Pappas GP, et al. Trial of an Aromatase Inhibitor in Lymphangioleiomyomatosis Group. A Phase II Clinical Trial of an Aromatase Inhibitor for Postmenopausal Women with Lymphangioleiomyomatosis. *Ann Am Thorac Soc*. 2017; 14(6): 919–928, doi: [10.1513/AnnalsATS.201610-824OC](https://doi.org/10.1513/AnnalsATS.201610-824OC), indexed in Pubmed: [28570161](https://pubmed.ncbi.nlm.nih.gov/28570161/).
91. McCormack FX, Inoue Y, Moss J, et al. National Institutes of Health Rare Lung Diseases Consortium, MILES Trial Group. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N Engl J Med*. 2011; 364(17): 1595–1606, doi: [10.1056/NEJMoa1100391](https://doi.org/10.1056/NEJMoa1100391), indexed in Pubmed: [21410393](https://pubmed.ncbi.nlm.nih.gov/21410393/).
92. Takada T, Mikami A, Kitamura N, et al. Efficacy and safety of long-term sirolimus therapy for Asian patients with lymphangioleiomyomatosis. *Ann Am Thorac Soc*. 2016; 13(11): 1912–1922, doi: [10.1513/AnnalsATS.201605-335OC](https://doi.org/10.1513/AnnalsATS.201605-335OC), indexed in Pubmed: [27513278](https://pubmed.ncbi.nlm.nih.gov/27513278/).
93. Neurohr C, Hoffmann AL, Huppmann P, et al. Is sirolimus a therapeutic option for patients with progressive pulmonary lymphangioleiomyomatosis? *Respir Res*. 2011; 12: 66, doi: [10.1186/1465-9921-12-66](https://doi.org/10.1186/1465-9921-12-66), indexed in Pubmed: [21600034](https://pubmed.ncbi.nlm.nih.gov/21600034/).
94. Kida Y, Burger CD. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N Engl J Med*. 2011; 365(3): 271–272, doi: [10.1056/NEJMc1106358](https://doi.org/10.1056/NEJMc1106358), indexed in Pubmed: [21774717](https://pubmed.ncbi.nlm.nih.gov/21774717/).
95. Young L, Lee HS, Inoue Y, et al. MILES Trial Group. Serum VEGF-D concentration as a biomarker of lymphangioleiomyomatosis severity and treatment response: a prospective analysis of the Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus (MILES) trial. *Lancet Respir Med*. 2013; 1(6): 445–452, doi: [10.1016/S2213-2600\(13\)70090-0](https://doi.org/10.1016/S2213-2600(13)70090-0), indexed in Pubmed: [24159565](https://pubmed.ncbi.nlm.nih.gov/24159565/).
96. Zhou Li, Ouyang R, Luo H, et al. Efficacy of sirolimus for the prevention of recurrent pneumothorax in patients with lymphangioleiomyomatosis: a case series. *Orphanet J Rare Dis*. 2018; 13(1), doi: [10.1186/s13023-018-0915-2](https://doi.org/10.1186/s13023-018-0915-2).
97. Yoon HY, Hwang JJ, Kim DS, et al. Efficacy and safety of low-dose sirolimus in lymphangioleiomyomatosis. *Orphanet J Rare Dis*. 2018; 13(1): 204, doi: [10.1186/s13023-018-0946-8](https://doi.org/10.1186/s13023-018-0946-8), indexed in Pubmed: [30428897](https://pubmed.ncbi.nlm.nih.gov/30428897/).
98. Gao N, Zhang T, Ji J, et al. The efficacy and adverse events of mTOR inhibitors in lymphangioleiomyomatosis: systematic review

- and meta-analysis. *Orphanet J Rare Dis.* 2018; 13(1): 134, doi: [10.1186/s13023-018-0874-7](https://doi.org/10.1186/s13023-018-0874-7), indexed in Pubmed: [30107845](https://pubmed.ncbi.nlm.nih.gov/30107845/).
99. Courtwright AM, Goldberg HJ, Henske EP, et al. The effect of mTOR inhibitors on respiratory infections in lymphangioleiomyomatosis. *Eur Respir Rev.* 2017; 26(143), doi: [10.1183/16000617.0004-2016](https://doi.org/10.1183/16000617.0004-2016), indexed in Pubmed: [28096282](https://pubmed.ncbi.nlm.nih.gov/28096282/).
 100. Parkhitko A, Myachina F, Morrison TA, et al. Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent. *Proc Natl Acad Sci U S A.* 2011; 108(30): 12455–12460, doi: [10.1073/pnas.1104361108](https://doi.org/10.1073/pnas.1104361108), indexed in Pubmed: [21746920](https://pubmed.ncbi.nlm.nih.gov/21746920/).
 101. Taveira-DaSilva AM, Jones AM, Julien-Williams PA, et al. Retrospective review of combined sirolimus and simvastatin therapy in lymphangioleiomyomatosis. *Chest.* 2015; 147(1): 180–187, doi: [10.1378/chest.14-0758](https://doi.org/10.1378/chest.14-0758), indexed in Pubmed: [25167325](https://pubmed.ncbi.nlm.nih.gov/25167325/).
 102. El-Chemaly S, Taveira-Dasilva A, Goldberg HJ, et al. Sirolimus and Autophagy Inhibition in Lymphangioleiomyomatosis: Results of a Phase I Clinical Trial. *Chest.* 2017; 151(6): 1302–1310, doi: [10.1016/j.chest.2017.01.033](https://doi.org/10.1016/j.chest.2017.01.033), indexed in Pubmed: [28192114](https://pubmed.ncbi.nlm.nih.gov/28192114/).
 103. Abdelwahab EMM, Pal S, Kvell K, et al. Mitochondrial dysfunction is a key determinant of the rare disease lymphangioleiomyomatosis and provides a novel therapeutic target. *Oncogene.* 2019; 38(16): 3093–3101, doi: [10.1038/s41388-018-0625-1](https://doi.org/10.1038/s41388-018-0625-1), indexed in Pubmed: [30573768](https://pubmed.ncbi.nlm.nih.gov/30573768/).
 104. Atochina-Vasserman EN, Abramova E, James ML, et al. Pharmacological targeting of VEGFR signaling with axitinib inhibits Tsc2-null lesion growth in the mouse model of lymphangioleiomyomatosis. *Am J Physiol Lung Cell Mol Physiol.* 2015; 309(12): L1447–L1454, doi: [10.1152/ajplung.00262.2015](https://doi.org/10.1152/ajplung.00262.2015), indexed in Pubmed: [26432869](https://pubmed.ncbi.nlm.nih.gov/26432869/).
 105. Bissler JJ, McCormack FX, Young LR, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med.* 2008; 358(2): 140–151, doi: [10.1056/NEJMoa063564](https://doi.org/10.1056/NEJMoa063564), indexed in Pubmed: [18184959](https://pubmed.ncbi.nlm.nih.gov/18184959/).
 106. Dabora SL, Franz DN, Ashwal S, et al. Multicenter phase 2 trial of sirolimus for tuberous sclerosis: kidney angiomyolipomas and other tumors regress and VEGF-D levels decrease. *PLoS One.* 2011; 6(9): e23379, doi: [10.1371/journal.pone.0023379](https://doi.org/10.1371/journal.pone.0023379), indexed in Pubmed: [21915260](https://pubmed.ncbi.nlm.nih.gov/21915260/).
 107. Davies DM, de Vries PJ, Johnson SR, et al. Sirolimus therapy for angiomyolipoma in tuberous sclerosis and sporadic lymphangioleiomyomatosis: a phase 2 trial. *Clin Cancer Res.* 2011; 17(12): 4071–4081, doi: [10.1158/1078-0432.CCR-11-0445](https://doi.org/10.1158/1078-0432.CCR-11-0445), indexed in Pubmed: [21525172](https://pubmed.ncbi.nlm.nih.gov/21525172/).
 108. Bissler JJ, Kingswood JC, Radzikowska E, et al. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet.* 2013; 381(9869): 817–824, doi: [10.1016/S0140-6736\(12\)61767-X](https://doi.org/10.1016/S0140-6736(12)61767-X), indexed in Pubmed: [23312829](https://pubmed.ncbi.nlm.nih.gov/23312829/).
 109. Yao J, Taveira-DaSilva AM, Jones AM, et al. Sustained effects of sirolimus on lung function and cystic lung lesions in lymphangioleiomyomatosis. *Am J Respir Crit Care Med.* 2014; 190(11): 1273–1282, doi: [10.1164/rccm.201405-0918OC](https://doi.org/10.1164/rccm.201405-0918OC), indexed in Pubmed: [25329516](https://pubmed.ncbi.nlm.nih.gov/25329516/).
 110. Goldberg HJ, Harari S, Cottin V, et al. Everolimus for the treatment of lymphangioleiomyomatosis: a phase II study. *Eur Respir J.* 2015; 46(3): 783–794, doi: [10.1183/09031936.00210714](https://doi.org/10.1183/09031936.00210714), indexed in Pubmed: [26113676](https://pubmed.ncbi.nlm.nih.gov/26113676/).
 111. Bee J, Fuller S, Miller S, et al. Lung function response and side effects to rapamycin for lymphangioleiomyomatosis: a prospective national cohort study. *Thorax.* 2018; 73(4): 369–375, doi: [10.1136/thoraxjnl-2017-210872](https://doi.org/10.1136/thoraxjnl-2017-210872), indexed in Pubmed: [28993539](https://pubmed.ncbi.nlm.nih.gov/28993539/).
 112. Taveira-DaSilva AM, Jones AM, Julien-Williams P, et al. Long-Term effect of sirolimus on serum vascular endothelial growth factor d levels in patients with lymphangioleiomyomatosis. *Chest.* 2018; 153(1): 124–132, doi: [10.1016/j.chest.2017.05.012](https://doi.org/10.1016/j.chest.2017.05.012), indexed in Pubmed: [28533049](https://pubmed.ncbi.nlm.nih.gov/28533049/).
 113. Ding Y, Yan S, Tian Y, et al. Primary abdominal lymphangioleiomyomatosis: report of a case. *World J Surg Oncol.* 2015; 13: 93, doi: [10.1186/s12957-015-0512-y](https://doi.org/10.1186/s12957-015-0512-y), indexed in Pubmed: [25888993](https://pubmed.ncbi.nlm.nih.gov/25888993/).
 114. Słodkowska J, Patera J, Breborowicz J, et al. Extrapulmonary lymphangioleiomyomatosis presented as the asymptomatic retroperitoneal tumours — two cases report. *Pol J Pathol.* 2006; 57(4): 205–207, indexed in Pubmed: [17285764](https://pubmed.ncbi.nlm.nih.gov/17285764/).
 115. Bhattacharyya A, Balogh K. Retroperitoneal lymphangioleiomyomatosis a 36-year benign course in a postmenopausal woman. *Cancer.* 1985; 56(5): 1144–1146, doi: [10.1002/1097-0142\(19850901\)56:5<1144::aid-cnrcr2820560531>3.0.co;2-f](https://doi.org/10.1002/1097-0142(19850901)56:5<1144::aid-cnrcr2820560531>3.0.co;2-f).
 116. Mboyo A, Flurin V, Foulet-Roge A, et al. Conservative treatment of a mesenteric lymphangioleiomyomatosis in an 11-year-old girl with a long follow-up period. *J Pediatr Surg.* 2004; 39(10): 1586–1589, doi: [10.1016/j.jpedsurg.2004.06.034](https://doi.org/10.1016/j.jpedsurg.2004.06.034), indexed in Pubmed: [15486914](https://pubmed.ncbi.nlm.nih.gov/15486914/).
 117. Szperek D, Szubert S, Zielinski P, et al. Malignant presentation of uterine lymphangioleiomyomatosis. *Taiwan J Obstet Gynecol.* 2015; 54(5): 603–607, doi: [10.1016/j.tjog.2015.08.015](https://doi.org/10.1016/j.tjog.2015.08.015), indexed in Pubmed: [26522119](https://pubmed.ncbi.nlm.nih.gov/26522119/).
 118. Radzikowska E, Przerwa KB, SkroAska P. Lymphangioma in patients with pulmonary lymphangioleiomyomatosis: results of sirolimus treatment. *Journal of Cancer Science & Therapy.* 2016; 8(9), doi: [10.4172/1948-5956.1000419](https://doi.org/10.4172/1948-5956.1000419).
 119. Tümay LV, Güner OS, Zorluoğlu A. An extrapulmonary manifestation of lymphangioleiomyomatosis: A rare case report. *Int J Surg Case Rep.* 2017; 41: 315–318, doi: [10.1016/j.ijscr.2017.10.057](https://doi.org/10.1016/j.ijscr.2017.10.057), indexed in Pubmed: [29132117](https://pubmed.ncbi.nlm.nih.gov/29132117/).
 120. Mohammadieh AM, Bowler SD, Silverstone EJ, et al. Everolimus treatment of abdominal lymphangioleiomyoma in five women with sporadic lymphangioleiomyomatosis. *Med J Aust.* 2013; 199(2): 121–123, doi: [10.5694/mja12.11567](https://doi.org/10.5694/mja12.11567), indexed in Pubmed: [23879512](https://pubmed.ncbi.nlm.nih.gov/23879512/).
 121. Rozenberg D, Thenganatt J. Dramatic response to sirolimus in lymphangioleiomyomatosis. *Can Respir J.* 2013; 20(6): 413–414, doi: [10.1155/2013/120376](https://doi.org/10.1155/2013/120376), indexed in Pubmed: [24137571](https://pubmed.ncbi.nlm.nih.gov/24137571/).
 122. Freitas CS, Baldi BG, Araújo MS, et al. Use of sirolimus in the treatment of lymphangioleiomyomatosis: favorable responses in patients with different extrapulmonary manifestations. *J Bras Pneumol.* 2015; 41(3): 275–280, doi: [10.1590/S1806-37132015000004553](https://doi.org/10.1590/S1806-37132015000004553), indexed in Pubmed: [26176526](https://pubmed.ncbi.nlm.nih.gov/26176526/).
 123. Hecimovic A, Jakopovic M, Pavlisa G, et al. Successful treatment of pulmonary and lymphatic manifestations of lymphangioleiomyomatosis with sirolimus. *Lymphology.* 2015; 48(2): 97–102, indexed in Pubmed: [26714374](https://pubmed.ncbi.nlm.nih.gov/26714374/).
 124. Cabeza L, Ruiz-Cobos M, Casanova Á. Resolution of thoracic and abdominal lymphangioleiomyomas in a patient with lymphangioleiomyomatosis treated with sirolimus. *Archivos de Bronconeumología (English Edition).* 2016; 52(6): 329–330, doi: [10.1016/j.arbr.2016.04.002](https://doi.org/10.1016/j.arbr.2016.04.002).
 125. Harari S, Spagnolo P, Cocconcilli E, et al. Recent advances in the pathobiology and clinical management of lymphangioleiomyomatosis. *Curr Opin Pulm Med.* 2018; 24(5): 469–476, doi: [10.1097/MCP.0000000000000502](https://doi.org/10.1097/MCP.0000000000000502), indexed in Pubmed: [29927757](https://pubmed.ncbi.nlm.nih.gov/29927757/).
 126. Ito T, Suno M, Sakamoto K, et al. Therapeutic effect of sirolimus for lymphangioleiomyomatosis remaining in the abdominopelvic region after lung transplantation: a case report. *Transplant Proc.* 2016; 48(1): 271–274, doi: [10.1016/j.transproceed.2015.12.021](https://doi.org/10.1016/j.transproceed.2015.12.021), indexed in Pubmed: [26915883](https://pubmed.ncbi.nlm.nih.gov/26915883/).
 127. Wahid S, Chiang PC, Luo HL, et al. Pelvic lymphangioleiomyomatosis treated successfully with everolimus: Two case reports with literature review. *Medicine (Baltimore).* 2017; 96(10): e4562, doi: [10.1097/MD.00000000000004562](https://doi.org/10.1097/MD.00000000000004562), indexed in Pubmed: [28272193](https://pubmed.ncbi.nlm.nih.gov/28272193/).
 128. Lecuelle D, Basille D, Renard C, et al. Highly effective sirolimus therapy for abdominal lymphangioleiomyoma. *Respir Med Res.* 2019; 75: 32–34, doi: [10.1016/j.resmer.2019.02.002](https://doi.org/10.1016/j.resmer.2019.02.002), indexed in Pubmed: [31262426](https://pubmed.ncbi.nlm.nih.gov/31262426/).
 129. Ussavarungsi K, Laroia AT, Burger CD. Low-dose sirolimus in retroperitoneal lymphangioleiomyomas. *Lung India.* 2019; 36(4): 349–352, doi: [10.4103/lungindia.lungindia_433_18](https://doi.org/10.4103/lungindia.lungindia_433_18), indexed in Pubmed: [31290423](https://pubmed.ncbi.nlm.nih.gov/31290423/).
 130. Klein M, Krieger O, Ruckser R, et al. Treatment of lymphangioleiomyomatosis by ovariectomy, interferon alpha 2b and tamoxifen — a case report. *Arch Gynecol Obstet.* 1992; 252(2): 99–102, doi: [10.1007/BF02389635](https://doi.org/10.1007/BF02389635), indexed in Pubmed: [1471918](https://pubmed.ncbi.nlm.nih.gov/1471918/).
 131. Yamashita S, Nakamura K, Shinozaki H, et al. Lymphangioleiomyomatosis suspected to be a gynecologic disease. *J Obstet Gynaecol Res.* 2011; 37(3): 267–269, doi: [10.1111/j.1447-0756.2010.01368.x](https://doi.org/10.1111/j.1447-0756.2010.01368.x), indexed in Pubmed: [21314806](https://pubmed.ncbi.nlm.nih.gov/21314806/).
 132. Basnet A, Shaaban H, Kessler W. A case of recurrent retroperitoneal lymphangioleiomyomatosis treated with progesterone therapy. *Urol Ann.* 2015; 7(1): 127–128, doi: [10.4103/0974-7796.148664](https://doi.org/10.4103/0974-7796.148664), indexed in Pubmed: [25657565](https://pubmed.ncbi.nlm.nih.gov/25657565/).
 133. Yoshizawa H, Kawai S, Hirota Y, et al. A case of uterine lymphangioleiomyomatosis in a young woman that was identified via peritonitis. *J Obstet Gynaecol Res.* 2019; 45(2): 482–486, doi: [10.1111/jog.13822](https://doi.org/10.1111/jog.13822), indexed in Pubmed: [30338891](https://pubmed.ncbi.nlm.nih.gov/30338891/).

Tomasz Sachański¹, Maciej Miodoński¹, Barbara Radecka^{2,3}

¹Department of Surgical Oncology with Breast Surgery Unit, Tadeusz Koszarowski Cancer Center in Opole, Poland

²Department of Oncology, Institute of Medical Sciences, University of Opole, Poland

³Department of Clinical Oncology, Tadeusz Koszarowski Cancer Center in Opole, Poland

Invasive breast cancer in ectopic axillary breast tissue — case report

Address for correspondence:

Lek. Tomasz Sachański
Opolskie Centrum Onkologii
ul. Katowicka 66a, 45-061 Opole
Tel: 606 898 677
+48 77 441 6073
e-mail: saszk73@gmail.com

ABSTRACT

Breast cancer is the most commonly diagnosed malignancy in women in Poland. Rare, unusual forms of breast cancer remain a diagnostic problem. The incidence of ectopic breast glandular tissue in the general population varies between 0.4 and 6%. The same abnormalities and diseases as in the anatomical mammary gland may develop in this tissue. Breast cancer may develop as well.

We present the case of a 36-year-old woman, who went to the doctor because of a nodule in the right armpit presented for 2 years. The patient was referred to a surgeon with suspicion of an epidermal cyst. The lesion was surgically removed and in the histopathological examination, the diagnose was: invasive breast cancer in ectopic glandular tissue. After imaging diagnostics, discussion of a multidisciplinary diagnostic and therapeutic team, the patient was offered a surgical procedure - widening of the excision margins to obtain oncological completeness and axillary lymphadenectomy. After the surgery, due to the results of the histopathological examination, complementary systemic treatment (chemo- and hormone therapy) and radiotherapy were used.

Doctors often do not consider the possibility of primary breast cancer occurring elsewhere than in the breast. Breast imaging does not always make it possible to diagnose the disease, and doctors performing and interpreting these tests often do not include primary armpit cancer in the differential diagnosis. This can cause a delay in diagnosis and worsen the prognosis.

Key words: rare cases of breast cancer, ectopic glandular tissue, additional breast, axillary tumor

Oncology in Clinical Practice
2021, Vol. 17, No. 1, 42–45
DOI: 10.5603/OCP.2020.0025
Translation: lek. Maciej Miodoński
Copyright © 2021 Via Medica
ISSN 2450-1654

Oncol Clin Pract 2021; 17, 1: 42–45

Introduction

Breast cancer is the most commonly diagnosed malignancy in women in Poland. It is also one of the biggest threats to the premature mortality of women [1]. The growing awareness of doctors of various specialties not only oncologists, allows more and more often to detect this disease at an early stage, also preclinical. Rare, unusual forms of breast cancer remain a diagnostic problem.

Ectopic breast glandular tissue occurs between 0.4 and 6% of the general human population [2]. Its presence is a consequence of the incomplete disappearance of the so-called mammary crests (*cretae mammae*) [3]. Such remaining additional glandular tissue is most often located within the axillary pits, but other locations, such as supra and subclavian region, subcutaneous region, and even anatomically distant locations such as perineum and anus are possible [4].

In such an additional gland, abnormalities and diseases characteristic of the anatomical mammary gland may occur. Cases of adenomas, fibro-cystic lesions, as well as breast cancer are reported [5]. Typically, the patient is not aware of the presence of such an incorrectly located additional glandular tissue until a palpable tumor is found, for example in the armpit.

A case report

A 36-year-old woman, without a significant medical history, was referred by a primary care physician to a general surgeon because of a nodule in the soft tissues of her right armpit for more than 2 years. The lesion has increased over the past few months. Due to the incriminating family history (mother's sister had breast cancer), the patient regularly checked her breasts during gynecological visits. Periodically performed breast ultra-

sound showed no abnormalities. The consulting surgeon found the relative movable tumor with a diameter of 2 cm and redness of the skin above it. The ultrasound examination of the armpit described a hypoechoic, irregular focal lesion with a diameter of 21 mm, involving skin and subcutaneous tissue, as well as, lymph nodes up to 8 mm in diameter next to the principal lesion. There was suspicion of the inflammatory epidermal cyst or inverted acne (*hidradenitis suppurativa*). The lesion was surgically removed.

In histopathological report, invasive cancer of special type (NST) with intermediate grade (G2) was identified, with the presence of angio- and neuro-invasion. The pathologist described the weaving of cancer in the dermis and subcutaneous tissue, and in the vicinity, structures corresponding to the residual weaving of the mammary gland. In summary, it was found that the whole picture corresponds to primary cancer originating from ectopic breast glandular tissue. Along with the major lesion, 4 lymph nodes were removed, in which metastatic lesions up to 3 mm in diameter were found with infiltration outside the lymph node capsule. The degree of pathomorphological severity was defined as pT1cN2a. In immunohistochemistry, the strong expression of estrogen and progesterone receptors, lack of HER2 receptor expression, and Ki67 proliferation index of about 30% were found. The cancer phenotype was defined as luminous B HER2 negative.

After diagnosis, the patient was referred to the Opole Oncology Center, where spectral mammography, chest X-ray and abdominal ultrasound were performed.

In spectral mammography (Fig. 1 and 2) no pathological strengthening foci were found both in breasts and axillary pits (the study was performed after a diagnostic excision). The results of the chest and abdominal imaging were normal. The patient's case was discussed during a multidisciplinary diagnostic and therapeutic team meeting. Due to the presence of cancer stuck to the edges of the surgical incision of the removed lesion and metastases to four lymph nodes with infiltration outside the node capsule, the patient was offered the surgery at first - widening the excision of the lesion with the removal of right axillary lymph nodes. In the pathomorphological examination of the postoperative material, the edges of the preparation were free of neoplastic lesions. There were found postoperative resorptive changes and ductal carcinoma in situ (DCIS) with an intermediate degree of differentiation present in individual ducts in the lodge area. In four of the twelve lymph nodes assessed, metastases of cancer up to 6 mm in diameter with infiltration outside the lymph node capsule were found. After surgery, the patient was offered complimentary treatment as part of a multidisciplinary medical consultation. Chemotherapy based on anthracyclines and taxoids was used, and then, according to the expression of steroid hormone receptors, pharmacological ovarian suppression and treatment with nonsteroidal aromatase inhibitors were included. The patient was also subjected to additional radiation therapy. The breast was irradiated together with the nodal field up to a dose of 50 Gy in 25 fractions with an additional dose of 10 Gy in 5 fractions after the tumor resection using 3D conformal radiotherapy with intensity-modulated radiation therapy (IMRT).

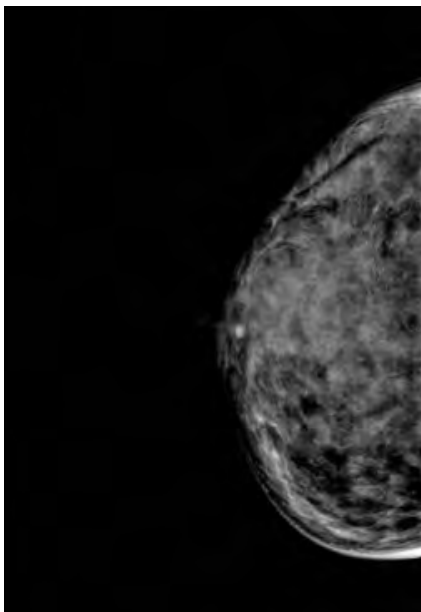


Figure 1. Contrast — enhanced spectral mammography of the right breast

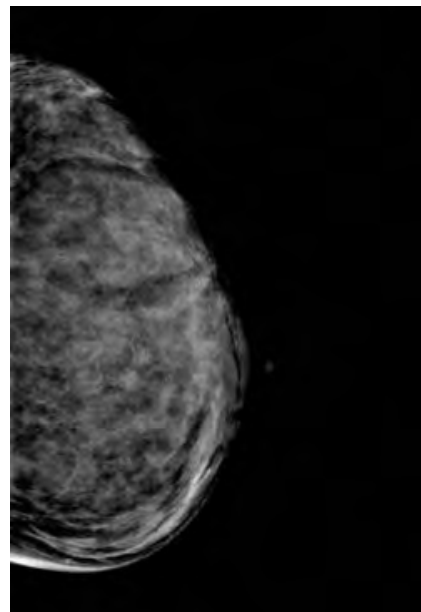


Figure 2. Contrast — enhanced spectral mammography of the left breast

Discussion

In case of a palpable tumor in the armpit, the most often taken into account are enlarged lymph nodes, which have been changed, reacted with inflammation or cancer. In differential, diagnostics should be considered such conditions as inflammation (abscess, boil, lipoma, inverted acne), epidermal cyst, as well as soft tissue tumors or peripheral nerve sheaths tumors [6, 7].

We should also remember about the possibility of ectopic breast glandular tissue. In terms of histological structure, one can distinguish an additional (supernumerary) breast and aberrant breast tissue [8]. Additional breasts most often occur in place of embryonic nipple combs, so-called "milky line" from armpits to the groin. The glandular structures of such an additional breast are ordered, with a secretory system, as well as with the possibility of an additional wart and areola. In the breast tissue of the split breast, the glandular structures are chaotic, with no ordered secretory system. They are usually located near the anatomical breast as an "islands" of glandular tissue [8].

Extrapodial glandular tissue undergoes changes under the influence of hormones, analogously to the anatomical mammary gland. It may develop various abnormalities and diseases such as fibro-cystic lesions, adenomas, papillomas, as well as breast cancers [9]. According to various authors, the incidence of breast cancer in ectopic breast glandular tissue accounts for 0.2 to 0.6% of all breast cancers [5]. Because of the rarity of the disease, doctors often do not consider the possibility of primary breast cancer outside the anatomical mammary gland. Imaging breast examinations do not always make it possible to diagnose the disease. This can cause a significant delay in diagnosis and worsen the prognosis.

The treatment of cancer in the ectopic breast glandular tissue within the armpit is generally subject to the same principles as the treatment of cancer of the anatomically located breast. However, some issues remain controversial, especially regarding the extent of surgical treatment. Some authors suggest mastectomy on the same side as the tumor in the ectopic tissue if the axillary lymph nodes are involved [10, 11]. Others argue that removal of the anatomical breast does not bring additional benefits to patients, and the prognosis is the same for local, radical removal of ectopic tissue and amputation [12, 13]. Therefore, the surgical treatment of choice is a wide excision of the lesion with surrounding tissues (including skin). Mastectomy is not indicated if breast imaging does not indicate cancer within it. However, it should be considered if the results of imaging tests are not clear and differential diagnosis does not allow for a reliable diagnosis [14].

In case of a primary lesion located in the ectopic tissue of the breast and clinically unchanged regional lymph nodes (which was not the case in this patient), lymph node surgery is necessary. Does sentinel node biopsy make sense in this case? Some authors show that metastases in axillary lymph nodes occurred in about 50% of the analyzed cases, and therefore very often, which could suggest the legitimacy of lymphadenectomy in such a situation [4, 10, 11]. However, several published papers indicate that lymphoscintigraphy allows accurate localization of sentinel lymph nodes. Therefore, it is possible to perform a sentinel node biopsy procedure and to avoid mutilating lymphadenectomy [16, 17]. This approach is particularly justified in cases of ectopic tissue location outside the armpit, where the absorbent flow down to the armpit on the same side is not obvious [17]. The choice of the site for radioisotope injection remains a technical issue. Will the retroareolar injection on the same side as the axillary tumor be appropriate? Considering lymphatic drainage and axillary location, it appears that peritumoral or surgical site biopsy is more appropriate in this case [17].

The scope of complementary radiotherapy also raises some controversy. Due to the rarity of this form of cancer, there is no clearly defined standard of treatment. Most authors suggest qualifying patients for complementary radiotherapy based on similar criteria as in the case of breast cancer in a typical location. There are differences regarding the scope of radiotherapy. Some authors consider it appropriate to irradiate the lodge after the removed tumor and axillary pit, others suggest covering the breast on the tumor side [18].

Systemic adjuvant treatment should be carried out in accordance with generally accepted recommendations for the treatment of patients with early breast cancer.

In the presented case, after a discussion within the multidisciplinary diagnostic and therapeutic team, a decision was made on supplementary radiotherapy for the lodge after the removed tumor and the armpit due to the involved lymph nodes and skin infiltration. Systemic treatment was adapted to cancer biology and the degree of pathomorphological severity.

Conclusions

Ectopic breast glandular tissue is often not included in routine mammography. Also, some physicians performing breast ultrasound examination do not take into account the possibility of cancer in the ectopic breast gland tissue. This causes delays in the diagnosis and treatment of additional breast cancer.

Conflict of interest

The authors report no conflicts of interest.

References

1. Jeziorski A, Rutkowski P, Wysocki W, et al. *Chirurgia onkologiczna t. 1*. PZWL, Warszawa. 2018: 19–20.
2. Gabriel A, Maxwell G. Breast embryology. 2018, <http://emedicine.medscape.com/article/1275146-overview>.
3. Shunke M, Schulte E, et al. *Atlas Anatomii człowieka t. 1*. Prometheus, Wrocław 2009.
4. Marshall MB, Moynihan JJ, Frost A, et al. Ectopic breast cancer: case report and literature review. *Surg Oncol*. 1994; 3(5): 295–304, doi: [10.1016/0960-7404\(94\)90032-9](https://doi.org/10.1016/0960-7404(94)90032-9), indexed in Pubmed: [7889223](https://pubmed.ncbi.nlm.nih.gov/7889223/).
5. Francone E, Nathan MJ, Murelli F, et al. Ectopic breast cancer: case report and review of the literature. *Aesthetic Plast Surg*. 2013; 37(4): 746–749, doi: [10.1007/s00266-013-0125-1](https://doi.org/10.1007/s00266-013-0125-1), indexed in Pubmed: [23620009](https://pubmed.ncbi.nlm.nih.gov/23620009/).
6. Kokot F, et al. Diagnostyka różnicowa objawów chorobowych. *Wyd. 3*. PZWL, Warszawa. 2007: 528–530.
7. Aymen F, Atef Y, Majed G, et al. Axillary Masse, is it an Ectopic Mammary Gland? *Breast Cancer: Current Research*. 2016; 1(2): 108, doi: [10.4172/2572-4118.1000108](https://doi.org/10.4172/2572-4118.1000108).
8. Georgiade NG, Georgiade GS, Riefkohl R, et al. *The breast: embryology, anatomy, and physiology*, Philadelphia: WB Saunders. 1990: 3–17.
9. Velanovich V. Ectopic breast tissue, supernumerary breasts, and supernumerary nipples. *South Med J*. 1995; 88(9): 903–906, doi: [10.1097/00007611-199509000-00002](https://doi.org/10.1097/00007611-199509000-00002), indexed in Pubmed: [7660204](https://pubmed.ncbi.nlm.nih.gov/7660204/).
10. Matsuoka H, Ueo H, Kuwano H, et al. [A case of carcinoma originating from accessory breast tissue of the axilla]. *Gan No Rinsho*. 1984; 30(4): 387–391, indexed in Pubmed: [6727040](https://pubmed.ncbi.nlm.nih.gov/6727040/).
11. Visconti G, Eltahir Y, Van Ginkel RJ, et al. Approach and management of primary ectopic breast carcinoma in the axilla: where are we? A comprehensive historical literature review. *J Plast Reconstr Aesthet Surg*. 2011; 64(1): e1–11, doi: [10.1016/j.bjps.2010.08.015](https://doi.org/10.1016/j.bjps.2010.08.015), indexed in Pubmed: [20934398](https://pubmed.ncbi.nlm.nih.gov/20934398/).
12. Tjalma WAA, Senten LLC. The management of ectopic breast cancer — case report. *Eur J Gynaecol Oncol*. 2006; 27(4): 414–416, indexed in Pubmed: [17009640](https://pubmed.ncbi.nlm.nih.gov/17009640/).
13. Cogswell HD, Czerny EW. Carcinoma of aberrant breast of the axilla. *Am Surg*. 1961; 27: 388–390, indexed in Pubmed: [13694314](https://pubmed.ncbi.nlm.nih.gov/13694314/).
14. Evans DM, Guyton DP. Carcinoma of the axillary breast. *J Surg Oncol*. 1995; 59(3): 190–195, doi: [10.1002/jso.2930590311](https://doi.org/10.1002/jso.2930590311), indexed in Pubmed: [7609527](https://pubmed.ncbi.nlm.nih.gov/7609527/).
15. Zhang S, Yu YH, Qu W, et al. Diagnosis and treatment of accessory breast cancer in 11 patients. *Oncol Lett*. 2015; 10(3): 1783–1788, doi: [10.3892/ol.2015.3388](https://doi.org/10.3892/ol.2015.3388), indexed in Pubmed: [26622750](https://pubmed.ncbi.nlm.nih.gov/26622750/).
16. Thorne AL, Jackson A, Yiangou C. The use of sentinel node biopsy in the treatment of cancer of an accessory breast. *Breast*. 2003; 12(2): 153–155, doi: [10.1016/s0960-9776\(02\)00266-7](https://doi.org/10.1016/s0960-9776(02)00266-7), indexed in Pubmed: [14659346](https://pubmed.ncbi.nlm.nih.gov/14659346/).
17. Gentilini O, Paganelli G, Trifiro G, et al. Sentinel node biopsy in ectopic breast cancer. *Nucl Med Commun*. 2003; 24(11): 1127–1128, doi: [10.1097/01.mnm.0000101603.64255.5f](https://doi.org/10.1097/01.mnm.0000101603.64255.5f), indexed in Pubmed: [14569165](https://pubmed.ncbi.nlm.nih.gov/14569165/).
18. Routiot T, Marchal C, Verhaeghe JL, et al. Breast carcinoma located in ectopic breast tissue: a case report and review of the literature. *Oncol Rep*. 1998; 5(2): 413–417, doi: [10.3892/or.5.2.413](https://doi.org/10.3892/or.5.2.413), indexed in Pubmed: [9468570](https://pubmed.ncbi.nlm.nih.gov/9468570/).