Precision oncology in the treatment of gynecological cancers

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1. ABBREVIATIONS

Abbreviation	Meaning	Page
ACT	adjuvant chemotherapy	7
AKT	Protein kinase B	6
ARID1A	The AT-rich interactive domain-containing protein 1A	70
ATM	ATM serine/threonine kinase	4
BARD1	BRCA1 Associated RING Domain 1	4
BRCA (1,2)	Breast Cancer gene (1,2)	4
BRIP (1,2)	BRCA1 Interacting Protein (1,2)	4
CA-125	cancer antigen 125	5
CCRT	concomitat chemoradiation	7
CDK12	Cyclin-dependent kinase 12	4
CGP	comprehensive genomic profiling	4
CHEK (1,2)	Checkpoint kinase (1,2)	4
CI	confidence interval	70
CNH	copy number high	6
CNL	copy number low	6
DNA	deoxyribonucleic acid	4
FANCL	Fanconi anemia complementation group L	4
FDA	The United States Food and Drug Administration	4
GA	genomic alteration	70
GIST	gastrointestinal stromal tumor	3
HER-2	Human epidermal growth factor 2	3
HPV	Human papillomavirus	6
HR	homologous recombination	8
$HR_{adjusted}$	adjusted hazard ratio	70
HRD	homologous recombination deficiency	8
HRR	homologous recombination repair	4
ICI	immune checkpoint inhibitors	7
IQR	interquartile range	70
LACC	locally advanced cervical cancer	7
LOH	loss of heterozygosity	4
MMR	mismatch repair	5
MSCT	multisliced computed tomography	5
MSS	microsatellite stable	4

MSI	microsatellite instability	4
mTOR	Mammalian target of rapamycin	6
Muts/Mb	mutations per megabase	4
NGS	next generation sequencing	3
NSCLC	non-small cell lung cancer	3
OS	overall survival	70
PALB2	Partner and localizer of BRCA2	4
PARP	Poly (ADP-ribose) polymerase	8
PD-L1	Programmed death-ligand 1	7
PET/CT	positron emission tomography and computed tomography	5
PFS	progression free survival	70
PI3K	Phosphoinositide 3-kinases	6
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	4
POLE	DNA Polymerase Epsilon, Catalytic Subunit	6
PTEN	Phosphatase and tensin homolog	6
RAD51B	DNA repair protein RAD51 Paralog B	4
RAD51C	DNA repair protein RAD51 Paralog C	4
RAD51D	DNA repair protein RAD51 Paralog D	4
RAD54L	DNA repair and recombination protein RAD54-like	4
RNA	ribonucleic acid	4
TC	paclitaxel and cisplatin	7
TCGA	The Cancer Genome Atlas	6
TKI	tyrosine kinase inhibitor	7
TMB	tumor mutational burden	4
VEGF	Vascular endothelial growth factor	8

2. INTRODUCTION

Twenty-first century brought revolutionary advancements in the fields of informational technologies and bioinformatics, as well as in molecular biology and medicine with unveiling and better understanding of the human genome. This has caused a paradigm shift in the establishment of postulates towards precision medicine resulting in a more individualized and targeted approach to the individual patient. Consequently, in order to optimize every day use of precison diagnostic and treatment procedures, a significant change needs to be introduced when approaching a patient with taking into account their known gene variations and their possible impact on the disease and the treatment outcomes, patient's comorbidities, general condition, as well as other aspects of an individual, such as lifestyle and environmental factors. Hence, precision medicine implies not only personalized treatment, but also a prompt and coordinated individual approach with a detailed and treatment-oriented diagnostic workup.

Oncology is one of the most dynamic fields of medicine with cancer as unique and specific disease for every single patient and, not less important, everlasting and both in time and space changing target. Therefore, it is most suitable for the implementation of precision medicine in everyday clinical practice. Of course, another, even more important, reason for a new diagnostic and treatment brakethroughs in oncology is high unmet need for further improvements in the outcomes which, in globaly speaking terms, is the most important public health problem of today. Furthermore, understanding the fundamental mechanisms of underlying carcinogenesis, its causal relation to gene alterations, and advances in molecular biology have made it possible to create novel therapeutic modalities, such as molecular targeted therapy and immunotherapy, which have generally better outcomes, significant improvement in patients' survival and, equally important, usually better quality of life. For instance, molecular targeted therapy is already accepted as the gold standard for first-line treatment in advanced or metastatic non-small cell lung cancer (NSCLC) [1], melanoma [2], gastrointestinal stromal tumor (GIST) [3], and recurrent ovarian cancer [4]. Immunotherapy with checkpoint inhibitors, on the other hand, is becoming the standard of care for many cancer types, including skin [5], lung [6], renal [7], and bladder cancer [8], and immunotherapy against specific antigens is standardized as a treatment for early or metastatic HER-2 positive breast cancer [9, 10], metastatic colorectal [11], gastric [12], ovarian [13], or cervical [14] cancer.

Despite the aforementioned scientifical and technological development, increased availability of modern technologies such as next generation sequencing (NGS), computing technology and evolution of tailored treatment, applicability of precision medicine in everyday clinical practice is still emerging and is one of hot topics. Moreover, proponents against its implementation are calling it „an illusion" and „a sobering idea" based on the results from two trials, one of which is a phase 2 SHIVA trial which discourages the use of „off-label" molecular-targeted therapy [15, 16]. However, the SHIVA trial was criticized for potential biases due to its design, administration of targeted therapy either as monotherapy in patients with more than one molecular alteration or incorrectly matching it for some patients [17]. On the other hand, several studies have shown favorable effects of the use of "off-label" molecular-targeted therapy with improved and almost doubled response rates and progression-free survivals [18-22]. All in all, the emphasis needs to be put on further research where classical randomized control trials will no longer suffice and where everyone will have to learn from and for every patient individually, creating a „self-learning

system“. Furthermore, to optimally implement precision medicine in everyday clinical practice there has to be improved access to the modern testings and the treatment, adequatly educated medical staff, established molecular tumor board comprised of professionals from different experties, proper data generation and trial designs with continuous monitoring and reporting which will hopefully lead to accelerated drug approvals and consequently benefit to many oncology patients. Following the abovestated, there are still a lot of unresolved issues regarding this matter and every effort is more than welcome to ensure optimal precision cancer care for all.

2.1 Comprehensive genomic profiling

In contrast to conventional testing, which uses single-target assays to potentially find one actionable gene alteration, comprehensive genomic profiling (CGP) by using next-generation sequencing (NGS), a targeted high throughput hybridization-based capture technology, provides detailed insight into tumor multiple gene specifics, expanding and personalizing diagnosis and treatment options available to every cancer patient [23].

For the study purposes, tumor specimens were obtained from surgery or biopsy samples of the primary disease or metastases and sent to a laboratory certified by the Clinical Laboratory Improvement Amendments and the College of American Pathologists (Foundation Medicine Inc., Cambridge, MA, USA) [24-25]. Formalin-fixed, paraffin-embedded tissue was sent as a block and one slide stained with hematoxylin and eosin or 10 unstained slides with one slide stained with hematoxylin and eosin. The minimum surface area was 25 mm^2, and the minimum tumor content was 20%, while the optimum was 30% of tumor nuclei, defined as the number of tumor cells divided by the total number of all cells with nuclei. Once the DNA was extracted, 50–1000 ng underwent whole-genome shotgun library construction and hybridization-based capture in order to detect alterations of 324 genes in total: 309 exons related to tumors, one promoter region, one non-coding RNA, and certain regions of introns in 34 frequently rearranged genes in tumors. The test also determined genomic signatures like microsatellite status, tumor mutational burden (TMB) and, in case of ovarian cancer, loss of heterozygosity (LOH) by assessing homologous recombination repair (HRR). Illumina® HiSeq 4000 was used to sequence hybrid capture-selected libraries to a high uniform depth. The typical median depth of coverage was >500×, with >99% of exons at coverage of >100×. The sequenced regions were analyzed for four different types of alterations: base substitution, deletion or insertion, copy number variation, and gene redistribution in a group of genes associated with tumor development. The microsatellite status (MSS or MSI) was based on genome-wide analysis of 95 microsatellite loci; TMB was determined by counting all synonymous and non-synonymous variants present at a 5% allele frequency or higher, with the total number presented as mutations per megabase (Muts/Mb) unit; and HRR mechanism was assessed for mutations in the 14 HRR genes, namely ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, and RAD54L [26].

Comprehensive genomic profiling is gradually being incorporated as a fundamental tool in the diagnostic workup of the various tumor types. Its application is one of the hot topics in recent years, with questions in matter such as cost, usefulness, and therapeutic benefit, as well as patient and social expectations [27–30]. The usage and value of molecular-targeted therapy outside its current indications are still being studied, but as previously mentioned it is already well-established as a

conventional treatment for many tumor types. While studies like phase 2 SHIVA trial discouraged the use of "off-label" molecular-targeted therapy due to its inferior progression-free survival when compared to standard care treatment [16], clinical studies like the MOSCATO trial have shown benefit and improved outcomes with targeted therapy but only in "hard-to-treat" patients [31]. Also, a number of trials have demonstrated the benefits of using molecular-targeted therapy "off-label," including enhanced and nearly doubled response rates and progression-free survivals [18–22]. The number of in-human studies investigating the dose escalation of targeted medication, such as the phosphatidylinositol 3-kinase-selective inhibitor alpelisib in patients with certain mutations like PIK3CA, has been rising quickly lately [32]. Furthermore, the FDA (Food and Drug Administration) has approved immunotherapy independent of the type of cancer for "tumor agnostic" biomarkers such as microsatellite instability and tumor mutational burden that are discovered as a result of new diagnostic techniques. Consequently, despite the arguments regarding the cost of CGP stated above, it is strongly advised to perform this “tumor agnostic” biomarkers in the absence of CGP, which entails less expensive but no less useful tests, such as immunohistochemical staining for mismatch repair status (MMR protein staining) [33].

Today we have more diagnostic tools than ever, more and more precise medications, and, paradoxically, less and less reliable data supporting their usage in specific patients. Additionally, with an anticipated, even more granular approach to a single patient and her or his tumor, it is highly likely that classical clinical trials will not be able to meet the demands of the ongoing advancement of oncology science. Therefore, the future of precision oncology research is going to be focused on real-world data and learning from every patient experience and tumor specificity across all tumor types generally, but especially in subtypes driven by targetable biomarkers.

2.2. Gynecological cancers

Gynecological cancers reffers to a term which encompasses malignancies of female reproductive system, such as vulva, vagina, cervix, uterus, ovaries and fallopian tubes with the latter three representing the vast majority of cases. In 2020, these entities made up to 15% of all cancer types diagnosed in women, affecting over 1,3 million of women, and are responsible for almost 15% of cancer related deaths resulting with over 600,000 women who succumb to the disease [34]. Furthermore, with their different distribution, uterine cancer as the most common gynecological cancer in developed countries and cervical cancer as the most common one in developing parts of the world and ovarian cancer present everywhere almost equally, they represent a major public and health burden worldwide [34]. In addition, uterine and cervical cancer are the only two entities with worsened overall survival in the USA over the last 20 years [35].

Nowadays, when approaching a patient with pathologically confirmed either cervical, uterine or ovarian cancer, an oncologist has to use properly and timely organized all of the available clinical, morphological and biochemical indicators of the extent of the disease, as well as novel technologies, in order to optimally personalize diagnostic and treatment workup. Depending on indications after clinical exam or after surgical procedures or in case of elevated biomarker cancer antigen 125 (CA-125) in ovarian cancer, morphological workup is needed. For instance, an ultrasound, multisliced computed tomography (MSCT) of abdomen and pelvis, magnetic resonance (MR) and/or thorax and even positron emission tomography and computed tomography (PET/CT) scan in cases of locally advanced cervical cancer [36]. Furthermore, novel technologies, such as comprehensive genomic profiling through next generation sequencing, are becoming widely used in

everyday clinical practice. Thus, CGP provides detailed insight into tumor specifities and alterations that are potentially targetable with some kind of therapy and with that represents the hallmark of precision medicine [23].

At the end of 2019, a CGP analysis of the tumor specimens provided by Foundation Medicine Inc. began in Croatia for patients diagnosed with metastatic disease as a part of the project for the development and implementation of precision oncology on a national level in Croatia [37]. Croatia, as one of transitioning countries, is ideal for assessment of cost benefit, real clinical impact of CGP on a national level, particularly in gynecological cancers and is representative for addressing the optimal treatment of cervical cancer.

2.2.1. Uterine cancer

Uterine cancer ranks first in incidence among invasive tumors of the female reproductive system in the developed countries due to its association with older age, better socio-economic status, and unopposed estrogen activity [38]. Unfortunately, 15–20% of patients present with or progress to metastatic disease with a 5-years survival rate of 16% [39]. The main treatment strategy for metastatic uterine cancer is chemotherapy or hormonal therapy with fewer than 12 months of the median overall survival [40]. According to the TCGA (The Cancer Genome Atlas) project in 2013, uterine/endometrial cancer is divided into four subgroups based on the genomic profiling of 373 endometrial cancer specimens [POLE ultra-mutated, microsatellite instability group, copy number low (CNL), and copy number high (CNH) groups][41]. The POLE ultra-mutated group, which consisted of 7% of tumors, and the microsatellite instability group of tumors (28% of tumors) are candidates for immunotherapy due to the high neoantigen load and consecutively optimal tumor microenvironment for enhanced cytotoxic T-cell response [41]. Improvement in outcomes of the CNL group (39% of tumors) may be in combination with hormonal therapy and the PI3K/AKT/mTOR pathway inhibitor and for the CNH serous-like group (26% of tumors) treatment with cell cycle regulators and the PI3K/AKT/mTOR pathway inhibitors [41].

Uterine cancer harbors a high level of gene alterations but is still insufficiently explored. It is ranked fourth in cancer incidence in Croatia with 778 women being diagnosed annually and having a mortality-to-incidence ratio of 0.26 [42]. Thus, we presented first-year CGP data on a country level for patients with newly diagnosed metastatic uterine cancer or whose initial disease had progressed during 2020 with primary goal of this project to assess a share of patients with opted targetable mutations, while the secondary goal was an assessment of the proportion of patients who have started with the CGP-guided therapy. Also, by defining and emphasizing potential opportunities as well as the problems we are facing in the precision oncology development and implementation of this specific field, the aim was to affirm the CGP of patients with metastatic uterine cancer in everyday clinical practice.

2.2.2. Cervical cancer

Cervical cancer ranks fourth in both cancer incidence and mortality among women, with approximately 604,000 newly diagnosed patients and an estimated 342,000 deaths worldwide in 2020. Furthermore, the burden of cervical cancer is not equally distributed. It is less common and less publicly important in developed parts of the world, whereas it is the most commonly diagnosed cancer as well as the leading cause of death in some developing parts of the world [34]. Even though cervical cancer is almost preventable now, due to primary (HPV vaccine) and secondary

(screening programs) prevention currently available, their unequal implementation and penetration in the different healthcare systems of countries worldwide could be one of the reasons for the aforementioned global inequality [43, 44]. The association of cervical cancer with lower-income areas in general, the fact that it affects a relatively younger population, the high mortality to incidence ratio, and inadequate implementation of existing prevention programs altogether make cervical cancer one of the major contributors to the global societal burden. The burden of cervical cancer creates an essential need for international intervention aiming to provide every woman worldwide with an equal chance to prevent and optimally treat this "underserved" disease [34, 45]. Unfortunately, a significant number of patients die, specifically, more than 50% of all newly diagnosed patients per year, underlining the absolute need for therapies with better outcomes [34]. In addition, standard treatment for locally advanced disease is concomitant application of cisplatin chemotherapy (CCRT) and radiotherapy with almost 40% of patients with local or distant recurrence of the disease [46-50]. Due to the latter, several strategies were explored in order to improve the outcomes, including application of adjuvant chemotherapy after CCRT resulting with inconclusive findings [51-55]. Thus, results from the phase III OUTBACK trial were eagerly awaited to establish a definite treatment for locally advancer cervical cancer (LACC). Since they were presented, adjuvant chemotherapy (ACT) is not recommended in the treatment of LACC [56]. Considering that adjuvant chemotherapy is treatment of choice for many other cancer types and that chemotherapy is standard treatment for recurrent or metastatic cervical cancer, which indicates its chemosensitivity, results from the OUTBACK trial should not represent the final verdict on this topic [57-64]. The review article we have recently published presents current state of knowledge regarding the use of adjuvant chemotherapy in locally advanced cervical cancer with critical appraisal of the OUTBACK trial, aiming to challenge its results and to, once more, raise a question about the optimal treatment of LACC.

In addition, high mortality rate also implies the need for research of novel treatment strategies, such as the tyrosine kinase inhibitors (TKIs) targeting angiogenic kinases, mTOR-inhibitors in PIK3CA mutated cancers, or immunotherapy with checkpoint inhibitors (ICI) in PD-L1 positive cancers [65]. In contrast to other tumor types, where we have recently witnessed significant improvements in the survival of metastatic patients, there were no significant breakthroughs regarding overall survival in the therapy of cervical cancer since the introduction of platinum and ifosfamide as a standard treatment regimen many years ago [66]. Recently, however, the incorporation of bevacizumab as a part of a first-line therapy option, together with cisplatin and paclitaxel (TC) as a chemotherapy backbone, has significantly increased the progression-free survival, response rate, and, most importantly, overall survival rate in metastatic or locally recurrent cervical cancer patient populations [67]. Based on the results of a registrational trial (GOG-240), bevacizumab is accepted as the treatment of choice when coupled with TC chemotherapy in the first-line setting of patients with advanced cervical cancer. Notwithstanding the significant results of the study, randomized controlled trials do not presume the same outcomes in the real-world setting when treating patients [68]. This difference in outcomes is possibly due to the absence of strict inclusion and exclusion criteria and, consequently, population diversity with a higher number of patients with comorbidities in real-world practice. Moreover, the organizational approach to regular work-ups and general oncological care, especially in the underserved parts of the world where the majority of cases are diagnosed, explain the difference between outcomes in real-world settings [69, 70]. Therefore, it is important to monitor the real-world efficacy and safety of the given drug to understand its actual

use and benefits in everyday clinical practice [71, 72]. Furthermore, this could be tremendously important for cervical cancer, where the burden of the disease is high in less-developed countries, since bevacizumab is a rather expensive drug. Hence, the aim of our study was to assess the real-world efficacy and safety of bevacizumab as a first-line treatment of advanced cervical cancer in the total population of Croatia, one of the transitioning countries as potential example for other similarly developed countries for implementation of bevacizumab in everyday clinical treatment of patients with metastatic or locally recurrent cervical cancer.

2.2.3. Ovarian cancer

Ovarian cancer is the eighth most common cancer diagnosed among women worldwide. While it usually occurs in women of older age, a significant number of patients are diagnosed at a younger age (≤55 years), especially women with positive family history. Furthermore, when defining public health importance, more than 70% of women are diagnosed with locally advanced or metastatic disease with an expected 5-year survival rate of less than 30% [73]. Due to its obscure clinical presentation, diagnosis at advanced stages, and high mortality rate, ovarian cancer is the most lethal cancer of the female reproductive system and thus represents one of the hot topics in oncology with a need for significant advances in the treatment. The last significant breakthrough in terms of chemotherapy administration occurred with the introduction of paclitaxel and carboplatin regimens at the end of 1990 [74]. Unfortunately, the introduction of immunotherapy directed against VEGF in combination with chemotherapy and as a maintenance treatment did not affect overall survival, despite a significant effect on progression-free survival [75–77]. Finally, targeted therapy with PARP inhibitors in patients with germline or somatic BRCA mutations has revolutionized therapy, statistically and clinically improving outcomes and increasing patient and societal expectations [78–80]. Since the introduction of the latter treatment, the determination of germline and somatic BRCA 1 and BRCA 2 status is mandatory in the diagnostic workup [81]. Additionally, in 2020, PARP inhibitors were approved for the treatment of ovarian cancer in patients with an established homologous recombination deficiency (HRD) status through BRCA or mutation of other genes involved in the HRD process [82]. HRD and consequent loss of heterozygosity, which represents the percentage of the tumor genome with a focal loss of one allele, lead to genomic instability and occur due to genetic or epigenetic inactivation of one or more HR pathway proteins, including BRCA 1, BRCA 2, RAD51C, ATM, PALB2, and BRIP1 [83–85]. A clinically significant LOH score with approved PARP inhibitor therapy was determined at a cut-off of ≥16 [81]. On the basis of these findings, diagnostic approach of ovarian cancer is dramatically changing, with molecular classification surpassing simple histological classification into type I and type II ovarian cancer, and targeted therapy is becoming the mainstay treatment for locally advanced or metastatic disease [86]. Thus, a determination of genomic instability or other potentially targetable mutations, along with BRCA 1 and BRCA 2, is a crucial component of the diagnosis and treatment management of these patients. Advanced technologies such as next-generation sequencing (NGS) are becoming more feasible and are used in daily clinical work, providing abovestated tumor specifics.

Ovarian cancer represents one of the major health burdens in Croatia due to its high mortality-to-incidence ratio (0.67) [42]. Furthermore, Croatia is among countries with the highest incidence and mortality of ovarian cancer in Europe [87]. Potential reason for the high mortality-to-incidence ratio lies in the late diagnosis and lack of proper treatments. For instance, in 2018, Croatia was one of the

countries with the lowest tier for PARP inhibitor uptake [88]. Ovarian cancer patients are treated with standard chemotherapy following surgery (or before when neoadjuvant therapy is indicated) or, in the case of initially metastatic disease, with platinum-based chemotherapy and paclitaxel every three weeks or dose-dense, +/− bevacizumab, or, recently, with PARP inhibitors, depending on the residual disease and BRCA status as well as the response to platinum therapy. In the treatment of recurrent disease, patients are also treated with standard chemotherapy based on platinum sensitivity, along with bevacizumab or with PARP inhibitors in cases of BRCA mutation. PARP inhibitors are given after response to platinum-based chemotherapy as a maintenance treatment. However, they are not indicated in cases of HRD or LOH as they are in some other European countries. As no established screening method is available, and the majority of women are consequently diagnosed in advanced stages with low survival rates, the diagnostic workup should receive special attention, particularly because all patients should have equal opportunities to be treated the same with already approved targeted therapies. CGP analysis was performed in patients with locally advanced or metastatic ovarian cancer on a national level. The aim of the study was to present the number of patients with targetable BRCA 1 and BRCA 2 mutations compared with the total number of patients whose CGP results revealed a need for targeted therapy with PARP inhibitors, as well as other potential targeted treatments, and to compare CGP with conventional testings for BRCA 1 and BRCA 2, and with that establish the position of the CGP early in the everyday diagnostics of ovarian cancer.

3. RESEARCH QUESTION AND OBJECTIVE OF JOINT ARTICLES

Following the abovestated, research question of these studies was to define the real position of precision oncology in everyday clinical practice of gynecological cancers.
Thus, to address the optimal treatment of advanced cervical cancer, the data was collected on a national level regarding the use of anti VEGF directed immunotherapy in the treatment of metastatic cervical cancer, which is currently standard treatment in combination with chemotherapy, but still unavailable in some developing countries. Whereas the review article critically evaluated the negative recommendation of the use of adjuvant chemotherapy in locally advanced cervical cancer with aim to define a need for more and further research in this field in order to improve rather week outcomes of patients with locally advanced cervical cancer.
Furthermore, to assess position and benefits of comprehensive genomic profiling in everyday clinical practice of metastatic uterine and locally advanced and metastatic ovarian cancer, the data was collected on a national level from all tested patients. Consequently, by highlighting the need for timely use of precise diagnostic and therapeutic technologies, targeted therapies and immunotherapy, as well as conservative treatment methods, the aim of these articles was to set path for personalized approach to a patient diagnosed with the cancer of female reproductive system.

4. SCIENTIFIC CONTRIBUTION OF PUBLISHED ARTICLES

The articles cover some of the hot topics in oncology recently. Altogether they contribute to the implementation of precision oncology in gynecological cancers which is especially important because they cover it from the perspective of its implementation in countries with lower socioeconomic status with relatively limited healthcare budgets. For example, they question optimal treatment of locally advanced and metastatic cervical cancer. Confirmation of the results from randomized controlled trial with real world data on a national level regarding the use of immunotherapy with bevacizumab in metastatic cervical cancer is particularly important and useful because majority of cases of cervical cancer are from developing countries and significant part of them don`t have bevacizumab as a reimbursed treatment. Meanwhile, the review article is challenging and critically presenting current negative recommendation regarding the use of adjuvant chemotherapy in locally advanced cervical cancer and with that contributes to raising once again the question about its use, emphasizing unequal patient distribution and proper dosage of treatment, with concluding that we need more randomized controlled trials on this matter before the final verdict.

In order to implement precision oncology in gynaecological cancers, the matter addressed in articles is also position of comprehensive genomic profiling early in the diagnostic and treatment procedure of locally advanced and metastatic ovarian and metastatic uterine cancer. Regarding the ovarian cancer, it is important in terms of treatment administration, with loss of heterozygosity and additional significant number of women who could have potential benefit from PARP inhibitors. Also, CGP in patients with metastatic uterine cancer is important because of potential treatment with immunotherapy with checkpoint inhibitors in one third of patients and other targeted therapies in vast majority of patients.

5. PUBLISHED ARTICLES

5.1. Real-World Efficacy and Safety of Bevacizumab in the First-Line Treatment of Metastatic Cervical Cancer: A Cohort Study in the Total Population of Croatian Patients

5.2. Precision Oncology in Metastatic Uterine Cancer; Croatian First-Year Experience of the Comprehensive Genomic Profiling in Everyday Clinical Practice

5.3. Comprehensive Genomic Profiling in the Management of Ovarian Cancer - National Results from Croatia

5.4. Is There a Place for Adjuvant Chemotherapy in the Treatment of Locally Advanced Cervical Cancer?

5.1. Real-World Efficacy and Safety of Bevacizumab in the First-Line Treatment of Metastatic Cervical Cancer: A Cohort Study in the Total Population of Croatian Patients

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Abstract

Background. Although today it is almost preventable, cervical cancer still represents a significant cancer burden, especially in some developing parts of the world. Since the introduction of bevacizumab in the 1st line treatment of metastatic disease, improvements of the outcomes were noted. However, results from randomized controlled trials are often hard to re-create in the real world setting. *Objective.* To assess the real-world efficacy and safety of bevacizumab as a 1st-line treatment of advanced cervical cancer. *Methods.* We conducted a retrospective cohort study on the total population of Croatian patients diagnosed with metastatic cervical cancer from 2016-2019 who were treated with bevacizumab in combination with cisplatin and paclitaxel (TCB) in a 1st-line. The comparison group was the consecutive sample of patients treated with chemotherapy alone. The primary endpoint was overall survival (OS). Secondary endpoints were progression-free survival (PFS), objective response rate, incidence of adverse events and the proportion of treatment discontinuation. *Results.* We enrolled 67 patients treated with TCB, and a control group of 62 patients treated with chemotherapy alone. The TCB cohort had significantly longer unadjusted OS, median 27.0 (95% CI 18.5; not calculable) months compared to 15.5 (10.7; 30.1) months in the chemotherapy-alone cohort. Adjusted OS was not significantly different. PFS was significantly longer for the TCB cohort, median 10.6 (95% CI 8.5; 15.4) months, than for the chemotherapy-alone cohort, median 5.4 (95% CI 3.9; 9.1) months, even after adjustment for baseline covariates ($HR_{adjusted}$=0.60; 95% CI 0.39; 0.94; p=0.027; false discovery rate <5%). *Conclusions.* In a real-world setting, TCB as a 1st-line treatment of metastatic cervical cancer was associated with longer

PFS, better objective disease control rate, and acceptable toxicity profile in comparison to chemotherapy alone. These results may indicate its utility and potential applicability in other parts of the developing world.

Introduction

Cervical cancer ranks fourth in both cancer incidence and mortality among women, with approximately 604,000 newly diagnosed patients and an estimated 342,000 deaths worldwide in 2020. Furthermore, the burden of cervical cancer is not equally distributed. It is less common and less publicly important in developed parts of the world, whereas it is the most commonly diagnosed cancer as well as the leading cause of death in some developing parts of the world [1]. Even though cervical cancer is almost preventable now, due to primary (HPV vaccine) and secondary (screening programs) prevention currently available, unequal implementation and penetration in the different healthcare systems of countries worldwide could be one of the reasons for the aforementioned global inequality [2, 3]. The association of cervical cancer with lower-income areas in general, the fact that it affects a relatively younger population, the high mortality to incidence ratio, and inadequate implementation of existing prevention altogether make cervical cancer one of the major contributors to the global societal burden. The burden of cervical cancer creates an essential need for international intervention aiming to provide every woman worldwide with an equal chance to prevent and optimally treat this "underserved" disease [1, 4]. Unfortunately, a significant number of patients die, specifically, more than 50% of all newly diagnosed patients per year, underlining the absolute need for therapies with better outcomes [1]. Also, it implies the need for research of novel treatment strategies, such as the tyrosine kinase inhibitors (TKIs) targeting angiogenic kinases, mTOR-inhibitors in PIK3CA mutated cancers, or immunotherapy with checkpoint inhibitors in PD-L1 positive cancers [5]. In contrast to other tumor types, where we have recently witnessed significant improvements in the survival of metastatic patients, there were no significant breakthroughs regarding overall survival in the therapy of cervical cancer since the introduction of platinum and ifosfamide as a standard treatment regimen many years ago [6]. Recently, however, the incorporation of bevacizumab as a part of a first-line therapy option, together with cisplatin and paclitaxel as a chemotherapy backbone, has significantly increased the progression-free survival, response rate, and, most importantly, overall survival rate in metastatic or locally recurrent cervical cancer patient populations [7]. Based on the results of a registrational trial (GOG-240), bevacizumab is accepted as the treatment of choice when coupled with TC chemotherapy in the first-line setting of patients with advanced cervical cancer. Notwithstanding the significant results of the study, randomized controlled trials do not presume the same outcomes in the real-world setting when treating patients [8]. This difference in outcomes is possibly due to the absence of strict inclusion and exclusion criteria and, consequently, population diversity with a higher number of patients with comorbidities in real-world practice. Moreover, the organizational approach to regular work-ups and general oncological care, especially in the underserved parts of the world where the majority of cases are diagnosed, explain the difference between outcomes in real-world settings [9, 10]. Therefore, it is important to monitor the real-world efficacy and safety of the given drug to understand its actual use and benefits in everyday clinical practice [11, 12]. Furthermore, this could be tremendously important for cervical cancer, where the burden of the disease is high in less-developed countries, since bevacizumab is a rather expensive drug. Hence, the aim of this study was to assess the real-world efficacy and safety of bevacizumab as a first-line treatment of

advanced cervical cancer in the total population of one of the transitioning countries, namely, Croatia.

Materials and Methods

Study design

We conducted a retrospective cohort study on the total population of patients diagnosed with metastatic cervical cancer between 2016 and 2020 in all Croatian oncology centers who were treated with bevacizumab in combination with cisplatin and paclitaxel backbone chemotherapy (TCB) in first-line therapy since its reimbursement status. The control group was the consecutive sample of patients treated with first-line chemotherapy alone for metastatic disease between 2014 and 2019. We conducted this real-world, multicentric study in six Croatian institutions: University Hospital Center Split, University Hospital Center Zagreb, Sestre Milosrdnice University Hospital Center in Zagreb and their Clinic for Tumors, and University Hospital Centers Rijeka and Osijek. The study was approved by the Ethics Committees of all participating institutions. Informed consent was obtained from all living patients before data collection. &e data were anonymized before the analysis, and the study was conducted in accordance with the World Medical Association Declaration of Helsinki of 1975, as revised in 2013 [13]. The study protocol was not preregistered, nor were the data reviewed centrally.

Participants

The targeted population was patients diagnosed with recurrent, locally advanced, and metastatic cervical cancer who were treated with TCB as a first-line setting from 2016 to 2020, starting from the time of reimbursement of bevacizumab in Croatia. We did not select the sample but collected the data on the total population treated with TCB. We selected a consecutive sample of patients from the control population. We enrolled patients who received first-line combination chemotherapy treatment for locally recurrent or metastatic disease. The sampling was stopped when the control sample size reached the size of the population treated with TCB. Because we planned to enroll the entire targeted population, we did not perform a power analysis before the start of the study.

Endpoints

The primary efficacy endpoint was the difference in overall survival (OS), defined as the time in months since treatment initiation to death from any cause. OS data in living patients were censored at the time of the last data collection. The secondary efficacy endpoints were the differences in progression-free survival (PFS), objective response rate, and disease control rate between the two cohorts. PFS was defined as the time in months since the initiation of therapy to the progression of the disease from any cause. PFS data in patients alive with no progression were censored at the time of the last exam. The objective response rate was estimated in compliance with the RECIST version 1.1 criteria as stable or progressive disease and partial or complete response. The disease control rate included complete and partial response and stable disease. Secondary safety endpoints were the incidence of treatment-related haematologic, nonhaematologic, or any adverse events of any grade and of grade 3 or 4 and the proportion of patients whose treatment was discontinued to control the adverse events. We defined the grades of adverse events according to the Common Terminology Criteria for Adverse Events v5.0 [14].

Treatment

Patients were administered the new standard line treatment for metastatic cervical cancer: TC chemotherapy protocol, which consisted of cisplatin at a dose of 50 mg per square metre of body surface area plus paclitaxel at a dose of 175 mg/m^2 and bevacizumab at a dose of 15 mg per kilogram of body weight. The therapy was administered at 21-day intervals until disease progression, unacceptable toxicity or complete response was noted. The control group of patients received the existing standard treatments for metastatic cervical cancer according to the physician's choice. The most common chemotherapy protocol used was TC with cisplatin at a dose of 50 mg per square metre of body surface area or carboplatin AUC 5 or 6 plus paclitaxel at a dose of 175 mg/m^2. Other protocols used were the combination of cisplatin at a dose of 100 mg/m^{2}applied on day 1 and 5-fluorouracil at a dose of 1000 mg/m^2 applied on days 1-5 of every 28-day cycle, the combination of ifosfamide 2000 mg/m^2 plus cisplatin 75 mg/m^2 every 21-day cycle, the combination of topotecan at a dose of 0.75 mg/m^2 on days 1-3 plus paclitaxel at a dose of 175 mg/m^2 on day 1 of every 21-day cycle and the combination of cisplatin at a dose of 70 mg/m^2 applied on day 1 and gemcitabine at a dose of 1250 mg/m^2 applied on days 1 and 8 of every 21-day cycle.

Statistical analysis

We performed all analyses in the population of patients who received at least one dose of 1st-line treatment for metastatic disease. We estimated the median OS and PFS using the Kaplan-Meier method with 95% confidence intervals (CIs). To assess the significance of differences in OS and PFS between the two cohorts, we used a two-sided log-rank test in the bivariable analysis and Cox proportional hazard regression in the multivariable analysis with adjustment for age at diagnosis, histology, ECOG performance status before the introduction of 1st-line treatment for metastatic disease, previous treatment with chemotherapy, and previous treatment with radiotherapy. We handled ties using the Efron method. To check the proportional hazard assumption, we assessed the consistency of the log HR over time by testing the non-zero slope of the generalized linear regression of the scaled Schoenfeld residuals on row time and on the log-time. We visually inspected the parallelism of log-log survival plots in the two cohorts. We calculated the significance of the differences between the two study groups in the objective response rate and safety outcomes using the chi-square (X^2) test. In the analysis of safety endpoints, we calculated relative risk with 95% CIs, and in the multivariable analysis we adjusted the relative risks for the treatment duration and for the aforementioned covariates using a Poisson regression with a robust variance estimator. We declared all missing data below the tables, and we did only the available cases analysis ("pairwise deletion") although we had no proof that the data were missing completely at random. We did not use any imputation method because the number of missing data was relatively low. We set two-tailed statistical significance at $p<0.05$ and calculated all CIs at the 95% level. We controlled the false positive rate using the Benjamini-Hochberg procedure with the false discovery rate set in advance at FDR<5%. We performed the statistical data analysis using StataCorp 2019 (Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

Results

We enrolled 67 patients diagnosed with metastatic cervical cancer who were treated with TCB in the 1st-line setting and 62 who were treated with chemotherapy alone. The two cohorts were of comparable age (Table 1), menopausal status, and body mass index. However, the TCB cohort had

a markedly better ECOG performance status before the initiation of 1st-line treatment for metastatic disease and less often had squamous cervical cancer or previous treatment with chemotherapy and radiotherapy. The duration of the 1st-line treatment of metastatic disease was somewhat longer in the TCB cohort. Overall, the median follow-up was 14.5 (interquartile range; IQR 8.6-20.5) months in the TCB cohort and 10.9 (3.9-26.4) months in the chemotherapy-alone cohort. The longest follow-up in the last recruited patients was 43.6 months in the TCB cohort and 50.1 months in the chemotherapy-alone cohort.

Efficacy endpoints

The median OS was 27.0 (IQR 18.5-not calculable) months in patients treated with TCB and 15.5 (IQR 10.7-30.1) months in the chemotherapy-only cohort (Table 2; Fig 1). This difference was statistically significant (log-rank test; X^2=5.05; p=0.025; FDR<5%). The unadjusted hazard ratio for death, with the chemotherapy-only cohort as the reference cohort, was HR=0.56 (95% CI 0.34 to 0.93); p=0.027; FDR<5%. After adjustment for age at diagnosis, histology, ECOG performance status before the introduction of 1st-line treatment for metastatic disease, previous treatment with chemotherapy and previous treatment with radiotherapy, the hazard ratio for death was no longer significant: HR=0.78 (95% CI 0.44 to 1.38); p=0.389; FDR>5%.The median PFS from the initiation of 1st-line treatment for metastatic disease was 10.6 (95% CI 8.5; 15.4) months in the TCB cohort and 5.4 (95% CI 3.9 to 9.1) months in the chemotherapy-only cohort (log-rank test, X^2=6.54; p=0.011; FDR<5%) (Table 2, Fig 1). The unadjusted HR was 0.60 (95% CI 0.41 to 0.89; p=0.011; FDR<5%), and the adjusted HR was 0.60 (95% CI 0.39 to 0.94; p=0.027; FDR<5%). Objective response rate, including complete response, and partial response, was not significantly higher in the cohort treated with TCB, 38/67 (57%), than in the cohort treated with chemotherapy alone, 24/59 (41%) (Chi-square test; $X^2(1)$=3.23; p=0.072; FDR>5%). The disease control rate, including complete response, partial response and stable disease, was significantly higher in TCB cohort, 52/67 (78%), than in the chemotherapy-alone cohort, 30/59 (51%) (Chi-square test; $X^2(1)$=9.89; p=0.002; FDR<5%).

Safety endpoints

The proportion of patients whose treatment was discontinued because of toxicity was not significantly different between the two cohorts (Table 2). Treatment discontinuation was experienced by 11/59 (19%) patients treated with TCB and 9/55 (16%) patients treated with chemotherapy alone (Chi-square test; $X^2(1)$=0.10; p=0.749; FDR>5%). Patients treated with TCB had significantly lower risk for treatment-related haematologic adverse events (RR=0.60; 95% CI 0.40; 0.90; p=0.007; FDR<5%). The relative risk remained significant after the adjustment for age at diagnosis, histology, ECOG performance status before the introduction of 1st line treatment for metastatic disease, previous treatment with chemotherapy, previous treatment with radiotherapy, and duration of the first-line treatment of metastatic disease using TCB or chemotherapy-alone (adjusted RR=0.51; 95% CI 0.32; 0.81; p=0.004; FDR<5%). The risk for non-haematologic treatment-related adverse events was not significantly different between the two cohorts (Table 2).

Discussion

The discrepancy in the numbers of diagnosed and successfully treated patients regarding the human development index (HDI) of countries, as well as within countries between developed and less-developed areas, puts cervical cancer patients in a rather “underserved” position [4, 15]. This is also

supported by the disparity in research funding among different cancer types. For instance, the parallel can be drawn with breast cancer, which is the most common cancer diagnosed among women. Breast cancer is 4 times more prevalent than cervical cancer but only contributes two times higher to cancer mortality, most likely due to the more than seven times higher research funding investment with a consequently significantly higher number of multiple treatment modalities [1, 16, 17]. Additionally, recent social network analysis has shown that breast cancer is the most frequent keyword used, representing 15% among all keywords, while cervical cancer is used only 2% of the time [18]. It is evident, from the aforementioned information about cervical cancer, that further efforts are needed in the promotion of primary and secondary prevention. Furthermore, enhancement of the existing treatment modalities is needed especially in the second-line setting considering that there is no standard treatment established and that the outcomes are still rather poor and such patients should be considered early for clinical trials regarding novel treatment strategies [19]. However, several significant improvements have been made considering the treatment of locally advanced disease as well as the treatment of metastatic disease with the application of TCB [7, 20]. Since the introduction of bevacizumab as a firstline treatment and the significant improvement in median OS by 3.7 months (HR 0.71), several studies have been conducted to assess its efficacy and safety in the real-world setting [7]. Among the first ones were studies conducted in Spain, Argentina, and British Columbia, and although all of them have shown outcomes from real-world bevacizumab similar to those from the registrational trial, there was no control group. &e previous studies were also conducted on a relatively small number of patients from single institutions and with a short period of median follow-up [21–23]. Recently, three studies were conducted in three different centers in China on a larger number of patients and with a control group. Two of these studies had similar results and toxicity profiles to the registrational trial [24, 25]. Meanwhile, the primary outcome of the third study was to assess the toxicity rate, and despite the benefit of bevacizumab, the combined treatment was not well tolerated due to higher grades of neutropenia, gastrointestinal fistula, and hypertension [26]. It is important to emphasize here that all three studies were performed in single institutions, leading to the potential bias of single institution quality of care on the presented outcomes. The results at the national level, with all patients treated included, define the “real” real-world evidence. Our results in the total Croatian population showed significantly higher PFS and OS among patients treated with TCB in comparison to the control group treated with chemotherapy only. Furthermore, our results have shown higher PFS and OS in comparison to the mentioned studies, in both their length and improvements such as considering control groups. Additionally, the toxicity profile of TCB in our patients was closest to the one from GOG-240, meaning that there was a higher incidence of hypertension and neutropenia, but it did not affect the treatment course or require significant therapy discontinuations. Considering the costs of bevacizumab treatment, the question arises about its cost-effectiveness for application in everyday clinical practice, especially in challenging financial medical environments where many cases are diagnosed. Our real-world study defines bevacizumab efficacy benefits similar to or above those from the registrational trial. Taking into account that results from randomized phase III trials are often difficult to repeat in general everyday clinical practice, strong recommendations should be made for all new drugs and treatments to be reviewed regarding their clinical benefit in terms of retrospective analysis in different setups, preferably on the country level and within different healthcare systems [8]. Recently, the loss of patent rights for bevacizumab (Avastin) has led to a significantly reduced price of the drug and thus better affordability in many healthcare systems. Our study, together with other real-world studies and the registrational trial, defines the true clinical

significance for bevacizumab in the therapy of recurrent or metastatic cervical cancer. While cost-benefit analysis in a developing world was questionable with rather high price of bevacizumab, recent loss of patent rights can, and most probably will, make this treatment more affordable to many underserved patients with recurrent or metastatic cervical cancer. Furthermore, estimation of the "Years of Life Lost" considering that cervical cancer affects a relatively younger population defines even more cervical cancer as underfunded and with absolute need for better and more affordable treatments [27, 28]. Hence, in addition to investment in primary and secondary prevention, investment in affordable treatments with significant clinical benefit should be supported on many different levels and should be given to otherwise underserved patients in many countries around the world.

Limitations of the study

The first limitation of our study was the lack of randomization into two study groups. For this reason, we cannot reliably rule out the effects of different unmeasured confounders, and the internal validity of our findings is lower than that in randomized controlled trials. At the same time, the real-world setting is the main strength of our study and the cornerstone of its generalizability to real-life populations. The second limitation was the larger number of missing data points for some variables. Data are routinely collected with different levels of rigor, reliability, and precision, and we could not control the basic qualities of electronic records. To minimize the negative effects of these limitations, we carefully collected all the data, checked for their inconsistencies, and cross-checked the suspicious entries in different records. The third limitation was the difference in the proportion of missing data between the two cohorts, although this difference was not large. We had more missing data in the cohort treated with chemotherapy alone than in the cohort treated with TCB. This was partially due to the different regulatory requirements for the recommendation of bevacizumab and other therapies and, consequently, the different levels of comprehensiveness of routinely collected data for patients treated with these two regimens. The fourth limitation was that we selected a consecutive and not the random sample of patients from the control population treated with chemotherapy alone; this could increase the risk of selection bias, but we cannot speculate about the direction or magnitude of the so-caused bias.

Conclusions

In the real-world setting, bevacizumab utilized as a first-line treatment for metastatic cervical cancer was associated with longer OS and PFS, a better objective disease control rate, and a similar toxicity profile to chemotherapy alone. These results may indicate its utility and potential cost-effectiveness in other parts of the developing world.

Table 1. Characteristics of patients and treatment

	Chemotherapy + bevacizumab (n=67)	Chemotherapy alone (n=62)
Age at diagnosis (years), median IQR)	51 (45-60)	56 (46-61)
Menopause, n (%)	45 (67)	38 (61)
Histology, n (%)		
squamous	46 (69)	51 (82)
adenocarcinoma	16 (24)	7 (11)
other	5 (8)	4 (6)
ECOG performance status, n (%) [a]		
0	38 (57)	15 (25)
1	24 (36)	29 (48)
2	5 (7)	16 (27)
Body mass index (kg/m^2), median (IQR)[b]	24 (22-29)	24 (22-27)
Previous treatment, n (%)		
chemotherapy	44 (66)	47 (76)
radiotherapy	45 (67)	48 (77)
Duration of targeted treatment (months), median (IQR)	4.3 (2.8-8.0)	3.7 (2.0-5.5)
Number of cycles, median (IQR)[c]	6 (5-11)	6 (3-6)
Follow-up (months), median (IQR)	14.5 (8.6-20.5)	10.9 (3.9-26.4)

Abbreviation: IQR = interquartile range (range between the 25th and 75th percentiles)

[a] ECOG performance status was missing for 2/62 (3%) patients treated with chemotherapy alone; [b] Body mass index was missing for 1/67 (1%) patients treated with bevacizumab and for 5/62 (8%) patients treated with chemotherapy alone; [c] Number of cycles was missing for 3/62 (5%) patients treated with chemotherapy alone

Table 2. Efficacy and safety assessment

	Chemotherapy + bevacizumab (n=67)	Chemotherapy alone (n=62)	p
Efficacy endpoints			
PFS (months), median (95% CI)	10.6 (8.5; 15.4)	5.4 (3.9; 9.1)	0.011*
Unadjusted HR (95% CI)	0.60 (0.41; 0.89)	1.00 referent	0.011*
Adjusted HR (95% CI) [a]	0.60 (0.39; 0.94)	1.00 referent	0.027*
OS (months), median (95% CI)	27.0 (18.5; n.c.)	15.5 (10.7; 30.1)	0.025*
Unadjusted HR (95% CI)	0.56 (0.34; 0.93)	1.00 referent	0.027*
Adjusted HR (95% CI) [a]	0.78 (0.44; 1.38)	1.00 referent	0.389
Objective response, n (%)[b]			
complete response (CR)	12 (18)	11 (19)	0.013*
partial response (PR)	26 (39)	13 (22)	
stable disease (SD)	14 (21)	6 (10)	
progressive disease (PD)	13 (19)	21 (36)	
could not be determined	2 (3)	8 (14)	
Objective response rate, n (%) [b]	38 (57)	24 (41)	0.072
Disease control rate, n (%) [c]	52 (78)	30 (51)	0.002*
Safety endpoints			
Treatment discontinuation because of toxicity, n (%) [d]	11 (19)	9 (16)	0.749
Treatment-related adverse events			
any grade	54 (81)	53 (85)	0.461
grades III-IV	33 (49)	41 (66)	0.053
Treatment-related, haematologic adverse events			
any grade	44 (66)	53 (85)	0.009*
grades III-IV	18 (27)	31 (50)	0.007*
Treatment-related, non-haematologic adverse events			
any grade	51 (76)	50 (81)	0.533
grades III-IV	22 (33)	21 (34)	0.901

Abbreviations: CI = confidence interval; PFS = progression-free survival; OS = overall survival; HR = hazard ratio; n.c. = not calculable

[a] Analysis was adjusted for: age at diagnosis, histology, ECOG performance status before the introduction of 1st-line treatment for metastatic disease, previous treatment with chemotherapy, previous treatment with radiotherapy; [b] Objective response rate includes complete and partial response; data were missing for 6/67 (10%) patients treated with TCB and 3/62 (5%) patients treated with chemotherapy alone; [c] Disease control rate includes complete and partial response and stable disease; [d] Data on treatment discontinuation because of toxicity were missing in 8 (12%) patients treated with TCB, and 7 (11%) patients treated with chemotherapy alone

* False discovery rate <5%

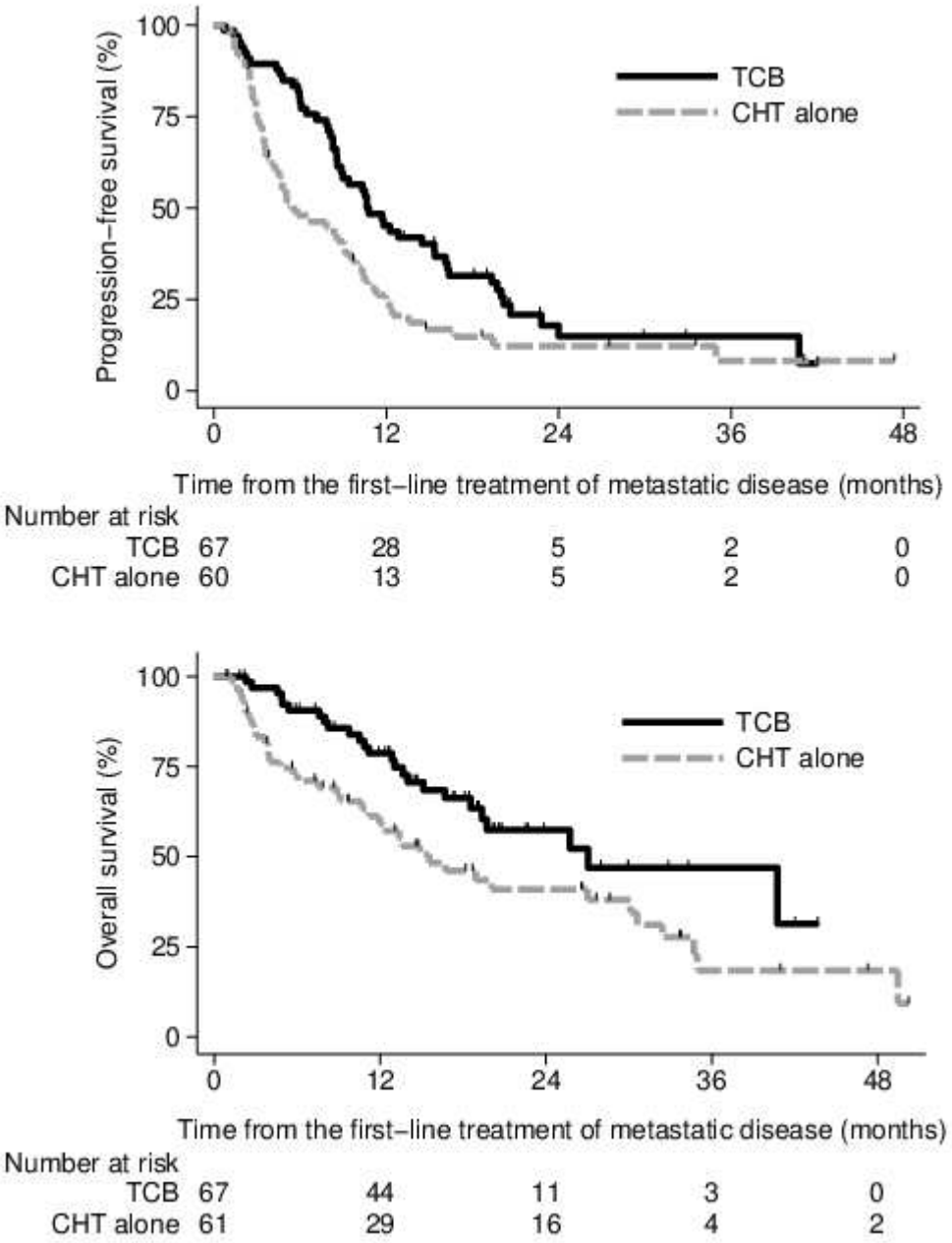

Figure 1. Kaplan-Meier curves of progression-free survival and overall survival from the introduction of first-line treatment for metastatic cervical cancer; TCB=bevacizumab in combination with cisplatin and paclitaxel backbone chemotherapy; CHT = chemotherapy

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

Dora Čerina: Speaker fees: Roche; **Višnja Matković:** Speaker fees and consulting: Astra Zeneca, Roche, Amgen, Stada; **Kristina Katić:** Speaker fees: Roche, AstraZeneca; **Ingrid Belac Lovasić:** Speaker fees and consulting: Amgen, Astellas, Astra Zeneca, BMS, Merck, Novartis, Pfizer, Roche, Sanofi, MSD; **Robert Šeparović:** Speaker fees and consulting: Pfizer, Novartis, Roche, MSD, Johnson & Johnson, Amgen, Pharmaswiss; **Ivana Canjko:** Speaker fees: Roche, MSD, Abbot, Takeda, Stada, Alvogen, Boehringer Inn, AstraZeneca; **Blanka Jakšić:** Speaker fees and consulting: Roche, Sanofi, Abbott, Alvogen, Sandoz, Janssen Cilag, Pliva; **Branka Petrić Miše:** Amgen, Astra Zeneca,BMS, Novartis, Pfizer, Roche, Sanofi, and MSD; **Marijo Boban:** Speaker fees and consulting: Abbott, Amgen, AstraZeneca, BMS, Boehringer Ingelheim, Merck, MSD, Pfizer, Roche, Servier, Takeda; **Eduard Vrdoljak:** Support for clinical trials and scientific projects: Pfizer, Roche, BMS, AZ, Speaker fees and consulting: Amgen, Astellas, Astra Zeneca, Boehringer Ingelheim, Johnson & Johnson, Novartis, Pharmaswiss, Pfizer, Roche, Sanofi, MSD, Merck; **Other authors** have no conflict of interest to declare.

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5.2. Precision Oncology in Metastatic Uterine Cancer; Croatian First-Year Experience of the Comprehensive Genomic Profiling in Everyday Clinical Practice

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Running title: Comprehensive genomic profiling of uterine cancer

Abstract

Comprehensive genomic profiling (CGP) is gradually becoming an inevitable part of the everyday oncology clinical practice. Its interpretation and optimal implementation of the results is one of the hot topics of the modern-day oncology. According to the recent findings, uterine cancer harbors a high level of gene alterations, but is still insufficiently explored. The primary goal of this project was to assess a proportion of patients with targetable mutation. Also, the aim was to define and emphasize potential opportunities as well as the problems we have faced in the in the first year of testing on the national level. We performed a multicentric, retrospective, nested cross-sectional analysis on the total population of Croatian patients with advanced/metastatic uterine cancer on whose tumors CGP was performed during 2020. CGP on 32 patients' tumor tissue revealed clinically relevant genomic alterations (CRGA) in 27 patients (84%) with a median of 3 (IQR 1-4) CRGA per patient. The most common CRGAs were those of phosphatide-inositol-3 kinases (PIK3) in 22 patients (69%), with 13/22 (59%) of those patients harboring PIK3CA mutation. The next most common CGRAs were ARID1A and PTEN mutations in 13 (41%) and 11 (34%) patients respectively. Microsatellite status was determined as stable in 21 patients (66%) and determined as highly instable in 10 patients (31%). High tumor mutational burden (≥10Muts/Mb) was reported in 12 patients (38%). CGP analysis reported some kind of targeted therapy for 28 patients (88%). CGP

determined clinically relevant genomic alterations in significant majority of patients with metastatic uterine cancer defining it as rich ground for further positioning and development of precision oncology.

Keywords: uterine cancer, precision oncology, genomic profiling, mutation

Introduction

Revolutionary advancement of diagnostics through optimal implementation of informational technologies and development of bioinformatics, combined with better understanding of the human genome and discovery of the comprehensive genomic testing, has led towards more individualized and targeted approach to the patient, making the first half of 21st century a time of the paradigm shift in the establishment of postulates of precision medicine. Consequently, dramatic changes are about to happen when approaching the patient, with taking into consideration his/her known gene alterations when choosing the treatment and their impact on response to it, comorbidities, general condition, as well as other aspects of an individual such as the lifestyle and environmental factors, and altogether with the aim to create optimal treatment strategy for every patient individually. Oncology, as one of the most propulsive branches of medicine, represents the most fruitful ground for implementation of precision medicine in everyday clinical practice. Definition of underlying causes of carcinogenesis and progress in the field of molecular biology has enabled development of novel treatment approaches such as molecular-targeted therapy and immunotherapy with improved outcomes and impact on patient`s survival. For instance, molecular-targeted therapy is already golden standard as the first line treatment in advanced or metastatic non-small cell lung cancer (NSCLC) [1], melanoma [2], gastrointestinal stromal tumor (GIST) [3] or as maintenance therapy in recurrent ovarian cancer [4]. On the other side, immunotherapy with checkpoint inhibitors is becoming the standard of care for many cancer types, such as skin [5], lung [6], renal [7] or bladder cancer [8], and immunotherapy against specific antigens is standardized as treatment for early or metastatic HER-2 positive breast cancer [9, 10], metastatic colorectal [11], gastric [12], ovarian [13] or cervical [14] cancer. Despite the above mentioned, conservative systemic approach is still the only treatment option for some human malignancies, including uterine cancer, which alongside cervical cancer, remains the only entity with worsened overall survival in the USA during the last 20 years [15]. Uterine cancer ranks first in incidence among invasive tumors of female reproductive system in the developed countries due to its association with older age, better socio-economic status and unopposed estrogen activity [16]. Unfortunately, 15-20% of patients presents with or progress to metastatic disease with 5-year survival rate of 16% [17]. As previously mentioned, the main treatment strategy for metastatic uterine cancer is chemotherapy or hormonal therapy with less than 12 months of the median overall survival [18]. According to the TCGA (The Cancer Genome Atlas) project in 2013, uterine cancer/endometrial cancer is divided into four subgroups based on the genomic profiling of 373 endometrial cancer specimens (POLE ultra-mutated, microsatellite instability group, copy number low (CNL) and copy number high (CNH) group)[19]. POLE ultra-mutated group which consisted of 7% of tumors and microsatellite instability group of tumors (28% of tumors) are candidates for immunotherapy due to high neoantigen load and consecutively optimal tumor microenvironment for enhanced cytotoxic T-cell response [19]. Improvement in outcomes of the CNL group (39% of tumors) may be in combination of hormonal therapy and

PI3K/AKT/mTOR pathway inhibitor and for the CNH serous-like group (26% of tumors) treatment with cell cycle regulators and the PI3K/AKT/mTOR pathway inhibitors [19]. At the end of 2019 comprehensive genomic profiling (CGP) provided by Foundation Medicine Inc. (FMI) became reimbursed in Croatia [20].

Considering the fact that uterine cancer harbors high level of gene alterations but is still insufficiently explored, the fact that it is ranked 4thin cancer incidence in Croatia with 778 women diagnosed annually and mortality to incidence ratio of 0.26 [21], here we present the first year CGP data on a country level for patients with newly diagnosed metastatic uterine cancer or whose initial disease had progressed during 2020. The primary goal of this project was to assess a share of patients with opted targetable mutations, while secondary was the proportion of patients who have started with the CGP-guided therapy. Also, by defining and emphasizing potential opportunities as well as the problems we are facing in the precision oncology development and implementation of this specific field, the aim was to affirm CGP of patients with metastatic uterine cancer in everyday clinical practice.

Methods

Project design

We performed a multicentric, retrospective, nested cross-sectional analysis on the total population of Croatian patients who were either newly diagnosed with metastatic uterine cancer or whose initial disease has progressed from January 1 to December 31, 2020, and on whose tumors CGP was performed. This analysis was nested within the baseline measurement of the cohort study aimed to assess the real-world utility of CGP, a next-generation sequencing approach that detects novel and known variants of the four main classes of genomic alterations and genomic signatures in order to provide prognostic, diagnostic and predictive insights that inform research or treatment decisions for individual patients across all cancer types. The obtained tumor specimen was sampled from surgery or biopsy of the primary disease or metastases and the formalin-fixed, paraffin-embedded tissue for the analysis was sent as a block and one hematoxylin and eosin stained slide or 10 unstained slides with one hematoxylin and eosin stained slide. Minimal surface area was 25 mm^2 and minimal tumor content was 20%, while optimal was 30% of tumor nuclei, defined as the number of tumor cells divided by total number of all cells with nuclei. In case of additional immunohistochemistry for PD-L1, 4 supplementary unstained slides were requested. The majority of CGP analysis was done through FoundationOneCDx and only for one patient with sarcoma FoundationOneHeme was performed and it was carried out in a Clinical Laboratory Improvement Amendments certified, College of American Pathologists accredited laboratory (Foundation Medicine Inc., Cambridge, MA, USA). Once the DNA was extracted, 50-1000 ng underwent whole-genome shotgun library construction and hybridization-based capture in order to detect alterations of 324 genes in total, of which 304 exons related with tumors, one promoter region, one non-coding RNA and certain regions of introns in 34 frequently rearranged genes in tumors, as well as determination of genomic signatures, such as tumor mutational burden (TMB) and microsatellite status. Illumina® HiSeq 4000 was used to sequence hybrid capture-selected libraries to high uniform depth. The typical median depth of coverage was >500x with >99% of exons at coverage >100x. The sequenced regions were analyzed for four different types of alterations- base substitution, deletion or insertion, copy number variation and gene redistribution in a group of genes

associated with the tumor development. The microsatellite status was based on genome wide analysis of 95 microsatellite loci, while TMB was determined by counting all synonymous and non-synonymous variants present at 5% allele frequency or greater and total number was presented as mutations per megabase (Muts/Mb) unit [22, 23, 24]. Depending on the results, patients were potentially administered CGP-guided therapy after progression to or unacceptable toxicity of the standard of care first-line or second-line systemic therapy and without having any approved or reimbursed therapy options for the treatment, in accordance to the multidisciplinary team`s decision. If patients were administered with CGP-guided treatment, the records of the course of the treatment were collected, as well as the occurrence of side effects and the patient's overall response. Also, there was radiological evaluation at the two-month intervals, in order to assess the effects of the targeted therapy and to make decision on its continuation or termination.

This analysis of real-world data was conducted in six Croatian institutions: University Hospital Centre Split, University Hospital Center Zagreb, Sestre Milosrdnice University Hospital Centre in Zagreb and their Clinic for Tumors, University Hospital Centers Rijeka and Osijek. The project was approved by Ethics Committees of all participating institutions. The informed consent was obtained from all patients before the data collection. Moreover, all patients signed the informed consent for the CGP analysis via FMI. The data file was anonymized before the analysis and the project was performed in accordance with the World Medical Association Declaration of Helsinki of 1975 as revised in 2013 [25].

Participants

The targeted population were patients initially diagnosed with metastatic uterine cancer or whose disease has progressed from initially diagnosed local or locoregional disease and on whose tumors CGP was performed in 2020. We planned to include the entire population of patients with metastatic uterine cancer who fulfilled the CGP criteria defined by Croatian Oncology Society: sufficient tissue for the CGP, good general health (ECOG performance status ≤2) and at least 12 months of life expectancy [20]. Hence, we did not perform the power analysis before the project start. Patients were administered with the first or second-line standard of care treatment for metastatic uterine cancer, chemotherapy or hormonal therapy, depending on their general condition, other comorbidities and physician`s choice. CGP-guided therapy was potentially administered after progression to or unacceptable toxicity of the standard of care first or second-line systemic therapy and without having any approved or reimbursed therapy options for the treatment, and in accordance to the multidisciplinary team decisions.

Endpoints

The primary endpoint was the proportion of patients having clinically relevant genomic alterations, defined as those with approved targeted therapy in patients' tumor type or approved in other tumor type, or with existing clinical trials available. The secondary endpoint was the proportion of patients having targetable mutations receiving designated therapy.

Statistical analysis

We described the data by percentages, medians and interquartile ranges (IQR) using StataCorp 2019 (Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

Results

Description of patients and previous therapy

During 2020, 32 patients with metastatic uterine cancer were presented to multidisciplinary teams and CGP was performed on their tumor tissue specimens. Median age was 65 (IQR 59-68) years with a total range from 44 to 79 years (Table 1). Majority of patients, 25 (78%) were in good general condition with ECOG performance status 0. The most common histological subtype was endometrial adenocarcinoma in 16 patients (50%). All patients received either chemotherapy or hormonal therapy as standard treatment for metastatic uterine cancer. Median number of prior lines of therapy for metastatic disease was 2 (IQR 1-3) (Table 1). Most common chemotherapy protocol used as first line treatment was combination of paclitaxel and carboplatin. While hormonal therapy comprised of megestrol acetate in the first line setting and then aromatase inhibitor afterwards.

Comprehensive genomic profiling

By CGP we found at least one genomic alteration (GA) in 31 (97%) of specimen. Clinically relevant genomic alterations (CRGA) were detected in 27 patients (84%) with a median of 3 (IQR 1-4) CRGA per patient (Table 2). The most common CRGAs reported were those of phosphatide-inositol-3 kinases (PIK3) in 22 patients (69%), with 13/22 (59%) of those patients harboring PIK3CA mutation. The next most common CGRAs were ARID1A and PTEN mutations in 13 (41%) and 11 (34%) patients respectively (Table 2~~, Figure 1~~). Thirty patients (94%) had genomic alterations without clinical significance with a median of 3 (IQR 1-5) GA per patient. The most common GA without clinical significance was TP53 mutation, reported in 15 patients (47%). Microsatellite status was determined as stable in 21 patients (66%) and determined as highly instable in 10 patients (31%). Median tumor mutational burden (TMB) was 5 (IQR 2-18) mutations per megabase (Muts/Mb) with the total range from 0 to 40. High TMB (≥10Muts/Mb) was reported in 12 patients (38%). After analysis of all CGP reports and all detected GA, some kind of targeted therapy was reported for 28 patients (88%), while for 4 patients (13%) there was no reportable therapeutic option. Targeted therapy approved for the patients' tumor type (on-label therapy) was reported in 1 patient (3%), while targeted therapy approved in other tumor type based on patients GA (off-label therapy) was reported in 26 patients (81%). Furthermore, targeted therapy without approval but also driven by patients GA was reported in 24 patients (75%). The vast majority of alteration-driven therapies encompassed those included in DNA repair such as PARP inhibitors, PI3-K/mTOR (phosphoinositide-3 kinase/mammalian target of rapamycin) and Ras/Raf/MEK (mitogen-activated protein kinase) inhibitors or immune check-point inhibitors (Table 3). Most common targeted therapies opted were mTOR inhibitors and immune check-point inhibitors. Four patients (12.5%) who have had disease progression on the given standard therapy and without further therapeutically valid options, the CGP-guided targeted therapy was opted based upon the MDT decision and compassionate use program availability.

Discussion

Results from the CGP analysis in our project have shown that vast majority of patients with metastatic uterine cancer harbors at least one genomic alteration, out of which significant proportion was clinically relevant. In contrast to the conventional testing, which by single-target assays,

discovers potentially one actionable gene alteration, comprehensive genomic profiling (CGP), by next-generation sequencing gives the detailed insight in tumor gene specifics and brings new dimension to the treatment options of every cancer patient, hence causing personalized and precise medicine to evolve. Consequently, CGP is gradually being integrated in the diagnostic workup of the different tumor types as a backbone diagnostic tool. However, questions that have arisen with CGP like cost, utility and clinical benefit and patients' and societal expectations were some of the hot topics during recent years [26–29]. As previously mentioned, molecular-targeted therapy is already established as a standard treatment in many tumor types, while its use and value outside of current indications is still under investigation. Clinical studies such as MOSCATO trial [30] have shown improved outcomes but only in the minority of "hard-to-treat" patients, while phase 2 SHIVA trial discourages the use of "off-label" molecular-targeted therapy due to unimproved progression free survival comparing it to the conventional treatment [31]. However, SHIVA trial was criticized for potential biases due to its design as well as targeted therapy that was administered either as monotherapy in patients with several molecular alterations or was incorrectly matched for some patients [32]. On the other hand, several studies have shown favorable effects of the use of "off-label" molecular-targeted therapy with improved and almost doubled response rates and progression free survivals [33–37]. Meanwhile, the number of in-human studies regarding the dose-escalation of targeted therapy, for instance phosphatidylinositol 3-kinase α-selective inhibitor alpelisib in patients with specific mutation such as PIK3CA, is rapidly increasing lately [38]. In addition, new diagnostic approaches led towards discovery of tumor genomic signatures such as microsatellite instability and TMB, and these are so-called "tumor agnostic" biomarkers for which FDA (Food and Drug Administration) approved immunotherapy regardless of cancer type. Despite abovementioned turmoil about the cost of CGP, it is strongly encouraged, especially in the low income countries, to do, if CGP is not available, less expensive but equally informative tests, such as immunohistochemistry staining for mismatch repair status (MMR protein staining) [39]. What we have at work today in oncology is the fact that we have more diagnostic capabilities than ever (like CGP), more and more precise drugs and, contradictorily, less and less valid evidence for their use in an individual patient. Furthermore, with expected, even more precise, granular approach to the single patient and her/his tumor we would, most probably end up with situation that classical clinical trials would not be able to address the needs of further development of oncology science. Consequently, real-world data, learning from every patient experience and every tumor specificity is about to become the backbone for the further researches in the field of precision oncology in all tumor types together and especially in subtypes driven by targetable biomarkers.

Our results have shown high mutation load of uterine cancer with at least one genomic alteration found in almost every patient tested, which is in accordance to the previous observations [40, 41]. Furthermore, a vast majority of patients (84%) had clinically relevant genomic alterations and the most common were PIK3CA, ARID1A and PTEN, which is similar to the existing findings of 93% [40] and 91% [41]of CRGAs, as well as the prevalence of the alterations. Both studies have shown potential clinical benefit from the administered CGP-guided therapy. However, study from Rodriguez-Rodriguez et al. observed targeted therapy in ovarian and uterine cancer, with only 25 patients with uterine cancer of all stages included in the study. Also, only nine patients were treated in accordance to the CGP with observed stable disease in two patients and partial response in four patients, but the treatment regimen was not stated [40]. On the contrary, study from Prendergast et al. included 74 patients with recurrent endometrial cancer with median age of patients of 61 year

and median number of prior chemotherapy lines 2 (range 1-4). The results of their study showed median number of CRGAs of 3 (range 0-7), MSI-high status reported in 18% of patients and median TMB of 24.3 (range 11.2-48) Muts/Mb per patient. Also, 24/74 (32%) patients have received a matched therapy according to the CGP results which consisted in the majority of patients of agents targeting PI3K/PTEN/mTOR pathway and immunotherapy (pembrolizumab). Objective responses were seen in 25% of patients, while nine patients have achieved stable disease with median duration of treatment 14.6 months and two out of six patients treated with immunotherapy have shown partial response, while others had stable disease and the median duration of the treatment was 17 months [41]. Although the study has several limitations, such as the small number of patients, comprehensive genomic profiling on archival specimens, not considering the tumor heterogeneity or possible changes of the molecular subtype of the recurrent endometrial cancer and only third of patients receiving targeted therapy, it is the first study that links CGP with clinical benefit in the patients diagnosed with recurrent endometrial cancer and suggests its potential benefit in the routine everyday clinical practice [41].

Our cross-sectional data of all tested patients on the country level have shown similarities with the results in the aforementioned studies. However, being the pilot year of the testing, there was only a small number of patients in general and particularly those receiving targeted therapy without enough time-length to assess its impact on the response or survival outcomes. Furthermore, there is discrepancy in the number of patients tested in each institution defining the learning curve in the new technology adaptation and potentially different approach to the value of CGP and its clinical use today. Moreover, different penetration of CGP in everyday clinical practice could be due to the different patient distribution, places of surgery and availability of archived or fresh tissues, as well as the organizational issues. Despite the above-mentioned limitations, majority of positive results speak in favor of our primary goal and have shown utility of CGP in everyday clinical practice of the patients diagnosed with metastatic uterine cancer. Also, our results show good compliance to the established protocol and adherence to the inclusion criteria for the comprehensive genomic profiling on the country level. The number of treated patients with uterine carcinoma in our analysis is rather small defining the same problem seen in other studies, lack of organized, structured approach to the CGP driven therapy. Namely, with existing health insurance setups in majority of countries and level of partnership between governmental administration and pharma industry it is difficult to foresee the faster and better implementation of treatment part of precision oncology development. We need more partnership as well as absolute monitoring and informing about performance of the given therapies according to the CGP at singe patient level. Considering that referred are the nested cross-sectional data, results of the treatment of our patients will be prospectively monitored during next two years and the outcomes of the precision oncology approach in the metastatic uterine cancer therapy will be carefully analyzed in the future.

In conclusion, our country based real-world data of the comprehensive genomic profiling of patients with metastatic uterine cancer, despite its limitations, represent significant resource for the estimation of value of CGP and personalized therapy based on its findings in everyday oncological practice and are important for further positioning and development of precision medicine in patients with uterine cancer.

Declarations

Data availability

The data used to support the findings of this project are available from the corresponding author upon request. However, systematized genomic alterations as well as main CGP findings with suggested therapies are provided as supplementary files; Main CGP findings and Systematized genomic alterations.

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Conflict of interest statement

Dora Čerina: Speaker fees: Roche; **Višnja Matković:** Speaker fees and consulting: Astra Zeneca, Roche, Amgen, Stada; **Kristina Katić:** Speaker fees: Roche, AstraZeneca; **Ingrid BelacLovasić:** Speaker fees and consulting: Amgen, Astellas, Astra Zeneca, BMS, Merck, Novartis, Pfizer, Roche, Sanofi, MSD; **Robert Šeparović:** Speaker fees and consulting: Pfizer, Novartis, Roche, MSD, Johnson & Johnson, Amgen, Pharmaswiss; **Ivana Canjko:** Speaker fees: Roche, MSD, Abbot, Takeda,Stada,Alvogen,Boehringer Inn, AstraZeneca; **Blanka Jakšić:** Speaker fees and consulting: Roche, Sanofi, Abbott, Alvogen, Sandoz, Janssen Cilag, Pliva; **Ana Fröbe**: Support for clinical trials/consulting: Amgen, Astellas, Janssen, Pfizer, Roche, Sandoz, and Sanofi; **Stjepko Pleština**; Speaker fees and consulting: Astra Zeneca, BMS, Amgen, Pfizer, Roche, Novartis, MSD, Merck, Servier; **Eduard Vrdoljak:** Support for clinical trials and scientific projects: Pfizer, Roche, BMS, AZ, Speaker fees and consulting: Amgen, Astellas, Astra Zeneca, Boehringer Ingelheim, Johnson & Johnson, Novartis, Pharmaswiss, Pfizer, Roche, Sanofi, MSD, Merck; **Other authors** have no conflict of interest to declare.

Author Contribution

Dora Čerina: Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Project administration; **Višnja Matković**: Investigation, Resources, Writing - Review & Editing; **Kristina Katić**: Investigation, Resources, Writing - Review & Editing; **Ingrid Belac Lovasić**: Investigation, Resources, Writing - Review & Editing; **Robert Šeparović**: Investigation, Resources, Writing - Review & Editing; **Ivana Canjko**: Investigation, Resources, Writing - Review & Editing; **Blanka Jakšić**: Investigation, Resources, Writing - Review & Editing; Ana Fröbe: Investigation, Resources, Writing- Editing and Response to review, Data systematization; **Stjepko Pleština**: Investigation, Resources, Writing - Review & Editing; **Žarko Bajić**: Formal analysis, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization; **Eduard Vrdoljak**: Conceptualization, Methodology, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision, Funding acquisition.

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5.3. Comprehensive Genomic Profiling in the Management of Ovarian Cancer - National Results from Croatia

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Abstract

Today, in the era of precision medicine, the determination of genomic instability or other potentially targetable mutations, along with BRCA 1 and BRCA 2, is a crucial component of the diagnosis and treatment management of advanced ovarian cancer. Advanced technologies such as next-generation sequencing (NGS) have enabled comprehensive genomic profiling (CGP) analysis to become more feasible for routine use in daily clinical work. Here, we present the results from the first two years of the analysis of patients with advanced ovarian cancer on a national level. The aim was to establish position of CGP in the daily clinical practice of treating ovarian cancer. We performed a multicenter, retrospective, cross-sectional analysis of the total population of Cro-atian patients who were either newly diagnosed with locally advanced or metastatic ovarian cancer, whose initial disease had progressed from January 1, 2020, to December 1, 2021, and whose tumors underwent CGP analysis. All 86 patients (100%) analysed with CGP had at least one genomic alteration (GA). The median LOH was 14.6 (IQR 6.8-21.7), with 35 (41%) patients having a LOH ≥16. We found a BRCA-positive status in 22 (26%) patients. Conventional testing, which detects only BRCA mutations, would have opted therapy with PARP inhibitors in 22 (26%) patients among our patients. Meanwhile, CGP revealed the need for PARP inhibitors in 35 pa-tients (41%). The results have identified a significantly higher number of women who would achieve a possible benefit from targeted therapy. Hence, we believe that CGP should be backbone diagnostic tool in management of ovarian cancer.

Keywords: advanced ovarian cancer; comprehensive genomic profiling; targeted therapy; precision medicine

Introduction

Ovarian cancer is the eighth most common cancer diagnosed among women worldwide. While it usually occurs in women of older age, a significant number of pa-tients are being diagnosed at a younger age (≤ 55 years), especially women with a fa-miliar background of ovarian cancer. Furthermore, when defining public health im-portance, more than 70% of women are diagnosed with locally advanced or metastatic disease, with an expected 5-year survival rate of less than 30% [1]. Due to its obscure clinical presentation, diagnosis at advanced stages and high mortality rate, ovarian cancer is the most lethal cancer of the female reproductive system and thus represents one of the hot topics in oncology with the need for significant advances in treatment. The last significant breakthrough in terms of chemotherapy administration occurred with the introduction of paclitaxel and carboplatin regimens at the end of 1990 [2]. Unfortunately, the in-troduction of immunotherapy directed against VEGF in combination with chemo-therapy and as a maintenance treatment did not affect overall survival, despite a sig-nificant effect on progression-free survival [3-5]. Finally, targeted therapy with PARP inhibitors in patients with germline or somatic BRCA mutations has revolutionized therapy, statistically and clinically improving outcomes and increasing patient and so-cietal expectations [6-8]. Since the introduction of the latter treatment, the determina-tion of the germline and somatic BRCA 1 and BRCA 2 status is mandatory in the diag-nostic workup [9]. Additionally, during 2020, PARP inhibitors were approved for the treatment of ovarian cancer in patients with an established homologous recombination deficiency (HRD) status through BRCA or mutation of other genes involved in the HRD process [10]. HRD and consequent loss of heterozygosity (LOH), which repre-sents the percentage of the tumor genome with a focal loss of one allele, lead to ge-nomic instability and occur due to genetic or epigenetic inactivation of one or more HR pathway proteins, including BRCA 1, BRCA 2, RAD51C, ATM, PALB2 and BRIP1 [11-13]. A clinically significant LOH score with approved PARP inhibitor therapy was determined at a cut-off of ≥16 [8]. Based on these findings, a shift in the paradigm for the approach to diagnosing ovarian cancer is occurring, with molecular classification surpassing the simple histological classification into type I and type II ovarian cancer, and targeted therapy is becoming the mainstay treatment for locally advanced or metastatic disease [14]. Thus, a determination of genomic instability or other poten-tially targetable mutations, along with BRCA 1 and BRCA 2, is a crucial component of the diagnosis and treatment management in these patients. Advanced technologies such as next-generation sequencing (NGS) are becoming more feasible and used in daily clinical work. NGS provides insights into all exons of cancer-related genes and identifies four main classes of genomic alterations: base substitutions, insertions or de-letions, gene rearrangements and copy number variations. In addition to the main ge-nomic alterations, CGP assays also determine their patterns and provide information regarding genomic instability or so-called “genomic scarring” by detecting tumor mu-tational burden (TMB), microsatellite instability (MSI) and loss of heterozygosity (LOH) through a complex computational analysis. Today, in the era of precision on-cology and following the expansion of targeted therapy and immunotherapy, several CGP assays have been approved by the U.S. Food and Drug Administration (FDA) for diagnostic, prognostic and therapeutic purposes, one of which is FoundationOneCDx (Foundation Medicine Inc., Cambridge, MA, USA) [15-17]. CGP is becoming more available and widely used, but the question of its accurate applicability, utility and cost benefit remains.

Ovarian cancer represents one of the major health burdens in Croatia due to its high mortality to incidence ratio (0,67), which puts Croatia among countries with highest mortality and incidence in

Europe [18, 19]. Potential reason for high mortality to incidence ratio lays in the late diagnosis and lack of proper treatments. For instance, in 2018, Croatia was one of the countries with lowest tier for PARP inhibitors uptake [19]. Ovarian cancer patients are treated with standard chemotherapy following sur-gery (or before when neoadjuvant therapy is indicated) or in the case of initially meta-static disease, with platinum-based chemotherapy and paclitaxel three-weekly or dose dense, +/- bevacizumab or from recently PARP inhibitor, depending on the residual disease and BRCA status as well as response to platinum therapy. In the treatment of recurrent disease, patients are also treated with standard chemotherapy based on platinum sensitivity, along with bevacizumab or with PARP inhibitors in case of a BRCA mutation. PARP inhibitors are given after response to platinum based chemo-therapy as a maintenance treatment. However, they are not indicated in case of HRD or LOH like in some other European Countries.

At the end of 2019, a CGP analysis of the tumor specimens provided by Founda-tion Medicine Inc. (FMI) has begun in Croatia for patients diagnosed with metastatic disease as a part of the project for the development and implementation of precision oncology on a national level in Croatia [20]. The first results and experiences are in the process of being analyzed, with the recently proven utility of CGP analysis in patients with metastatic uterine cancer [21].

As no established screening method is available and the majority of women are consequently diagnosed in advanced stages with low survival rates, the diagnostic workup should receive special attention. Particularly, because all patients should have equal opportunities to be treated the same, with already approved targeted therapies. Due to the CGP analysis provided by FMI, we present the results from the first two years of testing patients with locally advanced or metastatic ovarian cancer on a na-tional level here. The aim of this study was to present the number of patients with tar-getable BRCA 1 and BRCA 2 mutations compared to the complete number of patients whose CGP results revealed a need for targeted therapy with PARP inhibitors, as well as other potential targeted treatments, and to establish the position for the CGP of ovarian cancer in daily clinical practice.

2. Materials and Methods

2.1. Project design

We performed a multicenter, retrospective, cross-sectional analysis of the total population of Croatian patients who were either newly diagnosed with locally ad-vanced or metastatic ovarian cancer, whose initial disease had progressed from Janu-ary 1, 2020, to December 1, 2021, and whose tumors underwent CGP analysis. The analysis was performed through FoundationOneCDx for all patients and was con-ducted in a certified Clinical Laboratory Improvement Amendments, College of American Pathologists accredited laboratory (Foundation Medicine Inc., Cambridge, MA, USA) [15-17]. The obtained tumor specimen was sampled from surgery or biopsy of the primary disease or metastases. Formalin-fixed, paraffin-embedded tissue was sent as a block and one hematoxylin and eosin stained slide or 10 unstained slides with one hematoxylin and eosin stained slide. Minimal surface area was 25 mm2 and mini-mal tumor content was 20%, while optimal was 30% of tumor nuclei, defined as the number of tumor cells divided by total number of all cells with nuclei. Once the DNA was extracted, 50-1000 ng underwent whole-genome shotgun library construction and hybridization-based capture in order to detect alterations of 324 genes in total, of which 309 exons related with tumors, one promoter region, one non-coding RNA and

certain regions of introns in 34 frequently rearranged genes in tumors. Illumina® HiSeq 4000 was used to sequence hybrid capture-selected libraries to high uniform depth. The typical median depth of coverage was >500x with >99% of exons at cover-age >100x. The sequenced regions were analyzed for four different types of alterations; base substitution, deletion or insertion, copy number variation and gene redistribution in a group of genes associated with the tumor development. The microsatellite status was based on genome wide analysis of 95 microsatellite loci, while TMB was deter-mined by counting all synonymous and non-synonymous variants present at 5% allele frequency or greater and total number was presented as mutations per megabase (Muts/Mb) unit, while homologous recombination repair (HRR) mechanism is assessed for mutations in the 14 HRR genes, ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, and RAD54L [17]. De-pending on the results, patients were potentially administered CGP-guided therapy in accordance with the approved (on-label) standard treatment of care available in Croatia.

This real-world analysis was conducted in five Croatian institutions: University Hospital Centre Split, University Hospital Center Zagreb, Clinic for Tumors Sestre Mi-losrdnice, University Hospital Centers Rijeka and Osijek. The project was approved by the ethics committees of all participating institutions. Informed consent was obtained from all patients for the CGP analysis and data collection. The data file was anony-mized before the analysis, and the project was performed in accordance with the World Medical Association Declaration of Helsinki of 1975 as revised in 2013 [22].

In accordance with the journal's guidelines, we will provide our data for the re-producibility of this study in other centers if such is requested.

2.2. Participants

We planned to include the entire population of patients who fulfilled the CGP cri-teria defined by the Croatian Oncology Society: sufficient tissue for the CGP, good general health (ECOG performance status ≤2) and at least 12 months of life expectancy [(20]). Hence, we did not perform the power analysis before starting the project. Pa-tients were administered the first-line standard of care treatment for locally advanced or metastatic ovarian cancer, depending on their general condition, other comorbidi-ties and physician's choice. CGP-guided therapy with PARP inhibitors was adminis-tered to patients with BRCA 1 or BRCA 2 mutation after the initial response to stand-ard of care first- or second-line systemic therapy, in accordance with the existing re-imbursement restrictions for PARP inhibitors in Croatia, as well as multidisciplinary team decisions.

2.3. Endpoints

The primary endpoint was to present and compare the proportion of patients carrying a BRCA 1 or BRCA 2 mutation with the proportion of patients having HRD or LOH, for which targeted therapy with PARP inhibitors was chosen. Moreover, in order to further investigate comprehensive genomic profiling, we have compared its results with the conventional testing for BRCA in a single institution in the same period of two years, from January 2020 to December 2021. Conventional testing was performed ei-ther from blood or paraffin-embedded tissue.

Comprehensive genomic profiling is approved by FDA and has underwent many validations [23, 24]. However, to confirm its results, we have explored and compared their compatibility with locally performed BRCA testing and immunohistochemistry testing for p53 mutation, among patients from a single institution.

The secondary endpoint was to present other clinically relevant genomic altera-tions detected (CRGA), which were defined as those with approved targeted therapy for the patients' tumor types or approved to treat other tumor types, or with existing clinical trials available. Also, we did comparison of the CGP results based on the his-tological subtypes.

2.4. Statistical analysis

We described the data as percentages, medians and interquartile ranges (IQRs) using StataCorp 2019 software (Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

3. Results

3.1. Description of patients and previous therapy

From January 1, 2020, to December 1, 2021, 86 patients with locally advanced or metastatic ovarian cancer were presented to multidisciplinary teams, and CGP was performed on their tumor tissue specimens. The median age was 59 (IQR 52-66) years, with a total range from 39 to 80 years (Table 1). The majority of patients, 71 (87%), were in good general condition with an ECOG performance status of 0. The most common histological subtype was high-grade serous cancer in 69 patients (80%). All patients received chemotherapy either in the neoadjuvant, adjuvant or metastatic set-ting as a standard treatment for locally advanced metastatic ovarian cancer. Nineteen (22%) patients were newly diagnosed with metastatic ovarian cancer (Table 1). The median number of prior lines of therapy for metastatic disease was 1 (IQR 0-1) (Table 1). The most common chemotherapy protocol used as the first-line treatment was a combination of paclitaxel and carboplatin in 84% patients.

Table 1. Characteristics of patients before comprehensive genomic profiling

	All patients (n=86)
Age at the time of diagnosis, median (IQR)	52 (52-66)
Metastatic disease at the initial diagnosis	19 (22)
FIGO stage at diagnosis †	
I*	3 (3)
II*	6 (7)
III	58 (67)
IV	19 (22)
Histological subtypes †	
serous carcinoma	
low grade	8 (9)
high grade	69 (80)
carcinosarcoma	2 (2)
microcellular carcinoma	2 (2)
clear cell carcinoma	1 (1)
mixed types	
endometrial + clear cell carcinoma	1 (1)
granulosacell tumour	1 (1)
steroid cell tumour	1 (1)
malignant seal ring cells	1 (1)
Number of patients receiving previous chemotherapy	
neoadjuvant	18 (21)
adjuvant	49 (57)
Number of previous treatment lines for metastatic disease	
0	43 (50)
1	26 (30)
2	14 (16)
3	2 (2)
7	1 (1)
ECOG performance status before CGP	
0	71 (83)
1	13 (15)
not determined	2 (2)

Data are presented as the numbers (percentages) of patients if not stated otherwise.
Abbreviations: IQR, interquartile range; CGP, comprehensive genomic profiling
Data were missing for the date of metastatic disease and number of previous treatment lines for metastatic disease in 1 (3%) patient.
† The total is <100% due to a rounding error.
* CGP was performed upon progression.

3.2. Comprehensive genomic profiling

All 86 patients (100%) analyzed using CGP had at least one genomic alteration (GA). Clinically relevant genomic alterations (CRGAs) were detected in 73 (85%) patients, with a median of 2 (IQR 1-3) CRGAs per patient (Table 2). The most common CRGAs reported were the functional loss of the tumor suppressor p53 encoded by the TP53 gene in 48 (56%) patients. The next most common CGRAs were those of phosphatide-inositol-3 kinases (PIK3) in 14 (17%) patients, KRAS (Kirsten rat sarcoma virus) in 13 (15%) and NF 1 (encodes neurofibromin) or NF 2 (encodes merlin) mutation in 10 (12%) patients (Table 2). Genomic alterations without clinical significance were detected in 69 (80%) patients with a median of 2 (IQR 1-3) GAs per patient. The microsatellite status was determined to be highly unstable in only 1 (0.01%) patient and was not determined in 3 (0.03%) patients. The median tumor mutational burden (TMB) was 3 (IQR 0-4) mutations per megabase (Muts/Mb), with a total range from 0 to 18. A high TMB (≥10 Muts/Mb) was reported in only 2 (0.02%) patients. The median loss of heterozygosity (LOH) was 14.6 (IQR 6.8-21.7), with 35 (41%) patients having LOH ≥16. The LOH status was not determined for 5 (0.06%) patients. We found a BRCA-positive status in 22 (26%) patients, with 15 (17%) patients carrying the BRCA1 mutation and 7 (8%) patients carrying the BRCA2 mutation. Altogether, 18 of 22 (81.7%) patients carrying a BRCA mutation had LOH ≥16.

Table 2. The results of comprehensive genomic profiling

	All patients (n=86)
Genomic alterations	
any genomic alteration	86 (100)
clinically relevant	73 (85)
clinically not relevant	69 (80)
Number of genomic alterations, median (IQR)	
total number	2 (1-3)
clinically relevant	2 (1-3)
clinically not relevant	2 (1-3)
Number of clinically relevant genomic alterations †	
0	13 (15)
1	20 (23)
2	20 (23)
3	14 (16)
4	8 (9)
5	11 (13)
Clinically relevant genomic alterations	
BRCA	22 (25)
BRCA 1	15 (17)
BRCA 2	7 (8)
TP53	48 (56)
PIK3 pathway	14 (17)
KRAS	13 (15)
NF 1/2	10 (12)
MYC	9 (10)

SOX2	7 (8)
PTEN or FGFR 1/2	5 (6)
CCND1/2 or AKT2 or ARID1A	4 (5)
CHEK2 or TSC1/2 or ERBB2	3 (4)
PDGFR A/B or AURKA or MDM2 or MET or ATM or NRAS or CDK12 or STK11	2 (2)
RICTOR or PALB2 or SMARCA4 or CTNNB1 or PTCH1 or BRAF or MTAP or AXL or MAP2K1 or KIT or NTRK2 or SMO	1 (1)
Loss of heterozygosity (LOH)	
median (IQR)	14.6 (6.8-21.7)
LOH ≥ 16	35 (41)
not determined	5 (6)
Microsatellite status	
stable	82 (95)
high instability	1 (1)
not determined	3 (4)
Tumour mutational burden (TMB), median (IQR)	3 (0-4)
Tumour mutational burden (TMB)	
not high	81 (94)
high (≥10 mutations/Mb)	2 (2)
not determined	3 (4)

Data are presented as the numbers (percentages) of patients if not stated otherwise.
Abbreviations: CGP, comprehensive genomic profiling; IQR, interquartile range
† The total is < 100% due to a rounding error.

3.2.1. Difference of CGP results regarding histological types

Considering that 80% of patients had high-grade serous ovarian cancer, subanal-ysis of CGP regarding the histological subtype was performed. Patients were separated into two groups, high-grade serous vs low-grade serous and other histological types. Markedly lower prevalence of clinically relevant mutations was found among the second group with also noted difference in BRCA status and LOH, Table 3.

Table 3. Difference of CGP results regarding histological types

	High-grade serous (n=69)	Low-grade serous + other types (n=17)
Genomic alterations		
any genomic alteration	69 (100)	17 (100)
clinically relevant	61 (88)	12 (71)
clinically not relevant	65 (94)	12 (71)
BRCA	21 (30)	1 (6)
BRCA 1	14 (67)	1 (6)
BRCA 2	7 (33)	0 (0)
Loss of heterozygosity (LOH)		
median (IQR)	16.4 (11.6-22.5)	2 (0.5-6.8)
LOH ≥ 16	34 (49)	1 (7)
not determined	2 (3)	3 (18)

Data are presented as the numbers (percentages) of patients if not stated otherwise.
Abbreviations: CGP, comprehensive genomic profiling; IQR, interquartile range

3.2.2. Comprehensive genomic profiling vs. conventional testings

Conventional testing for BRCA was performed from blood or paraffin-embedded tissue. Comparing the CGP results with conventional testings performed in the same period of two years, from January 2020 to December 2021, in a single institution, the clinically relevant difference was found with higher number of patients having BRCA mutations after CGP analysis. Moreover, CGP provided information regarding LOH, resulting in 27% more patients in total who would potentially have benefit from PARP inhibitors, Table 4.

Table 4. Comprehensive genomic profiling vs. conventional testing for BRCA

	CGP results (n=33)	Conventional testing (n=49)
Testing from blood	0 (0)	31 (63)
Testing from tissue	33 (100)	18 (37)
BRCA	12 (36)	9 (18)
BRCA 1	9 (27)	7 (14)
BRCA 2	3 (9)	2 (4)
Loss of heterozygosity (LOH)		
median (IQR)	15.7 (8.85-21.9)	
LOH ≥ 16	15 (45)	
not determined	2 (6)	49 (100)

Data are presented as the numbers (percentages) of patients if not stated otherwise.
Abbreviations: CGP, comprehensive genomic profiling; IQR, interquartile range

For the same group of patients coming from a single institution, we have per-formed internal validation of CGP results through determination of BRCA status and immunohistochemistry confirmation of TP53 status. BRCA status was determined lo-cally for 9 patients and matching with

CGP results was 100%. While, immunohisto-chemistry for TP53 was performed locally in 20 patients with 18 of them (90%) having same results as CGP.

After an analysis of all CGP reports and all GA reports, some type of targeted therapy was chosen for 56 (65%) patients. Targeted therapy approved for the patients' tumor type (on-label therapy) was reported in 41 (48%) patients, while targeted ther-apy approved in other tumor types based on patients' GA (off-label therapy) was re-ported in 55 (64%) patients. All of the on-label alteration-driven therapies were in-cluded in the DNA repair mechanism with PARP inhibitors, such as olaparib, ni-raparib, and rucaparib. The same group of patients had the most common off-label therapy as well, which was also the PARP inhibitor talazoparib. Moreover, other most common alteration-driven off-label therapies were those encompassing PI3K/mTOR (phosphoinositide-3 kinase/mammalian target of rapamycin) and Ras/Raf/MEK (mi-togen-activated protein kinase) mutations, with mTOR and MEK inhibitors as the most frequently used targeted therapy. GCP-guided targeted therapy with PARP inhibitors was administered to 14 (16%) patients based upon the indication, clinical need, MDT decision and reimbursement status of the therapy.

4. Discussion

4.1. Summary of Main Results

The results from the CGP analysis in our study showed that all patients with lo-cally advanced or metastatic ovarian cancer harbored at least one genomic alteration. Additionally, the molecular profile of our group of patients is similar to previous find-ings from ovarian cancer, particularly to The Cancer Genome Atlas (TCGA) compre-hensive profiling (12). Conventional testing in the ovarian cancer diagnostic workup using single-target assays that detect only BRCA mutation would have potentially in-dicated targeted therapy with PARP inhibitors in 22 (26%) patients among our group of tested patients. Results presented in Table 4 from a single institution show that con-ventional testing is less sensitive than CGP, particularly regarding the somatic muta-tions. While, CGP performed using next-generation sequencing revealed a need for targeted therapy with PARP inhibitors in 35 patients (41%), resulting in a clinically significant number of patients who would potentially benefit from already approved treatment options. Furthermore, the results of the CGP analysis provided information on other potential targetable mutations and, as a result, have led to the discovery of more patients who would potentially benefit from targeted therapy.

4.2. Results in the Context of Published Literature

As mentioned previously, significant advances have occurred with modification of the surgical approach, administration of TC chemotherapy and the introduction of immunotherapy and targeted therapy [2-8]. Despite the aforementioned improve-ments, ovarian cancer treatment outcomes are still rather unsatisfactory compared to some other cancer types due to the diagnosis at advanced stages and inherent biological specificities [25]. In recent years, a focus on medicine, particularly in oncology, has been placed on an individual patient, emphasizing the need for treatment person-alization. Precision medicine, in a full sense, comprises a timely and organized indi-vidual approach, extensive and treatment-oriented diagnostic workup and personal-ized therapy [26].

Hence, by providing more detailed insights into the specific tumor genes and “genomic scarring” of each patient, CGP represents the next step towards precision oncology and enables the potential discovery of the next breakthrough re-garding targeted alteration-driven therapy for individuals and a possible significant effect on the outcomes of women diagnosed with advanced ovarian cancer.

Although NGS was already recognized as fundamental for precision medicine, its limitations, such as interpretation of the results, were also described, and we are still in the quest to define its optimal position and application in daily clinical practice [27, 28]. Subsequently, several trials involving targeted therapy were conducted and produced controversial results, with some trials, such as MOSCATO and SHIVA, re-porting negative outcomes with targeted-based therapy, while several other trials ob-tained positive results with targeted therapy having an approximately double effect on the investigated outcomes [29-34]. Meanwhile, due to its specific genetic back-ground, more than 15% of ovarian carcinoma patients carry germline mutations, and an additional 5-11% of patients carry somatic mutations in either the BRCA1 or BRCA2 genes. Including other HRD gene mutations, up to 50% of patients have HRD with existing excellent treatment opportunities with PARP inhibitors, making ovarian cancer an ideal tumor for an upfront CGP analysis [10, 25]. In addition, targeted therapy, as well as obligatory biomarker detection as a part of the diagnostic process, has already been established in ovarian cancer management [25, 35]. As a result, The European Society for Medical Oncology (ESMO) recommends the routine use of NGS in the ovarian cancer workup [36].

Furthermore, one can argue that with more precise diagnostics, classical clinical trials will no longer suffice for drawing conclusions regarding treatment, and we will have to learn for and from every patient individually. Hence, the importance and em-phasis is placed on real-world data and clinical experience to determine the real effi-cacy and toxicity, where we already have positive feedback for the use of PARP inhib-itors in ovarian cancer [37, 38].

4.3. Strengths and Weaknesses

The limitations of our study, which may affect the interpretation of the results, in-clude its retrospective design and the relatively small number of patients. The retro-spective design of the study may lead to patient selection and subsequent bias in the study results.

On the other hand, the study presents a national experience of all consecutive pa-tients tested, and our results define the potential importance of CGP in the daily clini-cal practice of treating patients with ovarian cancer.

4.4. Implications for Practice and Future Research

The study results present a real-world data on a national level and as such have a great validity for clinical practice and for setting position of CGP analysis in everyday diagnostic and treatment management of locally advanced ovarian cancer. Also, they bring a new perspective of personalized and precise treatment, in which therapy is tailored individually for patients according to their CGP findings. Hence, new re-searches from this field could result with the reimbursements of novel treatment strategies.

5. Conclusions

Here, we present the two-year experience of CGP in the ovarian cancer diagnostic workup. Based on the results that indicate a significantly higher number of women would achieve a possible benefit from targeted therapy, CGP should be integrated into the diagnostic workup of locally advanced and metastatic ovarian cancer as a back-bone diagnostic tool.

Author Contributions: Dora Čerina: Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, and Project administration; **Višnja Matković**: Investigation, Resources, and Writing - Review & Editing; **Kristina Katić**: Investigation, Resources, and Writing - Review & Editing; **Ingrid Belac Lovasić**: Investigation, Resources, and Writing - Review & Editing; **Robert Šeparović**: Investigation, Resources, and Writing - Review & Editing; **Ivana Canjko**: Investigation, Resources, and Writing - Review & Editing; **Žarko Bajić**: Formal analysis, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, and Visualization; and **Eduard Vrdoljak**: Conceptualization, Methodology, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision, and Funding acquisition.

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5.4. Is There a Place for Adjuvant Chemotherapy in the Treatment of Locally Advanced Cervical Cancer?

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Abstract

Findings on the efficacy of adjuvant chemotherapy (ACT) of locally advanced cervical cancer (LACC) after the concurrent chemoradiation (CCRT) therapy were inconsistent, and the OUTBACK trial was expected to shed some light regarding the topic. Its results on ACT in LACC were negative, with the conclusion of not to use it. The objective of this review was to present the inconsistencies of previous studies, along with the OUTBACK trial in more detail, and to rethink whether its results provide an unambiguous and definite answer to the optimal position of ACT in the treatment of LACC. To critically appraise the OUTBACK trial and understand the consequences of its results, we used only randomized controlled studies (RCTs) on ACT in LACC that have been included in high-quality systematic reviews and meta-analyses. We calculated the pooled prediction intervals using a random effects meta-analysis of all published randomized studies including the OUTBACK trial. After combining the OUTBACK trial with the results of four previous randomized trials, the pooled hazard ratio for overall survival benefit of CCRT + ACT was 0.95 (95% CI 0.75; 1.20). The pooled hazard ratio of the four previous trials was 1.00 (95% CI 0.69; 1.44). The OUTBACK trial improved the precision of the pooled estimate, but the clinical heterogeneity and the consequent prediction intervals are still very wide, and with 95% reliability, we can expect that if the new study, using a similar approach to the ACT, on a randomly selected patient population from the presented five trials is conducted, its hazard ratio for overall survival after ACT would be between 0.47 and 1.93. In conclusion, there is an absolute need for further research in order to optimally define the position of ACT in the treatment of LACC.

Keywords: uterine cervical neoplasms; locally advanced cervical cancer; concurrent chemoradiation; adjuvant chemotherapy

Introduction

Each year, cervical cancer (CC) affects approximately 0.6 million women worldwide, with more than half of those unfortunately succumbing to the extent of the disease [1]. This high mortality to incidence ratio is at least partly a consequence of CC's unequal global distribution. CC is the most commonly diagnosed gynecological cancer and the leading cause of cancer death among women in developing parts of the world [1]. The social weight of CC is increased by the fact that a majority of the women are being diagnosed at a relatively young age and with locally advanced disease [2]. Due to all of the above, CC constitutes a major global health and societal burden, with an underemphasized need to improve its well established and proven primary and secondary prevention, as well as timely and optimal treatment.

Standard treatment for locally advanced cervical cancer (LACC) remains the concomitant application of cisplatin chemotherapy and radiotherapy [3,4,5,6,7]. Nonetheless, after completion of primary CCRT, 30–40% of patients present with local or distant recurrence of the disease [3,4,5,6]. In an attempt to improve still unsatisfactory outcomes in LACC therapy, several treatment strategies were explored, including the application of concomitant polychemotherapy [8], higher doses of cisplatin [9], surgery following CCRT [10], neoadjuvant chemotherapy before CCRT [11], and adjuvant (consolidation) chemotherapy (ACT) after CCRT [12,13,14,15,16]. The latter has caused great turmoil because findings on the efficacy of ACT of LACC after the CCRT therapy were inconsistent, and the OUTBACK trial was expected to shed some light regarding the topic. Considering its results were negative, here we claim that OUTBACK trial should not be the last RCT undertaken regarding this topic but that further research is required [17]. Moreover, having in mind the above mentioned CC's global distribution and societal impact, we presume facing two challenges when developing adjuvant chemotherapy in the field of LACC. In order to develop sustainable, widely applicable adjuvant chemotherapy, the same should consist of generic, easily obtained cytostatic drugs. Consequently, the first challenge is to be active and improve existing outcomes significantly, and the second one is to be affordable and with that, available to many underserved patients in the developing world.

The real question is what makes CC so special that adjuvant therapy does not work [18,19]? It certainly is a unique cancer, because it is preventable, detectable, and treatable. Perhaps its uniqueness lies in its resistance to adjuvant chemotherapy? Locally advanced stages of a vast majority of other cancer types, regardless of histological subtype, are effectively treated with adjuvant chemotherapy, which contributes to a clinically relevant longer overall survival (OS) [20,21,22,23,24,25]. On the other hand, when approaching recurrent or metastatic CC, it is implied to use chemotherapy as a treatment backbone [26,27]. The response rates achieved by standard chemotherapy regimens in a first-line adjuvant or metastatic treatment setting of breast, colon, and lung cancer do not exceed 50% [28,29,30,31]. The results of the first-line chemotherapy regimens used in the treatment of metastatic uterine cervical squamous cell carcinoma are similar [32,33]. Moreover, the true chemosensitivity of one tumor is defined in the neoadjuvant setting, where the reported response rates in CC range from 80–85% [11,34]. According to the above stated facts, it seemed justified to hypothesize that adjuvant or consolidation chemotherapy will have its benefit on CC as well.

Until 2020, there were only four published RCTs of CCRT + ACT efficacy in LACC compared to CCRT alone, with one inconclusive review and meta-analysis [13,14,15,16,35,36]. The two most

recent meta-analyses were by Horeweg et al. One was published this year and it incorporated the results from the fifth RCT, the OUTBACK trial [17,18,19]. In general, current data and knowledge strongly discourages the use of adjuvant chemotherapy in LACC [19].

The objective of this review was to present the inconsistencies of previous studies, along with the OUTBACK trial in more detail, and to rethink whether its results provide an unambiguous and definite answer to the optimal treatment of LACC.

2. Materials and Methods

This review presents current state of knowledge regarding the use of adjuvant chemotherapy in locally advanced cervical cancer with critical appraisal of the OUTBACK trial. To critically appraise the OUTBACK trial and understand the consequences of its results, we used only the comparable studies that have been included in the high-quality systematic review by Tangjitgamol et al. [36] and meta-analysis by Horeweg et al. [18], who already assessed their risks of bias. We additionally searched for RCTs published after 5 September 2020, i.e., from the date covered by Horeweg et al.'s systematic review [18]. Eligible studies were randomized controlled trials of radiotherapy with concurrent chemotherapy followed by ACT in the treatment of LACC FIGO stage IB-IVA in women ≥18 years of age and ECOG performance status ≤2, with no neoadjuvant therapy and OS as the primary or secondary outcome. The outcome we focused on was OS because this was the OUTBACK trial's primary outcome. In two studies, Lorvidhaya et al. [14] and Kim et al. [15], we had to calculate the hazard ratios (HR) by the Parmar [37] and Tierney [38] methods because they were not originally published, but in both cases, we checked the results of our calculations with the results obtained by Horeweg et al. [18]. We calculated the pooled estimates of HR using the random effects model with a restricted maximum likelihood method and weighted the studies inversely to their variances. We decided in advance that we would use the random effects model because the core of our hypothesis was clinical heterogeneity, although we erroneously expected that the five RCTs would be more methodologically homogenous. For the pooled estimate, in addition to confidence intervals (CI), we calculated the prediction interval (PI). We calculated all CIs and PIs at the 95% level. The number of RCTs was too small for the quantitative analysis, e.g., meta regression, of the possible causes of inconsistencies, and we performed only the qualitative synthesis. We performed the statistical data analysis and drew the forest plot using StataCorp 2019 (Stata Statistical Software: Release 16. College Station, TX, USA: StataCorp LLC).

3. Results

3.1. Previous Trials

The only RCT with statistically significantly better PFS (3-year PFS of 74.4% vs. 65%, HR 0.68) and OS (3-year OS with HR 0.68) in the CCRT + ACT arm (Table 1, Figure 1) was performed by Dueñas-González et al. [13]. This RCT, which controlled the balance of a relatively large number of predictive factors by minimization randomization with a concealed allocation of participants but no blinding, was performed in Mexico, Argentina, India, Panama, Bosnia and Herzegovina, Peru, Thailand, Pakistan, and Australia. Respecting the mortality-to-incidence ratio, from the perspective of the targeted population, this is the most relevant study on ACT in LACC conducted to date. The study had a reasonably short enrollment period of approximately two years, a relatively high

proportion of patients in the CCRT + ACT arm who received at least one dose of ACT (86%), and the second-best ACT completion rate (77%). The key weakness of this otherwise well-designed study was the difference in the initial CCRT treatment between its two study arms: six cycles of cisplatin 40 mg/m2 in the CCRT alone arm, and six cycles of concurrent cisplatin 40 mg/m2 and gemcitabine 125 mg/m2 in the ACT arm of the study. In a later ACT protocol, patients received two additional cycles of cisplatin 50 mg/m2 with gemcitabine 1000 mg/m2. All patients received the same dose of RT, 50.4 Gy to the entire pelvic region in 28 fractions of 1.8 Gy/d, 5 days a week, over the 6 weeks of chemotherapy. Furthermore, after completion of CCRT, the majority of patients (93%) underwent low- or intermediate-dose rate brachytherapy (BCT) with cesium-137. A BCT dose of 30 to 35 Gy was delivered to point A to result in a cumulative dose of 80 to 85 Gy combining RT and BCT, and cumulative RT and BCT dose to point B (the pelvic wall) was 55 to 65 Gy. The ACT arm has started with adjuvant chemotherapy two weeks after BCT [13]. In addition to this regimen of RT and BCT, ACT arm also received the combination chemotherapy (cisplatin and gemcitabine) concomitantly with RT, unlike CCRT arm which received only monocisplatin concomitantly. Consequently, in this study, patients from ACT arm received different, combinational chemotherapy concomitantly with RT, as well as adjuvantly resulting in difficulties to define or measure impact of both of them on the final OS results. Higher toxicity rate of the combinational chemotherapy could have caused the difference in the discontinuation rate between these two study arms. In this sense, the dropout patterns in the Dueñas-González et al. study may have been somewhat different than in the other studies.

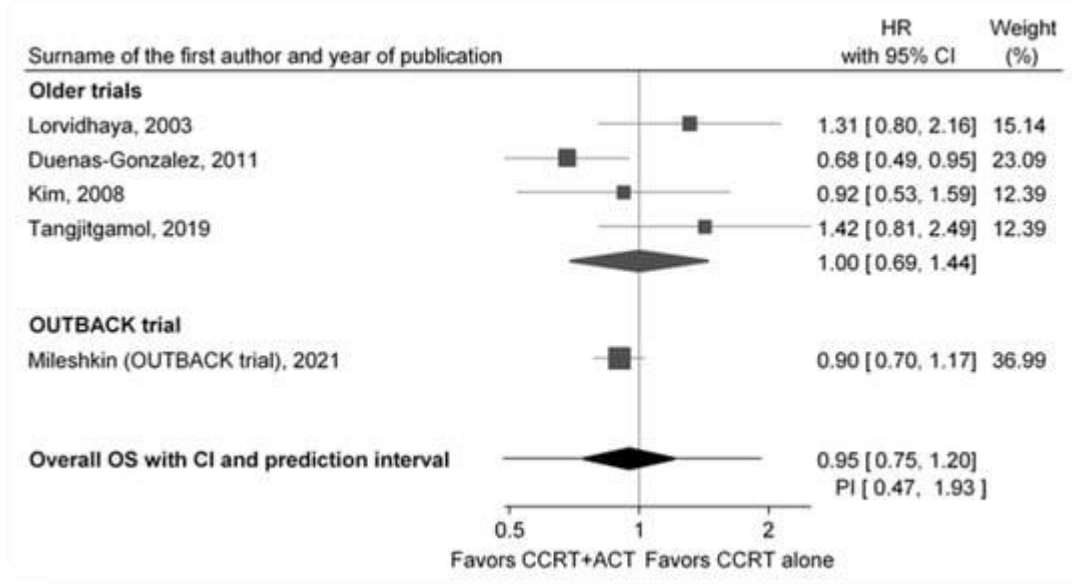

Figure 1. Forest plot of the effects of CCRT + ACT compared to CCRT alone on overall survival; gray squares represent each study hazard ratio (HR) for dying from any cause and whiskers represent 95% confidence intervals (CI); the size of the squares represent the weight of the study inverse to the study variance; black diamonds represent the pooled hazard ratio for older trials and for all trials (older and OUTBACK trial) calculated using a random effects model with a restricted maximum-likelihood method; whiskers from the lower "overall" diamond represent 95% prediction interval (PI); studies are sorted by the year of the end of enrollment.

Table 1. Overview of included randomized controlled trials sorted by year of end of enrollment.

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	Lorvidhaya [14]	**Dueñas-González [13]**	**Kim [15]**	**Tangjitgamol [16]**	**Mileshkin OUTBACK [17]**
Year of publication	2003	2012	2008	2019	2021
Country	Thailand	Multiple[a]	Korea	Thailand	Multiple[b]
Outcomes					
The main result favors ACT	no	yes	no	no	no
Overall survival (HR)	1.41[c]	0.68	0.92[c]	1.42	0.90
(95% CI)	(0.79; 2.16)	(0.49; 0.95)	(0.53; 1.59)	(0.81; 2.49)	(0.70; 1.17)
Randomization	stratified[e]	minimization[d]	stratified[f]	stratified[g]	stratified[h]
Concealed allocation	no	yes	no	not clear/yes	no
Masking/blinding	no	no	no	outcome ass.	no
Patients					
Number of patients randomized	230 / 233	259 / 256	78 / 77	130 / 129	461 / 465
Enrollment (start-end year)	1988-1994	2002-2004	1998-2005	2015-2017	2011-2017
Duration of enrollment (years)	6	2	7	2	6
Patients median age (years)	50 / 48[i]	45 / 46	58 / 57	49 / 50	46 / 45
Range of patients age (years)	< 65	22-68 / 18-70	36-75 / 34-73	23-68 / 26-68[j]	21-99 / 22-88
Disease					
Stage (%)					
IB1 (all node+), IB2, IIA	0 / 0	0 / 0	0 / 0	0 / 0	33 / 33
IIB	43 / 50	62 / 61	67 / 75	65 / 62	43 / 43
IIIA	1 / 1	< 1 / < 1	6 / 3	1 / 3	0 / 0
IIIB	55 / 49	36 / 37	22 / 17	31 / 35	24 / 24[k]
IVA	0 / 0	2 / 2	5 / 5	3 / 0	-
Median tumor diameter (cm)	n.a.	6 / 6	5 / 5[l]	5 / 5	5 / 5
Histology (%)					
Squamous cell carcinoma	90 / 88	93 / 94[m]	96 / 95	77 / 76	83 / 79
Adenocarcinoma	6 / 9	7 / 6	3 / 3	20 / 22	15 / 17
Adenosquamous carcinoma	1 / 0	-	1 / 3	2 / 2	3 / 4
Small-cell carcinoma	3 / 3	-	0 / 0	0 / 0	0 / 0
Positive pelvic lymph nodes	yes	n.a.	yes	yes	yes
Para-aortic lymph nodes > 1 cm	yes	no[n]	no	no	no
Previous chemotherapy or RT	no	no	no	yes[o]	yes[p]
Intervention (%)					
Completed CCRT	95	n.a.	73	80	83
Received at least one ACT dose	n.a.	86	n.a.	77	78
Completed CCRT+ACT	92	77	65	65	62
CCRT in control arm (cycle x DRUG mg/m^2 or AUC)	2 x MIT 10 2 x FU 300 mg/day	6 x CIS 40	6 x CIS 30	6 x CIS 40	5 x CIS 40
CCRT in ACT arm	2 x MIT 10 2 x FU 300 mg/day	6 x CIS 40 6 x GEM 125	2 x CIS 20 2 x FU 1000	6 x CIS 40	5 x CIS 40
ACT protocol	3 x FU 200 mg/day	2 x CIS 50 2 x GEM 1000	1 x CIS 20 1 x FU 1000	3 x PAC 175 3 x CAR 5	4 x PAC 155 4 x CAR 5
Follow-up					
Median follow-up (months)[r]	89	46	39	27	60

Data are presented in CCRT+ACT arm / in control CCRT only arm if not stated otherwise

Abbreviations: OS, overall survival; HR, hazard ratio; CI, confidence interval; CCRT, concurrent chemoradiation; ACT, adjuvant (consolidation) chemotherapy; RT, radiotherapy; n.a., not availa-ble; CIS, cisplatin; GEM, gemcitabine; FU, fluorouracil; PAC, paclitaxel; CAR, carboplatin; A, AUC; MIT, mitomycin C

[a] Mexico, Argentina, India, Panama, Bosnia and Herzegovina, Peru, Thailand, Pakistan, Austral-ia; [b] Australia, New Zealand, USA, Saudi Arabia, Canada, China, Singapore; [c] HR was calculated by Parmar (37) and Tierney (38) methods; [d] Minimization using Pocock and Simon algorithm [56], balancing disease stage (IIB vs. III-IVA), tumor diameter (<5 cm vs. ≥ 5 cm), study center (not clear, probably 9 that is one per country), radiation equipment (cobalt-60 vs. linear accelerator), age (<55 vs. ≥ 55 years); [e] Stratified for six study centers; [f] Stratified for tumor stage; [g] Mixed block with stratification for disease stage (IIV vs. III-IVA) and histopathology (squamous vs. adenocarcinoma or adenosquamous carcinoma); [h] Stratified for nodal status, par-ticipating site, FIGO stage, age, planned extended-field radiotherapy; [i] Mean instead of median; [j] Interquartile range instead of range; [k] Including IIIB and IVA; [l] Estimated from categories (≤ 4; 4.1-6; 6.1-8; ≥ 8.1) weighted by frequencies; [m] Including squamous cell, poorly differentiated and adeno/squamous carcinoma; [n] Para-aortic lymph nodes > 1 cm were exclusion criteria, but 2.3% in ACT arm and 4.7% in CCRT alone arm had at least one; [o] Previous chemotherapy was not an ex-clusion criterion, but all patients had newly diagnosed cervical cancer, so the previous chemo-therapy/radiotherapy were allowed only for other cancers; [p] But not for cervical cancer; [r] Round-ed down to the last full month

The oldest RCT was performed by Lorvidhaya et al. in Thailand, from 1988 to 1994, and it found, although not significantly, a better effect for CCRT alone (HR 1.42 was calculated by Parmar [37] and Tierney [38] methods) (Table 1, Figure 1) [14]. Randomization was stratified for the six study centers included, and consequently, two study arms were not perfectly balanced for the disease stage. Patients randomized to the CCRT + ACT arm had less frequent stage IIB and somewhat more frequent stage IIIB disease than the patients in the CCRT alone arm. Patients have received conventional RT which consisted of external RT and BCT. External RT was given to the whole pelvis in dose of 40–50 Gy with a midline shield to give the pelvic lymph nodes a dose of up to 50 Gy. A parametrium dose of up to 60–66 Gy was added to the involved side, depending on the extent of parametrial involvement, while BCT was given either high or medium dose rate, according to the standard in each center. The high-dose rate was 700–750 cGy at point A; two times per week for 2 weeks (four applications). The medium dose rate was a single application of 2500–2800 cGy to point A or two applications of 1400–1750 cGy to point A. The total dose at point A was 68–80 Gy. In other relevant characteristics, the patients in the two study arms were well balanced. Although the median age of patients enrolled in the Lorvidhaya et al. study was the second-highest compared to the other four RCTs, age above 65 was an exclusion criterion in this study. The distribution of age was not properly reported, nor is it an analysis of the possible moderating effect of age on OS. The most questionable part of this study was the chosen ACT protocol, monochemotherapy with 5FU, which many consider as not optimal for adjuvant therapy of LACC.

The smallest study with the longest enrollment period of seven years was conducted by Kim et al. in Korea from 1998 to 2005, with 78 patients in CCRT + ACT and 77 in the CCRT alone arm [15]. The study found no statistically significant longer OS and PFS in the CCRT + ACT arm (4-year OS of 70% vs. 67%, HR 0.92; 4-year PFS of 67% vs. 66%) (Table 1, Figure 1). Although the Kim et al. study had a randomization stratified for tumor stage, probably due to the relatively smaller samples, the final allocation resulted in a certain level of disbalance comparable to that from the Lorvidhaya et al. study (Table 1). In the initial CCRT arm, a relatively lower dose of cisplatin was administered: six cycles with 30 mg/m2. RT comprised of external irradiation to the whole pelvis of 41.4–50.4 Gy in 23–28 fractions plus high-dose rate (HDR) BCT (30–35 Gy in 6–7 fractions) to point A, together with a parametrial boost. One of the important limitations of the Kim et al. study was the low completion rate: 73% for CCRT alone, and 65% in the CCRT + ACT arm. However, the most important limitation is the fact that two out of the three cycles of chemotherapy based on the cisplatin and 5-fluorouracile (5FU) were given concomitantly with the external part of the radiotherapy. Therefore, this is not exactly the study of adjuvant chemotherapy, but more of a two types of concomitant chemoradiotherapy schedule.

The Tangjitgamol et al. study, conducted between 2015 and 2017 in Thailand, found no statistically significant benefit of ACT on OS (Table 1, Figure 1) [16]. Moreover, among the five studies compared, this study resulted in the least favorable results for ACT with 3-year OS of 69.5% in the CCRT + ACT arm vs. 80.1% in the CCRT arm and 3-year PFS of 63.4% in the CCRT + ACT arm vs. 66.6% in the CCRT arm (HR 1.26). The key limitations of this very well designed and executed RCT were its low completion rate (65%) and rather small number of randomized patients for a phase III trial, although rationally founded. While systemic recurrences were significantly lower in the ACT arm of the study, 5.4% vs. 10.1% ($p = 0.029$), defining the expected efficacy of ACT in the therapy of LACC, the OS HR was 1.42 (95% CI = 0.81–2.49; $p = 0.221$). This was the only

RCT that masked the outcome assessment and the only one that used a standard six cycles of cisplatin 40 mg/m2 protocol as the CCRT in both study arms, while RT comprised of 45–50.4 Gy given in 25–28 fractions, 1.8–2 Gy/day, 5 days a week, and patients had high-dose rate BCT 6.0–7.5 Gy for 3–4 fractions.

3.2. Critical Appraisal of the OUTBACK Trial

3.2.1. Study Overview

The OUTBACK was an international phase III trial of ACT after CCRT, compared to CCRT alone, as the primary treatment for LACC (ClinicalTrials.gov identifier: NCT01414608). Eligible patients were women with LACC ≥ 18 years of age, FIGO 2008 stage IB1 (only node positive), IB2, II, IIIB, IVA with ECOG performance status 0–2. The study enrolled 739 patients from USA and Canada, 165 patients from Australia and New Zealand, and 15 patients from China, Saudi Arabia, and Singapore. In the CCRT + ACT arm, 461 patients were enrolled, 83% completed the CCRT treatment as planned, 78% received at least one ACT dose, and 62% completed the ACT treatment as planned. In the control, the CCRT alone arm, 465 patients were enrolled, and 84% completed the CCRT treatment as planned. The CCRT was the same in both arms, consisting of five cycles of cisplatin and external-beam RT for five weeks, then intracavitary brachytherapy. ACT began four weeks after CCRT, with four cycles of paclitaxel and carboplatin. The primary outcome was OS at the fifth year from randomization, and the median follow-up was 60 months. The secondary outcomes were progression-free survival, adverse events and patterns of disease recurrence [17]. The analysis that has been presented so far was Kaplan–Meier curves and an unadjusted log rank test. The OUTBACK trial found no statistically significant differences in OS between patients allocated to the two study arms. OS after five years was almost the same in the CCRT alone arm (71%) and in the CCRT + ACT arm (72%). The difference was <1% (95% CI -6; 7%) [17]. HR for OS was 0.91 (95% CI 0.70; 1.18) and for PFS 0.87 (95% CI 0.70; 1.08), and adverse events of grades 3 to 5 occurred in 81% of patients in the CCRT + ACT arm compared to 62% in the CCRT alone arm during the first year after the randomization. Finally, the patterns of disease recurrence were similar. The OUTBACK trial was properly designed and well executed.

3.2.2. External Validity

The OUTBACK trial's targeted population was defined precisely but not too narrowly [17]. The study enrolled 33% of patients with FIGO 2008 stage IB1 (only node positive), IB2 or IIA tumors. Compared to the other RCTs, the OUTBACK population had a less advanced disease stage at the time of enrollment, which could have had an effect in favor of the null hypothesis of no ACT-relevant additional benefit to the effects of CCRT. The OUTBACK trial sample allocation was not proportionate to the population sizes in different countries. Furthermore, nonwhite patients in the OUTBACK trial's ACT arm had two times higher odds for not even starting the targeted intervention.

3.2.3. Internal Validity

OUTBACK was an open-label trial with no concealed allocation nor masking for the treatment assignment of participants or those delivering treatment, and with no blinded outcome assessment [17]. This could jeopardize the OUTBACK trial internal validity to a certain extent. The stratified randomization was used. Stratification was done for nodal status, participating site, FIGO stage, age and planned extended-field radiotherapy. However, randomization was performed before CCRT, so

no stratification was conducted for completion of CCRT. The two study groups were well balanced at baseline in terms of age, ECOG performance status, geographical region, tobacco smoking, nodal involvement, extended field planned, FIGO stage, histology, and tumor diameter. Black participants were somewhat less prevalent in the CCRT + ACT arm. The planned initial treatment was the same in both arms, but the number of weekly cisplatin cycles was lower in the CCRT + ACT arm. This imbalance was accounted for by the sensitivity analysis, and no significant differences in OS or PFS in the CCRT + ACT arm were found between those who did and did not complete CCRT. In all other parameters of the initial CCRT treatment, participants from the two study arms were well balanced. No data was presented yet on the eventual differences between the two study arms in the treatment, other than the intervention of interest, especially of the treatment after disease progression, which could have influence on the OS results [40]. An important limitation of the study was the relatively low completion rate of the targeted intervention. ACT was administered in only 78% of participants assigned to the intervention arm, and 62% received all four ACT cycles as planned. In 2016, the OUTBACK trial protocol was amended to increase the sample size from 780 to 900, due to nonadherence with ACT, but at the 2021 Virtual ASCO Annual Meeting, from 4–8 June 2021, only the intention-to-treat analysis with Kaplan–Meier curves and unadjusted log rank test results were presented, with no sensitivity analysis or per-protocol analysis, which could help explain the effects of poor adherence with the ACT treatment. The duration of enrollment in the OUTBACK trial was six years.

3.3. Contribution of the OUTBACK Trial

After combining the OUTBACK trial results with the results of the four previous RCTs, using a random effects model with a restricted maximum-likelihood method, the overall HR for OS was 0.95 (95% CI 0.75; 1.20) (Figure 1). The pooled HR of the four previous studies was 1.00 (95% CI 0.69; 1.44). The OUTBACK trial, with its relatively large sample, markedly improved the precision of the pooled estimate. It also narrowed the PI from 0.23 to 4.28 in the previous four studies to the overall 0.47 to 1.93. However, the heterogeneity and the consequent PI are still very wide, and with the 95% reliability, we can expect that if the new study, with similar design, randomly selected from the population of the presented five RCTs, is conducted, the pooled HR for OS would be between 0.47 and 1.93.

4. Discussion

There are only five RCTs on ACT in LACC. All of them, including the OUTBACK trial, share some common weaknesses that could jeopardize their internal validity. The main limitation is the relatively low completion rate. No study was double-blinded, and only one properly masked the outcome assessment [16]. Only Dueñas-González et al. concealed the allocation and performed the adaptive randomization controlling for a large number of relevant prognostic factors [41]. Only the two newest studies had approximately comparable interventions [16,17], and the OUTBACK trial has not yet reported and controlled the post-ACT treatments that may have biased the findings on OS.

The OUTBACK trial was expected to establish a definite LACC treatment approach. Unfortunately, based on the results from previously published RCTs, including the two most recent by Tangjitgamol et al. and the OUTBACK trial which were two properly designed and conducted

trials, the current state of knowledge of adjuvant chemotherapy in the treatment of LACC does not recommend its use in everyday clinical practice. Now, the question is: do we need more RCTs or not? Do we have definitive answers on this topic or not? Can we once and for all close the subject of adjuvant chemotherapy in LACC?

4.1. Predictive and Prognostic Factors That Could Have Caused the Inconsistencies

We have discussed different predictive and prognostic factors as the possible causes of the inconsistencies among these five RCTs in terms of ACT effects on OS. However, having in mind the wide predictive intervals we presented earlier, all these interpretations also should be read as the proposal of factors used as a helpful guide in identifying subpopulations that could derive benefit from ACT.

4.1.1. Nonadherence

The ACT initiation and completion rates in the OUTBACK trail were low [42], but they were not relevantly lower than those in the Kim et al. [15] or Tangjitgamol et al. studies [16]. The OUTBACK and these previous two RCT completion rates were markedly lower than the 86% initiation and 77% completion of the two cycles of cisplatin and gemcitabine in Dueñas-González et al. [13] or the 92% completion of three cycles of oral 5-fluorouracil in the Lorvidhaya et al. study [14]. Undoubtedly, low adherence may jeopardize the internal validity of the trial if, as is certainly the case, it is not randomly distributed among participants, but with regard to this, the OUTBACK trial was not an outlier. What needs to be studied and understood are the differences in adherence between Kim et al. [15], Tangjitgamol et al. [16], and the OUTBACK trial [17,42] compared to Lorvidhaya et al. [14] and Dueñas-González et al. [13]. Tertiary, post hoc analysis of the OUTBACK trial found the highest multivariable, adjusted odds for not starting ACT in patients older than 60, non-Caucasian women, and patients with poor physical function self-rated on QLQ-C30. In the Tangjitgamol et al. study, 74% of all reasons for not even starting the ACT treatment were the patients' or their physicians' decisions [16]. Loss to follow-up, protocol violation, hematologic toxicity, and progression combined accounted for 26% of the reasons for not starting the ACT, while patients' decline of further treatment was not occurring at all as the reason for incompletion of the initial CCRT treatment. Furthermore, one of the possible causes for the premature discontinuation of ACT could be the socioeconomic background and health insurance status of the patients. Hence, these are the parameters that also should be monitored in the OUTBACK trial and all future trials. The reported initiation and completion rates in OUTBACK, Kim et al., and Tangjitgamol et al. studies were markedly lower than in some observational studies [43,44,45]. In our personal practice, the adherence for completion of CCRT was 100%, while four to six cycles of ACT were received by 80% of patients [46,47,48].

4.1.2. Stage of the Disease

Firstly, it is well known that the relative and absolute benefit of adjuvant chemotherapy is larger for more advanced stages of local disease irrespective of the type of tumor, cervix included [20,21,22]. Dueñas-González et al. found the better ACT effects in stage III or IV adenocarcinoma [41]. The comparable RCTs on patients with higher stages of LACC performed by Tangjitgamol et al. in 2019 found the significantly lower rate of systemic recurrences in the ACT arm (paclitaxel plus carboplatin) than in the CCRT arm (5.4% vs. 10.1%; $p = 0.029$), although no significant differences in overall or locoregional recurrences or three-year PFS or OS [16]. Comparably, the RCT

performed by Dueñas-González found a significantly lower distant failure rate in the ACT arm (8.1% vs. 16.4%; p = 0.005) [13]. So did Tang et al., 2012 (14.3% vs. 23.6%; p < 0.005), but with one additional cycle of neoadjuvant cisplatin and paclitaxel in the ACT arm [49]. A recent meta-analysis by Horeweg et al. found the benefit of ACT after CCRT on distant-metastasis-free survival as well, although no significantly longer PFS or OS [18]. Moreover, the median tumor diameter in the Dueñas-González et al. trial was larger than in the OUTBACK trial and all three other RCTs. However, the Lorvidhaya et al. trial enrolled the largest proportion of patients with stage IIIB disease and found the second-worst effects of ACT on OS.

It is important to once again emphasize that the OUTBACK trial enrolled patients with Stage IB1 only in the case of a nodal positivity, which would, according to the current classification, upgraded them into Stage III1C, consequently with higher risk for both locoregional and distant failure [50]. However, from the available data, it is not clear how many of these patients could actually be upgraded nor type of the diagnosis of the nodal involvement. Hence, they are considered as patients with lower disease stage, alongside with patients with Stage IB2 and IIA. Although it represents a relevant difference compared to previous studies (33% of total patient population is more than in other RCTs), it will allow the authors of the OUTBACK trial to analyze the effects of ACT in the lower stages of LACC, which may prove particularly valuable from the perspective of our main conclusion. On the contrary, it should be considered a weakness of the OUTBACK trial. Namely, a higher proportion of patients with a lower stage of the disease could result in higher rates of noninitiation of ACT. It could motivate physicians and patients not to initiate ACT at all and/or not to complete the planned intervention, because the relative importance of toxicity is larger in less severe illness, and the perception of need for ACT may be lower in patients with a less severe disease stage.

Furthermore, the stage migration, in time and place, based on the diagnostic infrastructure, the quality of radiology and multidisciplinarity in general, is one of the most important factors in defining the outcomes of the patients enrolled in the RCTs, as well as in the everyday clinical practice. Generally speaking, older RCTs and trials that were conducted in less resourced medical environments tend to have under-staged patients and consequently worse outcomes. Following that statement, newer RCTs, especially the OUTBACK trial which is mostly conducted in well-resourced medical systems, should be more precise, and have less impact of potential stage migration on the real results of adjuvant chemotherapy in cervical cancer.

4.1.3. Regional Differences

Given the sample allocation by country, the OUTBACK study can be considered the first international RCT conducted in developed countries. Due to the rather significantly large discrepancies between the relative sample and population sizes in particular countries and marked differences in mortality-to-incidence ratios among the three groups of countries, the OUTBACK trial results should probably be reported without the results from China, Saudi Arabia, and Singapore. Additional analysis of the Dueňas-González et al. trial has indicated differences in the ACT effects in different, less developed countries [41]. Due to discrepancies in incidence and mortality, disparities regarding the treatment availability across many developing countries, and considering the described allocation of the OUTBACK trial sample, new RCTs are absolutely needed in order to properly understand the possible effects of ACT globally.

4.1.4. Treatment

Regarding the intervention, the most similar study or perhaps the only one similar enough to the OUTBACK trial was the Tangjitgamol et al. study [16]. It may seem confusing that these two studies found such different results, albeit in the end, they came to the same conclusion. In both studies, the cisplatin regimen in CCRT was changed to paclitaxel and carboplatin in ACT. The carboplatin-based regimen was shown to be noninferior to the cisplatin-based regimen in metastatic or recurrent CC [51,52], but the Kitagawa et al. study found significantly shorter OS in patients treated with paclitaxel and carboplatin if they had not received prior cisplatin. Initial CCRT in both the abovementioned trials were cisplatin-based, but still, in the available literature on ACT in LACC, there is no example of a paclitaxel-cisplatin ACT regimen to assess the possible effects of this switch to carboplatin. Therefore, special attention should be raised on the subject of the chemotherapy chosen for the OUTBACK trial. While four cycles of ACT are in line with what could be recommended in other oncology areas, especially after the CCRT part of the therapy, the intensity of ACT is not according to the widely accepted and used standards [53]. The usual dose of paclitaxel in a three-week schedule is 175 mg/m2, and the usual dose of carboplatin is AUC 6 [53]. Moreover, questionable is the decision to switch from the cisplatin used in the CCRT part of the protocol to the carboplatin in the ACT. Consequently, when you add two potentially detrimental things (low adherence in the ACT arm of the study and rather nonconventional dose intensity), you can argue why per protocol analysis is needed as well as why the OUTBACK trial should not be considered the definitive answer on the efficacy of ACT in the CC field.

4.1.5. Duration of Enrollment and Follow-Up

The length of the study enrollment period is also a potential reason for looking deeper into the results of the study. Six years is a rather long period, when treatment patterns could change and influence the study results [54]. The OUTBACK trial's enrollment period was no longer than in Kim et al. [15] or Lorvidhaya et al. [14], which lasted seven and six years, respectively, but it was three times longer than in Dueñas-González et al. [13] or in Tangjitgamol et al. [16], which lasted for two years. The OUTBACK trial had the second-longest follow-up period of 60 months. In addition to the fact that longer follow-up is a value in itself, it also is associated with many risks to the internal validity of OS as an outcome. Namely, the longer the follow-up is, the higher the frequency of additional therapy after the end of ACT, and this therapy was not yet described in the OUTBACK reports, nor were the overall survivals adjusted for its effects.

4.2. Future Directions

Taking into account that ACT is a cornerstone in the treatment of many solid cancers, that the results of chemotherapy in the treatment of metastatic CC are quite comparable to the results in other types of cancers, and that there is a rather weak level of evidence and a small number of RCTs performed in not so optimal conditions, we think that there is still no definitive answer regarding the efficacy and safety of CCRT + ACT on LACC. CCRT + ACT has a potential role to further improve control of the disease, especially a distant one. When designing new regimens for the successful treatment of LACC, we must take into consideration patient and disease specificities, treatment cost and feasibility, due to the fact that the majority of cases of CC are diagnosed in undeveloped countries. Furthermore, novel RCTs with properly designed, widely applicable, treatment strategy for assesment of adjuvant chemotherapy, should be carried out in regions from which majority of targeted population of patients come from, such as countries with lower income

and socioeconomic status. We have to change the underserved title for CC, invest more in the ACT research, and publish more studies in well-defined populations. The results of the OUTBACK trial are not negative, i.e., HR 0.86 for PFS and HR 0.90 for OS with early and constant separation of the PFS and OS Kaplan–Meier curves, which defines the possibility that a significant number of patients do derive benefit from ACT. CC patients' inherent specificities (low socioeconomic background, lower education status, less than optimal health insurance level, less adherence to the suggested therapy) do not help in our quest to solve the issue of the value of ACT. Nevertheless, all these problems should not discourage us but on the contrary, generate more and more well designed and founded trials globally that will, once and for all, define the position of ACT in the LACC treatment approach.

We cannot end this report without stressing once again the lack of investment in CC research, both clinical and basic. On ClinicalTrials.gov, as of 15 November 2021, there were 358 breast cancer registered studies not yet recruiting and not mentioning ACT, and 122 comparable CC studies, but 27 breast cancer studies that mention ACT and only two dealing with ACT in CC in the future. Moreover, when comparing to the breast cancer, the age-adjusted worldwide incidence of CC in 2020 in ≥20-year-old females was 22.1 per 100,000, which was 28% of the breast cancer incidence. However, due to the mortality rate of 12.1 in CC compared to 22.6 in breast cancer, the mortality-to-incidence ratio of CC is almost two times larger than in breast cancer (0.55 vs. 0.28, respectively). Additionally, the CC mortality rate is highly inversely associated with country income level. In low-income countries, the age-standardized mortality rates for CC and breast cancer are almost equal, and the incidence of breast cancer is only slightly higher, whereas in the high-income countries, the incidence of breast cancer is markedly larger than the incidence of CC in comparison to mortality (Table 2). In addition, it can be argued that not only are CC patients underserved, but also CC as a cancer in general. Regarding the total number of manuscripts published between 2016 and 2020 and indexed in MEDLINE (PubMed), reporting on the results of randomized controlled trials (RCT) and mentioning ACT in the title, abstract or keywords represented 2% of all RCT in CC, while representing 18% in breast cancer [55]. Unfortunately, this translates into slow development of the field, leading to unsatisfactory treatment outcomes, especially in LACC and, together with a lack of successful treatment options for metastatic disease, to an unacceptable mortality rate. Instead of traditional, variable-oriented analysis, we think that a future, person-oriented analysis such as finite mixture modeling or latent class analysis is necessary, which will subsequently lead to the recognition of subpopulations of patients with different profiles of predictive and prognostic factors and different expected ACT effects. In our opinion, such analysis is more than needed, especially in the era of precision oncology, as it represents a combination of the traditional approach based on population averages (including the prediction or prognosis modeling) and personalized oncology focused on the individual.

Table 2. Breast and cervical cancer epidemiology 2020, in ≥ 20-year-old females, age-standardized rates per 100,000 [55].

	Worldwide	Income levels				Continent					
		Low	Low middle	Upper middle	High	Asia	Europe	Northern America	Latin America	Africa	Oceania
Incidence											
Breast	79.7	56.2	51.6	73.3	135.0	61.3	123.8	149.0	86.5	67.8	146.3
Cervix	22.1	39.7	28.2	21.2	13.9	21.1	17.7	10.2	24.7	42.7	16.8
Ratio cervix to breast	0.28	0.71	0.55	0.29	0.10	0.34	0.14	0.07	0.29	0.63	0.11
Mortality											
Breast	22.6	30.5	24.5	20.2	21.5	19.9	24.7	20.9	22.5	32.3	24.5
Cervix	12.1	29.0	17.7	10.8	4.2	11.7	6.3	3.5	12.6	29.4	7.7
Ratio cervix to breast	0.54	0.95	0.72	0.53	0.20	0.59	0.26	0.17	0.56	0.91	0.31
Mortality-to-incidence ratio											
Breast	0.28	0.54	0.47	0.28	0.16	0.32	0.20	0.14	0.26	0.48	0.17
Cervix	0.55	0.73	0.63	0.51	0.30	0.55	0.36	0.34	0.51	0.69	0.46
Ratio cervix to breast	1.93	1.35	1.32	1.85	1.90	1.71	1.78	2.45	1.96	1.45	2.74

4.3. Limitations

The main limitation of our analysis could be considered that the results of the OUTBACK trial have not yet been published in their final form. In our manuscript, all interpretations of possible causes of inconsistencies of the OS in different populations were bivariable, while no predictive or prognostic factor truly exists in the isolation from other factors. The number of relevant RCTs was too small for valid multivariable meta-regression analysis, but the individual patient data should be analyzed in this way by the authors of particular trials. The OUTBACK trial authors conducted a multivariable analysis of the patients' characteristics associated with not starting ACT [42]. The overall effects, i.e., differences in OS between subpopulations of patients, should be analyzed in a comparable way. We did not take into account RT parameters, although they may affect OS. To critically appraise the OUTBACK trial, we have compared it to the studies with quite different interventions. In our search of the number of manuscripts indexed in MEDLINE, and reporting the results of RCT mentioning and not mentioning ACT, we did not check whether each record really reported on ACT or just mentioned it in some other role. For this reason, all the figures we presented are exaggerated. However, there is no reason to believe this exaggeration is different between manuscripts on breast or cervical cancer, and our estimates may be considered the best-case scenario.

5. Conclusions

Due to the relatively small number of RCTs, their methodological diversities, particularly in terms of intervention, and after including the OUTBACK trial in the analysis, our conclusion is that its results should not represent the final verdict and close the subject of ACT in LACC. Moreover, there is an absolute need for further research in order to optimally define the position of ACT in the treatment of LACC.

Author Contributions

D.Č.: Conceptualization, Methodology, Investigation, Data Curation, Writing—Original Draft, Writing—Review and Editing, Project Administration; T.B.J.: Investigation, Writing—Review and Editing; M.B.F.: Investigation, Writing—Review and Editing; K.T.: Investigation, Writing—Review and Editing; Ž.B.: Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Writing—Original Draft, Writing—Review and Editing, Visualization; E.V.: Conceptualization, Methodology, Investigation, Writing—Original Draft, Writing—Review and Editing, Supervision, Funding Acquisition. All authors have read and agreed to the published version of the manuscript.

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The authors confirm that the data supporting the findings of this study are available within the article.

Conflicts of Interest

Dora Čerina, Tihana Boraska Jelavić, Matea Buljubašić Franić, Krešimir Tomić, Žarko Bajić, and Eduard Vrdoljak declare that they have no competing interests.

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6. SUMMARY OF JOINT ARTICLES

Together, articles cover important topic of implementation of precision oncology in everyday clinical practice of gynecological cancers. They address availability and utility of CGP, its position in daily work, as well as the optimal treatment for both locally advanced and metastatic cervical cancer. The greatest value is that they were, aside from the review article, conducted on a national level and they provide real-world data on the matter abovestated. Moreover, first study confirmed phase III randomized control trial results in the real-world setting, showing that chemotherapy plus bevacizumab is optimal treatment for metastatic cervical cancer. These patients had significantly longer unadjusted OS with a median of 27.0 (95% CI 18.5; not calculable) months, compared to 15.5 (10.7; 30.1) months in patients only treated with chemotherapy. Also, PFS was significantly longer for the chemotherapy plus bevacizumab cohort, with a median of 10.6 (95% CI 8.5; 15.4) months, than for the chemotherapy-alone cohort, with a median of 5.4 (95% CI 3.9; 9.1) months. In addition, better objective disease control rate and a similar toxicity profile was observed. These results may indicate utility and potential cost-effectiveness of bevacizumab in other parts of the developing world where it is still not reimbursed. On the other hand, review article deals with the optimal treatment of locally advanced cervical cancer and brings current state of knowledge regarding the topic. It also challenges results of the OUTBACK trial and through calculation of pooled hazard ratio, which is improved when results from OUTBACK are combined with results from other RCT`s (0.95 vs 1.00), indicates that there is high proability that subgroup of patients derives benefit from adjuvant chemotherapy and that further research is needed. The other two studies, also conducted on national level, present real-world data of application and utility of comprehensive genomic profiling. One was conducted in patients with metastatic uterine cancer and it proves high mutational load of uterine cancer, with at least one genomic alteration found in almost every patient tested. Also, the vast majority of patients (84%) had clinically relevant genomic alterations, and the most common were PIK3CA, ARID1A, and PTEN. Furthermore, high tumor mutational burden (TMB; ≥10Muts/Mb) was reported in 12 patients (38%), while highly unstable microsatellite status was reported in 10 patients (31%). This points out that one third of patients would potentially have benefit from treament with checkpoint inhibitors. Regarding alteration-driven therapies, some kind of targeted therapy was reported for 28 patients (88%). Second study with CGP analysis was conducted in patients with locally advanced or metastatic ovarian cancer and it showed that all patients had at least one genomic alteration. The median loss of heterozygosity was 14.6 (IQR 6.8–21.7), with 35 patients (41%) having an LOH ≥ 16 and positive BRCA status was found in 22 patients (26%). Conventional testing, which detects only BRCA mutations, would have opted for therapy with PARP inhibitors in 22 (26%) of our patients, while CGP revealed the need for PARP inhibitors in 35 patients (41%). The results identified a significantly higher number of women who would achieve a possible benefit from targeted therapy. Altogether, articles set path for personalized approach to a patient diagnosed with the cancer of female reproductive system.

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8. SAŽETAK

Znanstveno-tehnološki napredak te razvoj preciznih dijagnostičko-terapijskih metoda, kao i ciljanih lijekova doveo je do fundamentalnih promjena u pristupu bolesniku te je upravo pojedinca smjestio u fokus medicine. Samim tim podatci iz stvarne kliničke prakse postaju sve važniji i do novih saznanja se dolazi učeći od svakog bolesnika. Personalizacija dolazi najbolje do izražaja u onkologiji koja je trenutno najpropulzivnija grana medicine. Unatoč tome, postoje oprečna mišljenja o aplikaciji precizne medicine u svakodnevnom onkološkom kliničkom radu. Proveli smo nekoliko studija na razini države kod bolesnica dijagnosticiranih s ginekološkim tumorom kako bismo ispitali stvarnu poziciju precizne medicine u onkologiji. Ginekološki tumori, točnije tumor vrata maternice, tijela maternice te jajnika, predstavljaju značajan javno-zdravstveni problem u svijetu. Cilj studija je bio opisati personalizirani pristup bolesnici oboljeloj od tumora ženskog spolnog sustava. Dvije studije su pokazale korist sveobuhvatnog genskog profiliranja kod bolesnica s lokalno uznapredovalim i metastatskim rakom jajnika te metastatskim rakom maternice. Sukladno nalazu SGP-a trećina bolesnica s metastatskim tumorom maternice bi imala potencijalnu korist od imunoterapijskog liječenja inhibitorima kontrolnih točaka te je otkriveno gotovo 30% više bolesnica s tumorom jajnika koje bi imale potencijalnu korist od terapije PARP inhibitorima. Nadalje, studija koja je također provedena na državnoj razini je podatcima iz stvarne kliničke prakse dokazala kako je optimalno liječenje metastatskog raka vrata maternice kemoterapija uz bevacizumab i time je potvrdila rezultate registracijske kliničke studije faze III. Navedeno je iznimno važno za druge zemlje u razvoju koje nemaju bevacizumab odobren u navedenoj indikaciji. Naposljetku, pregledni članak donosi trenutna saznanja o liječenju lokalno uznapredovalog raka vrata maternice te donosi kritički osvrt na OUTBACK studiju čiji su rezultati etablirali negativan stav o adjuvantnoj kemoterapiji u liječenju istog. Analizom rezultata OUTBACK studije i izračunom združenog omjera rizika svih randomiziranih kliničkih studija, evidentno je kako postoji podskupina bolesnica koja bi potencijalno imala korist od primjene adjuvantne kemoterapije. Stoga njegovi rezultati nebi smjeli biti definitivni te postoji potreba daljnjih istraživanja kako bi se došlo do optimalne strategije liječenja lokalno uznapredovalog raka vrata maternice.

9. SUMMARY

Due to the scientifical and technological evolution, development of precise diagnostic-therapeutic tools, such as comprehensive genomic profiling, as well as targeted therapy, approach to patient is dramatically changing and medicine is turning towards an individual. Also, the emphasis is put on the real-world data and learning from every patient individually. This personalization is most noticeable in oncology, as it is the most propulsive branch. However, its application in everyday clinical practice is still debatable. In order to question the real position of precision medicine in oncology, we conducted several studies on a national level in patients diagnosed with gynecological cancer. Gynecological cancers, precisely uterine, cervical and ovarian cancer, present the major public and health burden worldwide. The aim was to set path for personalized approach to a patient diagnosed with the cancer of female reproductive system. Two studies have shown utility of CGP as the backbone diagnostic and decision-making therapeutic tool for locally advanced and metastatic ovarian and uterine cance. CGP revealed one third of patients with metastatic uterine cancer who would potentially have benefit from immunotherapy with checkpoint inhibitors and it revealed almost 30% extra patients in ovarian cancer who would potentially benefit from PARP inhibitors. One study conducted on a national level as well has proved the results from phase III RCT in the real-world setting, regarding the optimal treatment of patients with metastatic cervical cancer, which is bevacizumab and chemotherapy. This is particularly important for other developing countries where bevacizumab is still not reimbursed. Meanwhile, review article presents current knowledge regarding the treatment of locally advanced cervical cancer with critical appraisal of the OUTBACK trial which results affirmed negative recommendation on adjuvant chemotherapy. Challenging its results and after calculating pooled hazard ratio from all RCT`s, there is subgroup of patients who would potentially have benefit from adjuvant chemotherapy. Thus, there is absolute need for further research in order to optimally address matter of adjuvant chemotherapy in locally advanced cervical cancer.

10. CURRICULUM VITAE

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