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With this excitement we are ready for the next publications of **2023** and once again we need your precious help, to be able to grow even more ***ARO*** in the name of scientific excellence.

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NARRATIVE REVIEW

EWING SARCOMA: A REAPPRAISAL

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ABSTRACT: Ewing sarcoma (ES) is an aggressive sarcoma of bone and soft tissues arising predominantly in children and young adults. Current management of primary ES relies on a multimodality approach, coupling intensive cytotoxic drugs regimens with surgery and/or radiotherapy. The combination of primary site control and possible metastatic disease resulted in increased survival rates in localized disease, at the expense of substantial acute and long-term toxicity. Contrarily, the prognosis is still dismal in the metastatic setting, especially for patients with recurrent ES. Indeed, the lack of an effective treatment strategy after a first-line chemotherapy represents an unmet clinical need. The aim of this study is to examine the current treatment options and discuss potential future perspectives that could answer to the key issues in the management of ES.

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Impact statement: This study reports the current management of Ewing sarcoma on a multimodal approach, coupling intensive cytotoxic drugs regimens with surgery and/or radiotherapy. It examines the current options and discuss potential future prospective.

Key words: *Ewing's sarcoma; sequential therapy; multidisciplinarity; new perspectives.*

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INTRODUCTION

ES is a rare tumor developed in bone and in soft tissue. The incidence ranges from 0,1-1 cases/million. The peak of incidence is between infancy and early adulthood (5-24 years old) (1-4). However, adults and elderly people can also be affected, mainly in extraosseous tissues. ES is considered in most situation a systemic disease from the beginning (5, 6) which, in absence of therapy, lead the patient to death in 90% of cases for metastatic disease. Despite multimodal and multidisciplinary approaches, metastatic disease occurs in 30% of the patients predominantly in the lungs (70-80%), bone marrow and bone (45-49%), soft tissue, liver, and brain (5-8). The cell of origin of ES is not well recognized, however the mesenchymal stem cell is the most accepted between geneticist and pathologists (9-14).

The most important molecular event in ES's cells is the chromosomal translocation between ETS and FET genes, with a reciprocal translocation (t 11;22) (q24; q12). The result is EWS/FLI 1 oncogenic gene fusion (15, 16). The product of EWS/FLI 1 gene fusion leads to gene regulation with activation and repression of thousands of other genes (15-18).
Other chromosomal variant is copying member variation and point mutations (STAG2, TP53, Rb1) but their real meaning is not definitively known (15, 19, 20) (**Table 1**).
The 2020 World Health Organization (WHO) classification of sarcomas calls "Ewing Sarcoma Family" the same nosological entity with similar morphological aspects, but with multiple chromosomal abnormalities: they have different prognosis and

Table 1. *Genes and genes variation related to ES.*

	GENES	FUSION PROTEINS	PREVALENCE
EWING sarcoma	FET-ETS	EWSR1-FLI1	85%
		EWSR1-ERG	10%
		EWSR1-FEV	<1%
		EWR1-ETV1	<1%
		EWSR1-ETV4	<1%
Ewing like sarcoma	FET (no ETS)	EWSR1-SP3	<1%
		EWSR1-PATZ1	<1%
		EWSR1-SMARCA5	<1%
		EWSR1-POU5F1	<1%
		EWSR1-NFATc2	<1%
		FUS-NFATc2	<1%
Ewing like sarcoma	no FET	BCOR / $CCNB_3$	<1%
		BCOR / $MAML_3$	<1%
		BCOR / ITD_5	<1%
		ZC3H7B-BCOR	<1%
		CIC / $FOXO_4$	<1%
		CIC / DUX_4	<1%
Atypical Ewing sarcoma	FET-ETS	FUS-ERG	<1%
		FUS-FEV	<1%

susceptibility to the therapy (see **Table 1**) (15, 16). Although ES is a high-grade tumor, it is characterized by low mutational burden. This is of fundamental importance in the therapeutic strategy.

GENERAL CONSIDERATION IN THERAPY

In case of osseous or extraosseous mass, a plain X ray of the segment, followed by CT scan and/ or MRI of the anatomical area is recommended. If the suspicion is confirmed a tru-cut or an incisional biopsy is mandatory. If ES diagnosis is confirmed a complete disease staging with whole body CT scan, PET CT scan is required. Some protocols suggest bone marrow biopsy to exclude bone marrow dissemination.

ES family recognize a localized in confront with a disseminated, metastatic disease. All decision about the ES therapeutic management should be taken in a multidisciplinary multitask group including at least Radiologist, Pathologist, Orthopedic, Surgeon, Radiotherapist and Oncologist (16, 21, 22).

The prognosis of the localized disease is determined by many factors: age and Performance Status of the patient, tumor volume (>200 ml), tumor site (better in the acral part of the skeleton than in the axial part; better osseous than extraosseous) (16, 22).

Following the guidelines (16, 22) in localized ES the most effective strategy involves a general approach with chemotherapy to reduce both the volume of the primary tumor and the risk of micrometastasis.

The local phase include surgery -± radiotherapy. Surgery is the preferred approach and leads to a higher local control of the disease. The surgical margin is a fundamental marker of radicality. Margins must be wide enough for oncological control but must respect the function either in osseous or in extraosseous ES (22, 23). Despite ES radiosensitivity, radiotherapy as unique modality of local treatment results in a high incidence of local recurrence and an increased risk of long-term toxicity (secondary bone tumor, osteoporosis, bone fractures).

An exclusive radiotherapy treatment can be considered in non-operable disease: large volume (>200 ml), pelvic or spine localization, poor histological response to neoadjuvant chemotherapy or extraosseous localization. The recommended dose is 54-55 Gy to the tumor in fractionated schedule (24-27). Proton beam therapy is a new radiotherapy technique that seems to increase the percentage of local control and reduce the risk of toxicity, particularly in the youngest patients, but more data on proton therapy compared with traditional radiotherapy are needed (22, 28).

As demonstrated by many studies (16, 22, 29) perioperative chemotherapy with neoadjuvant and adjuvant approach is strongly suggested. Before 1980 ES was treated solely with surgery and radiotherapy and about 95% of the patients died from disease (30). The neoadjuvant/adjuvant strategy has a double end point: better local control and reduction of metastatic relapse.

With the combination including Vincristine, Doxorubicin, Ciclophosphamide, (VDC) ± Ifosfamide and Etoposide, five years overall survival in localized disease increased from zero to 50-80% (31-33). The addition of Ifosfamide and Etoposide (EI) to VDC regime increased 5 years survival to 60-70% always in localized disease (34-38). Some recent European trials assessed efficacy and safety of other regimens such as VIDE (Vincristine, Ifosfamide, Doxorubicin and Etoposide) as induction therapy followed by VAI (Vincristine, Actinomycin D, Ifosfamide) or VAC (Vincristine, Actinomycin D, cyclophosphamide) as consolidation (see **Figure 1**) (39). Some attempts were done with dose density schedule recycling every 14 days. A higher percentage of local response were recorded, but with a very high level of toxicities.

Similarly high-dose chemotherapy with bone marrow or stem cell transplant did not increase the percentage of event free survival (EFS).

In conclusion the duration of chemotherapy (at least 30 weeks) is more important than the dose intensity or dose density (40). In **Table 2** we resume the most common sequences in ES trials.

METASTATIC DISEASE

The prognosis of refractory disease (less than 90% of tumor necrosis after chemotherapy in local disease) or recurrent/metastatic ES remains very severe. The 5 years survival is 22% for recurrent ES and is less than 10% for metastatic disease.

Two different population are recognized:

1. poor responders to chemotherapy after local treatment (<90% necrosis).
2. de novo metastatic disease or relapse.

To date no standard treatment has been defined for metastatic disease and the role of surgery in local relapse or limited pulmonary metastasis (1-2 nodules) is not completely understood.

Many combinations including drugs not used in neoadjuvant/adjuvant setting are proposed: Temozolomide + Irinotecan (46), Topotecan + Ciclophosfamide (47), Docetaxel + Gemcitabine (48), High-dose Ifosfamide (49).

All these drugs have been evaluated in phase II trials and only once comparative study is available (49).

Objective responses range from 0 to 60 % but they don't translate into a longer survival, especially for

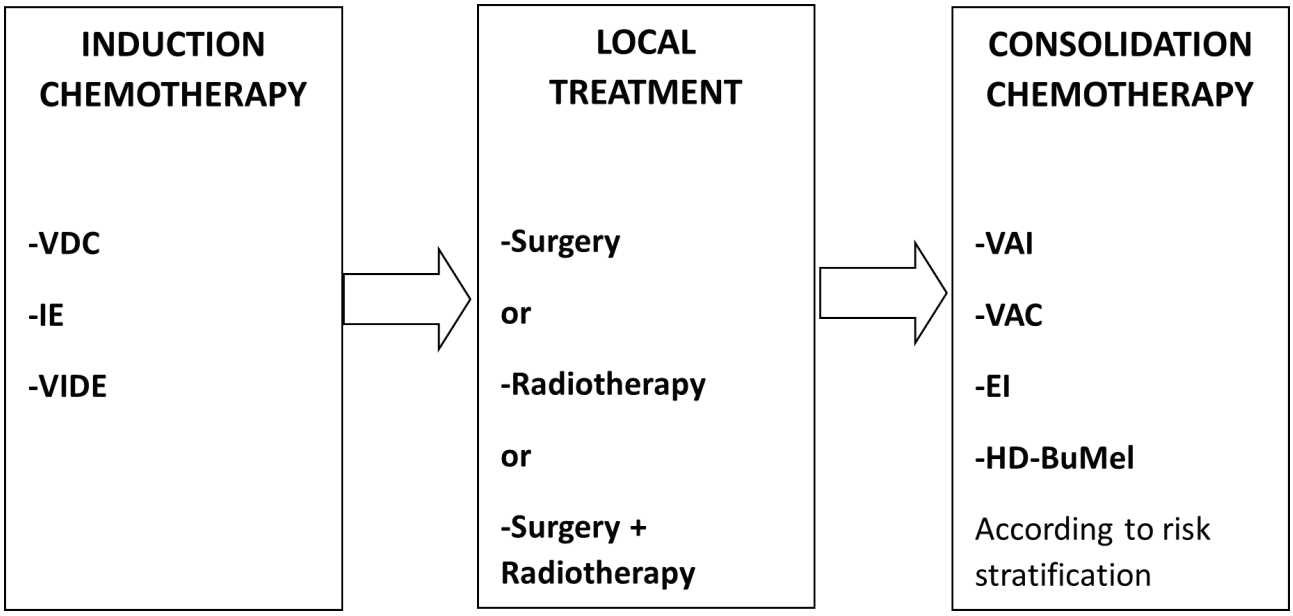

Figure 1. *Sequential therapy in localized ES.*
Abbreviations: VDC: Vincristine/Doxorubicin/Cyclophosphamide; IE: Ifosfamide/Etoposide; VIDE: Vincristine/Ifosfamide/Doxorubicin/Etoposide; VAI: Vincristine/Actinomycin D/Ifosfamide; VAC: Vincristine/Actinomycin D/Cyclophosphamide; HD-BuMel: High Dose Busulfan/Melphalan and Stem Cell Rescue.

Table 2. *The most important trials in localized ES treatment.*

AUTHOR/TRIAL NAME	YEAR	CHEMOTHERAPY	RESULTS
Grier/INT-0091 (41)	2003	VDC *vs*. VDC/IE	5 y EFS 54% *vs*. 69% with addition of IE in localized ES
Kolb/MSK (42)	2003	VDC/IE with augmented Cyclophosphamide	4 y EFS 82% in localized ES
Granowetter/INT-0154 (43)	2009	VDC/IE with augmented alkylating doses vs standard VDC/IE	No improvement in outcomes with augmented doses
Womer/AEWS0031 (44)	2012	cVDC/IE	6 y EFS improved to 73% from 65% with compressed chemotherapy for localized ES
Dirksen/Euro-E.W.I.N.G.99 and Ewing 2008 (45)	2019	VAI vs VAI/HD-Bu-Mel	No clear benefit from BuMel in high-risk ES
Brennan/Euro Ewing 2012 (29)	2022	VIDE induction +VAI/VAC (or VIA/HD-BuMel) *vs*. VDC/IE induction + IE/VC (or VAI/HD-BuMel)	3 y EFS 67% *vs*. 61% in the VDC/IE induction group

Abbreviations: VDC: Vincristine/Doxorubicin/Cyclophosphamide; IE: Ifosfamide/Etoposide; cVDC: compressed VDC; VAI: Vincristine/Actinomycin D/Ifosfamide; HD-BuMel: High Dose Busulfan/Melphalan and Stem Cell Rescue; VIDE: Vincristine/Ifosfamide/Doxorubicin/Etoposide; VAC: Vincristine/Actinomycin D/Cyclophosphamide.

early recurrences (<2 years from the end of adjuvant chemotherapy). A group apart is the solitary lung metastasis that removed can guarantee 5 years survival in the 30% of patients.

The European study rEECur (49) with multistage design comparing Temozolomide + Irinotecan, Topotecan + Ciclophosfamide, High-dose Ifosfamide and Docetaxel + Gemcitabine showed that High-dose Ifosfamide is the most effective treatment in Overall Survival (OS). Sites of recurrences were replapsed in the primary site (15%), pleuropulmonary (34%), another site or multiple (51%). High-dose Ifosfamide did better: EFS 5.7 months and OS 16.8 months were the results. Better survival was recorded in teens <14 years old.

NON-CHEMOTHERAPY DRUGS

At present no target therapies are recognized as part of active treatment in relapsed ES.

Cabozantinib 40 mg/m^2 in children and 60 mg/m^2 in adults was investigated in a French study (57). This drug is MET and VEGFR$_2$ inhibitor, two products of oncogene demonstrated in ES cancerogenesis.

Forty-five patients with relapsed/pre-treated ES were recruited. Ten patients had PR (25.6%) and 13 (38.4%) SD.

At 6 month follow up 25.6% of patients were progression free. Neutropenia, hand and foot syndrome, transaminases increase was recorded. Despite the positive results of this study Cabozantinib is not approved by European Medicine Agency (EMA) in pre-treated ES (**Tables 3, 4**).

IMMUNOTHERAPY

In such distressing panorama many efforts have been made for the implementation of immunotherapy in relapsed or metastatic ES. However, check point inhibitors (ICIs) (anti PD1, anti PDL1 and anti CLTA4) offered very poor results.

Tawbi (58) *et al*. in their study did not record any objective response. Few trials are still on going with different ICIs than Nivolumab.

Some T-cell based therapies are investigated in Clinical Trials against cell surface target as EGFR, HER$_2$, ROR$_1$, IGF$_1$R. To date, no drug has been approved for relapsed ES (**Table 3**).

INVESTIGATIONAL APPROCHES

The rationale for experimental alternative treatment is based on ES molecular pathogenesis, particularly on the implication of a unique molecular driver in cell transformation. The target inhibition of EWS-FLI1 has been showing promising results in a phase I/II trial investigating the molecule YK-4-279 (TK216), designed to bind ETS proteins directly, disrupt protein interactions, inhibit

Table 3. *Target therapy drugs.*

TARGET	DRUG
CD-99	Anti CD-99 mAb
PARP	PARP INHIBITORS
GD2	CAR anti GD2
VEGFR	Bevacizumab
IGF1R	Anti IGF1R
RANK	Zoledronic acid

Table 4. *Principal drug cited in different study to treat ES with results.*

DRUG	AUTHOR-YEAR	RESULTS
Figitumumab	Olmos-2010 (50)	2/16 PR
Figitumumab	Juergens-2011 (51)	14% ORR
R1507 anti IGF1R	Pappo-2011 (52)	10% ORR
Ganitumab	Tap-2012 (53)	6% ORR
Olaparib	Choy-2014 (54)	No ORR
Talazoparib + Temozolamide	Schafer-2020 (55)	No ORR
Talazoparib + Irinotecan ± Temozolamide	Federico-2020 (56)	1 CR – 4 PR
Cabozantinib	Italiano-2020 (57)	26% ORR

transcription factor function, and cause apoptotic cell death (59). Yet, the development of drug resistance through clonal selection is inevitable, theoretically limiting its long-term efficacy (60). In this context, preclinical data showed that cancer stem cells plasticity and tumor heterogeneity can be reverted by correcting the defective TARBP2-dependent microRNA maturation by exposure to the fluoroquinolone enoxacin (61). Riggi *et al*. in their recent study explored the potentially targeted molecular and epigenetic mechanism by which EWS and its partners promote cell transformation (62). The modification of chromatin structure and DNA accessibility to transcription factors seems to play a major role. EWS-FLI1 controls the epigenetic regulation of gene expression through different molecular pathway: the recruitment of the major ATP-dependent chromatin-remodeling complex SWI/SNF (switch/sucrose non-fragmentable), also known as BAF (BRG1/BRM-associated factor), which allows DNA accessibility; the reduction of DNA methylation and the inhibition of microRNAs that promotes differentiation (63). The encouraging data on the activity of KDM1A, a demethylase identified to regulated chromatin states through the removal of mono- and dimethyl group, from in vitro studies led to the active development of several small-molecule KDM1A inhibitors in different solid and hematological tumors (64). As KDM1A is overexpressed in ES tumors, directed KDM1A inhibition was first tested in vitro with the use of non-competitive KDM1A inhibitor SP-2509, showing a dramatic reversal of both the up- and downregulated transcriptional profiles of EWS/FLI and EWS/ERG accompanied by the induction of apoptosis and disruption of morphologic and oncogenic phenotypes modulated by EWS/FLI (65, 66). This evidence opened the door to dedicated currently ongoing Clinical Trial. A phase II Trial was designed to test the activity of the IGF-R targeted monoclonal antibody R1507 in patients with recurrent of refractory sarcomas. Although IGFR signaling pathway is implicated in the transformation of fibroblast and is known to support EWS/ETS-driven oncogenesis, its inhibition led to disappointed results (67, 68). Poly (adenosine diphosphate-ribose) polymerase inhibitors (PARPi) have been showing considerable efficacy in different clinical settings, especially in in tumors with homologous recombination deficiency. The high expression of PARP in ES cells led to test PARPi in clinic with scarce results with Olaparib. Trial testing the combination of chemotherapy with PARPi are still ongoing (69). Finally, encouraging early phase data comes from the innovative field of the Chimeric Antigen Receptor (CAR) cells therapies (70). In **Table 4** are resumed some of these studies.

CONCLUSIONS

To date, polychemotherapy remains the cornerstone treatment for ES both in local and metastatic/relapsed disease. The multidisciplinary approach has significantly improved OS for localized disease, with 60-70% 5 years survival using neoadjuvant/adjuvant therapies associated with surgery and or radiotherapy. On the contrary, despite the multimodal treatment, survival in metastatic disease is less than 10% at 3 years.
Characteristically ES is recognized for a specific translocation EWSR1 on chromosome 22 to site 1 gene (FLI1) on chromosome 11: the fusion EWS/FLI1 is fundamental for tumorigenesis in ES and theoretically it could be a specific target for a future effective therapy. Unfortunately, neither this specific translocation nor different mutations seems to be a reliable target for therapies. A great effort is required in ES, as well in other bone and STS, to identify the cell of origin, different and druggable molecular activities, not only in "time" ES but also in every subtype of round cell sarcomas family. Within the same morphological aspect multiple entities with different biological and clinical behavior have been recently described, and treatment strategies should be increasingly targeted. The ancient chemotherapy regimens included in VCD ± EI combination has allowed a great deal in localized disease, but the substantial toxicity of the standard therapies and the limited options for patients with recurred ES still represent an unmet need.

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REVIEW

ANIMAL SENTINELS AND CANCER REGISTRIES: STATE OF THE ART AND NEW PERSPECTIVES

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ABSTRACT: The concept that animals may serve as sentinels of environmental hazards is not new. Nowadays, it has been widely recognized the important role that pets have in the field of comparative oncology. Cancer epidemiologic data are the foundation for prevention and control; therefore, Cancer Registries represent a fundamental tool that systematically collects, and stores validated and comprehensive data. In Veterinary Medicine, cancer registries have unfortunately been sporadic, short-lived, and lacked communication and collaboration. Therefore, there is little up-to-date information available on the incidence of different types of cancer in companion animals anywhere in the world. The purpose of this review is to provide a brief overview of the currently and globally active veterinary cancer registries. Moreover, a special focus will be dedicated to a novel web-based cancer registration system implemented in the Department of Veterinary Medicine in Naples, Italy. This platform was designed and conceived for the implementation of the regional Animal Cancer Registry, for a constant evaluation of the frequency, the incidence and/or the prevalence of cancer cases in pets and for advancement in the field of veterinary comparative oncology.

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Impact statement: Animal Cancer Registries are a fundamental instrument for advancement in the field of veterinary comparative oncology. Epidemiologic data on cancer in companion animals can help to identify new environmental hazards.

Key words: *Animal Cancer Registry; comparative oncology; sentinel animals, web-based cancer database*

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INTRODUCTION

The presence of environmental contaminants in air, water and food may represent significant health risks for human population. Several environmental toxicants have been proven by scientific studies as important risk factor for chronic diseases and cancer. In some cases, solid scientific evidence has determined that the exposure to a specific environmental compound is the cause of a specific disease (*i.e.*, asbestos exposure and mesothelioma) (1). However, the causality between environmental hazards and diseases is often difficult to be proved. Several authors suggest that human epidemiologic studies may be usefully complemented by similar studies conducted on sentinel animal species in order to prevent and overcome confounding factors such as chronic low-dose exposure, multiple exposure routes, long latency periods and non-specific health outcomes (2, 3). In 1960, the publication of the book Silent Spring by Rose Carson firmly introduced the concept that the exposure to environmental hazards may determine adverse effects on the health of animal population as well as humans (4). Since both humans and ani-

mals may develop disease through exposure to environmental hazards, it has been suggested that animals, like the "canary in the coal mine" could serve as natural sentinels for human health providing crucial information about the relationship between environmental hazards and human health risk (2, 5-8). Due to differences in body weight and metabolism from humans, animals may be more susceptible than humans to particular hazards (2, 5-8). Animals also tend to be less mobile and to be exposed to higher levels of a given environmental hazard, compared to humans living nearby who may be actively modifying exposures through clothing, buildings, and dietary choices. Furthermore, animals usually have shorter life spans than humans and therefore may exhibit a shorter latency for development of an environmentally induced condition (2, 5-8). Significantly, cancer develops naturally in dogs within the environment they share with their human owners, therefore cancer initiation and progression in dogs are influenced by similar risk factors including age, diet, sex, household conditions and pollution (2, 3). Moreover, cancer in animals shares several traits with its human counterpart such as histological features, genetic alteration, biological behavior and, most importantly, cancer biology (2, 3). This concept is the foundation of comparative oncology, an emerging and quickly expanding field of research that has the purpose of studying cancer risk and tumor development across different species and to provide a suitable model for advancing of the understanding, diagnosis, and management of cancer in humans (6, 9). Alas, the application of Sentinel Animal Systems in obtaining data about environmental monitoring and human health risk is not free from uncertainties and ambiguity. One of the main limitations includes the results interpretation, which can be difficult and controversial if not compared objectively and reasonably to human health hazard risks (10). Moreover, the use of Sentinel Animal Systems is usually limited by the lack of standardized methods in researching programs, thus, on large scale, misleading and inadequate collection of data in well-structured and efficient database (10). Cancer data are the foundation for prevention and control; therefore, Cancer Registries (CR) represent a fundamental tool that systematically collect, and stores validated and comprehensive data that include patient personal information (sex, age, date and place of birth, residence) and cancer-related data such as the characteristics of the individual tumor (location, morphology, grading, stage and behavior) and eventually the availability of the screening status (11). This information is extremely useful for both clinicians and epidemiologists because they allow the analysis and the interpretation of data providing information that may be successfully used for health care planning and monitoring, for carrying out evaluations of the effectiveness of cancer screenings (11) and even more for the implementation of prevention measures. The sources from which the information about cancer patients is collected define three different types of Cancer Registries, each one with proper advantages and drawbacks. Hospital-based Cancer Registry (HCR) and Pathology-based Cancer Registry (PCR) collect all medical records from patients from a given hospital or diagnostic laboratory, respectively (12, 13). The information held by HCR and PCR are extremely important for improving the clinical management and human resources needed to support patient care and also to better understand the diagnostic capacity of the institution and country where they are located (12, 13). However, neither HCR or PCR are reflective of the area and/or the overall population from which the cases arise, thus several cancer cases may be missed affecting epidemiological studies and the estimation of tumor incidence rates (12, 13). Population-based Cancer Registry (PBCR) records all new cancer cases generated by hospital and pathology-based cancer registries in a well-defined and enumerated population (generally the population in a specific geographical area in which these cancers are occurring) (14). Population-based Cancer Registries are considered the gold standard in human cancer epidemiology (14), moreover they are extremely important in accessing to mortality information needed for the calculation of survival rates screening and the assessment of treatment programs efficacy in reducing cancer deaths. Currently, human cancer registries are regulated by law and their establishment and maintenance have been (and still are) complex and time-consuming (14). In Veterinary Medicine, cancer registries have unfortunately been sporadic, short-lived, and lacked communication and collaboration (9, 14-16). Therefore, there is little up-to-date information available on the incidence of different types of cancer in companion animals anywhere in the world (9). Several initiatives to set up veterinary cancer registries have been developed since the early 1960s (16), but numerous veterinary cancer registries have been discontinued for limited funding, for the non-mandatory nature of animal cancer case reporting, and for the serious issues in enumerating the background population (15). In recent years, we

are witnessing to a rising revived interest in veterinary cancer registries and several initiatives have been taken for the activation and/or implementation of regional, national and international databases for animal cancer registration (9).

The purpose of this review is to provide a brief overview of the current and active veterinary cancer registries, with a special focus on a novel web-based cancer registration system implemented in the Department of Veterinary Medicine and animal production of Federico II University of Naples to support the Campania Animal Cancer Regional Registry in Italy and that was designed and conceived as an integrated part of the regional Animal Cancer Registry.

ANIMAL CANCER REGISTRIES: INTERNATIONAL STATE OF THE ART

The Vet Cancer Registry is an international, free, and web-based data collection point for confirmed veterinary cancer cases that was initiated in 1994 with the development of the International Veterinary Brain Tumor Registry (VIBTR). This service is still active and cases with confirmed diagnoses have been registered from all over the world (16). In the United States, the Veterinary Oncology Market Committee from the Veterinary Cancer Society (VCS) has recently started a collaboration with large national laboratories to establish incidence data for a variety of neoplasms in pet animals (17). In 2013, the first Animal Cancer Registry in Latin America was created in Brazil with the Sao Paulo Animal Cancer Registry (RCA-SP) (18). The RCA-SP is a hospital- and web-based registry that provides clinicians with an electronic medical record system to collect and store pertinent cancer data connected to a central database (18).

In 2020, the Latin American Society of Veterinary Oncology (SLOVET) intends to launch, with Portuguese platform Vet-OncoNet, the first Latin American Veterinary Cancer Registry (17).

In November 2019, The University of Queensland established the ACARCinom network, the first Australia-wide registry of animal cancers that will generate accessible datasets for identifying patterns and trends of cancers in animal using retrospective data from the Veterinary Laboratory Services (17). In Kenya, a collaborative work is ongoing with the human Kenya National Cancer Registry investigating cancers affecting humans and dogs in Nairobi (19).

In the United Kingdom, a pathology-based animal tumor registry was set up within the Small Animal Veterinary Surveillance Network (SAVSNET) run by the University of Liverpool. The collected data derive from anonymous general practice, electronic health records or diagnostic pathology reports and comprise both the tumor description (type and location) and the animal (breed, neutering status and veterinary practice postcode) (15).

ANIMAL CANCER REGISTRIES: STATE OF THE ART IN EUROPE

In Norway, a cancer registration project was initiated for canine cancer in a defined geographical region in 1990 (16). This registry collects, stores and reports information about the geographical distribution, reproduction status, nutritional status, prior hormonal treatment, and concurrent diseases and includes both malignant and benign neoplasms. In the past, the Registry originally provided free histopathological evaluation to practitioners in the area when submitting cases for the registry, but in 1998 the register became a national register and free histopathology is no longer available for the practitioners (16).

In 2005, a new veterinary cancer registry was established at the Royal Veterinary and Agricultural University in Denmark (Danish Veterinary Cancer Registry). Veterinarians voluntarily submit data to this web-based data compilation. It is an incidence registry, and inclusion of a case is based on clinical information, cytology, diagnostic imaging and histopathology when available (16). In Portugal, a platform named Vet-OncoNet has been created in order to share information on companion animal tumors and, accordingly to One Health concept, to contribute to the research in prevention and therapy in animal and human oncology (20). This platform involves researchers from the departments of Population Studies, Veterinary Clinics, Pathology and Molecular Immunology at the Instituto de Ciências Biomédicas Abel Salazar (ICBAS) and the Department of Veterinary Public Health of the Instituto de Saúde Pública da Universidade do Porto (ISPUP). Vet-OncoNet is a replicable tripartite animal cancer database that uses business intelligence tools to optimize the process of capturing, treating, and reporting animal cancer data to a national level in three interfaces: ACR (animal cancer registry, pathology-based), COR (clinical oncology registry, vet practice-based) and RFR (risk

factor registry, owner-based) (20). Furthermore, being aware of the role of animals within the family and as possible sentinels of environmental risks to cancer in humans, the network built an interface (Pet-OncoNet) dedicated to owners and a database (RFR) that receives information regarding pets and owners' daily habits (20).

In Spain, the first national pet cancer registry project is currently being constructed at the School of Veterinary Sciences of the University of Las Palmas de Gran Canaria (17).

The Swiss Canine Cancer Registry comprises diagnostic records of dogs provided by 3 veterinary diagnostic laboratories in Switzerland (the Vetsuisse Faculty Institute for Veterinary Pathology Zürich, the Vetsuisse Faculty Institute for Animal Pathology Bern, and the Zyto/Histo Diagnostik private veterinary diagnostic laboratory) (16). The canine population data originated from the Swiss animal registration database Animal Identity Service ANIS; the registration of resident dogs and the deregistration in case of death or permanently leaving Switzerland has been mandatory since 2007 (17, 21).

ANIMAL CANCER REGISTRIES: STATE OF THE ART IN ITALY

In Italy, the Animal Tumor Registry (ATR) has been an increasing reality throughout the national territory (22). The first Registry was established in the province of Genoa in 2008 (23), but twelve active ATRs are now officially identified, distributed between northern and southern Italy and operating in limited territorial areas and coordinated by the Veterinary and Comparative Oncology Reference Center (CE.R.O.VE.C.) of the Genoa section of the Experimental Zooprophylactic Institute of Piedmont, Liguria, Valle d'Aosta and Genoa. Animal Cancer Registry of the provinces of Venice and Vicenza of the Veneto Region started in 2005 (24). In 2017, incidence data were obtained in Piedmont, a well-delimited geographical area in northwest Italy (25). The RTA of Rome has been active since 2009, through current research projects of the IZS Lazio and Tuscany (IZSLT) in the field of oncology and environmental epidemiology, carried out on pilot areas in the province of Rome. From 2018, all Lazio Region was involved (26). In 2014, the Umbria Region activated the Animal Cancer Registry that involve veterinarians, the Department of Veterinary Medicine of the University of Perugia and the Experimental Zooprophylactic Institute of Umbria and Marche (IZSUM) for diagnosis by double-blind reading and for data processing (22, 27). The same approach and methodology were implemented by Marche Region in 2015 (28). In 2013, Animal Cancer Registry started in both Sardinia and Sicily Regions (22).

ANIMAL CANCER REGISTRY IN CAMPANIA REGION, ITALY

The area of Naples in Campania region, in Italy, is experiencing the dramatic consequences of more than two decades of extensive illegal dumping and burning of mixed waste of urban as well as industrial origin. The illegal and criminal practice of waste burning caused an international toxic-waste scandal that sadly renamed the agricultural landscape of Italy's Campania region as "Land of Fires". In this scenario, the potential risks for environmental, human and animal health prompted the Italian authorities to establish, in 2010, the Regional Centre for Veterinary Urban Hygiene (CRIUV) as the first example in Italy of integration and synergy among the veterinary public health system (ASL), Zooprophylactic Institute and Federico II University of Naples. The mission of the Centre is articulated in several activities aiming to develop strategies for the assessment of risk exposure to environmental pollution in animals and, subsequently, in human population. The CRIUV is the principal site of the Animal Cancer Registry in Campania region (29-31) and regulated its cooperation with the National Center for Veterinary and Comparative Oncology (CEROVEC).

The ACR of Campania region, in southern Italy, was established in 2011 by a regional law as a surveillance and research unit within the CRIUV. The principal aims of Campania ACR are: 1) to collect data and estimate companion animals' cancer incidence on the whole regional territory, 2) understand the natural history of cancer occurrence in pet animals, and 3) eventually compare animal and human cancer incidence data.

Campania ACR aims to be a Population-based Cancer Registry at least for canine tumors; since registration of dog is mandatory and official demographic registry of the canine population established in the Campania region is used as primary source to estimate the amount of the canine population. To date, the total number of dogs registered in Campania is 1.287.747 (32) which represent 1/4 of the human population that an estimated

5.624.420 people in Campania (33). The strategies to prevent possible biases related to failure to comply with the registration obligation and failure to notify deaths are not yet put in place, therefore the accuracy of the registration remains uncertain. Campania ACR recognizes two reference veterinary anatomic pathology laboratories for data collection, namely the anatomic pathology laboratory of the Department of Veterinary Medicine and Animal Production of Federico II University of Naples and the laboratory of the Istituto Zooprofilattico Sperimentale del Mezzogiorno based in Campania Region.

Campania ACR is supported by public funds that allow to provide discount for cytological and histopathological evaluation to veterinary practitioners and veterinary laboratories working in the registry's interest areas. Moreover, all veterinary practitioners in Campania region are constantly informed about the ACR activities by informative campaigns and continuing education courses promoted by veterinary public health services and veterinary professional associations. All veterinary practitioners are invited to submit any suspected neoplasm from animals living in Campania region. A standardized sample submission form is specifically designed for the registration of tumor cases and is available on the website of the two reference laboratories. The sample submission form has blank fields to fill with animal specific information including species, age, sex, breed, neutered/spayed status, and identification number. Residence address of the owner is also included. Tumor data include the tissue, organ and anatomical site, number of samples (number of slides in case of cytology) and the type of biopsy, date of excision, clinical stadiation of the tumor and any related and relevant historical and clinical information. Formalin fixed samples are routinely processed, paraffin embedded and stained with Haematoxylin and Eosin (HE) for histological examination. Immunohistochemistry is also performed in case of poorly differentiated neoplasm. Tumors are classified according to the most recent available classification systems of tumors of domestic animals and coded according to the World Health Organization's International Classification of Disease for Oncology (ICD-O) (9) to eventually facilitate comparisons with existing human and animal cancer registries. This kind of classification and coding is used by Campania ACR and other regional ACR and it is the same used in human medicine according to WHO rules.

In 2020, a web-based database system (Piattaforma myClinical) was set up for the Diagnostic Service of Pathology and Animal Health (DIPSA) of the Department of Veterinary Medicine in Naples, Italy and implemented with specific features that permit the communication among veterinary practitioners and pathologists. The database was designed for the collection and management of information regarding animal cancer patients in Campania; the database was also integrated with a quality-control based data recording that assigns each new case its own code (ID number), thus avoiding duplicating case registrations. Information including species, breed, age, topographic localization of the tumor, clinical and histological characteristics of cancer can be systematically recorded, collected and consequently used for epidemiological analysis. Importantly, the pathologists that analyze the samples have the possibility to insert a morphological diagnosis and to categorize the neoplasms using the last recognized classification system easily and directly for canine tumors associated to the last edition of the International Classification of disease for Oncology (ICD-O) (9). Furthermore, the database is connected to a geographic information system (GIS) that permits the creation of maps providing a spatial distribution of neoplastic diseases. The same web-based database is currently used to collect and store information of cancer animal patients from Molise (since 2020) (34) providing and sharing data to its respective regional animal cancer registry. In our opinion, the herein described platform has multiple applications and can be potentially used to compare animal cancer data and human cancer data laying the fundamentals for a substantial advance in the study of veterinary comparative oncology. Recorded information can be used for the implementation of the Regional Cancer Registry, for a constant evaluation of the frequency, the incidence and/or the prevalence of cancer cases in pets and to compare data with human cancer registries.

PIATTAFORMA myClinical: DATA COLLECTED IN TWO YEARS ACTIVITY (2020-2022)

Based on data collected so far and only using those case reports for which at least some essential information (species, breed, age, sex and neutered/spayed status, topography, and morphological diagnosis) are available, frequencies of tumor topographies and main morphological diagnosis

are described, considering age, breed and sex. Seven age classes are defined (0-3 years, 4-5 years, 6-7 years, 8-9 years, 10-11 years, 12-13 years, and 14 years and more) while breeds are distinct in purebred and not purebred. A declaration of one breed was accepted as reported, while a declaration comprising two breeds (*i.e.*, shepherd-cross, or shepherd-boxer-cross) was categorized as not purebred. To investigate malignancy, each tumor group was divided into benign (behavior code 0-2) and malignant (behavior code 3-9) according to the ICD-O classification (9). When one animal is diagnosed with more than one tumor type or location, these are collected as separate events. This descriptive analysis of data takes into consideration age at diagnosis, sex, neutered/spayed status, tumor location and morphological diagnosis. In the absence of suitable denominators, the proportion of cases for a specific category of the tumor over the total number of the tumors (*e.g.*, the number of malignant mammary tumors over the total number of malignant tumors) is evaluated and expressed as a percentage of all tumors. Also, some comparisons between groups are made. From January 2020 to October 2022 DIPSA database collected 5740 histologic and cytologic diagnosis of which 3318 resulted to be neoplastic diagnosis; specifically, 2937 (89%) are histological diagnosis and 381 (11%) are cytological diagnosis. Regarding the species, 2941(89 %) neoplasms are from dogs, 345 neoplasms (10%) are from cats and 32 (1%) neoplasms are from other species (hamster, horse, and rabbit). Most of the dogs are female (55%) and most of males (90%) and females (67%) are not neutered/spayed (**Figure 1a**). 1264 dogs (43%) are mixed breed, and the most frequent breeds are Labrador retriever (4.8%, n. 141), German shepherd (4.2%, n. 123) and Boxer (2.6%, n. 76) (**Figure 1b**). The most represented age range are between 8 and 11 years for female dogs and 10-13 y for male dogs (**Figure 1c** and **d**). The mean age at first diagnosis is similar in both sexes, with small differences in neutering status and cancer behavior: neutered/spayed dogs were slightly older at first diagnosis than entire ones, and malignant tumors were firstly diagnosed in slightly older dogs (**Table 1**).

Malignant tumors represent 78% of the total cases collected in cat (N = 269) and 54% of the total cases collected in dog (n = 1588) (**Figure 2a**). Epithelial (56%), and mesenchymal (30%) tumors are the most represented tumors in dogs, whereas epithelial tumors (65%) and hematopoietic tumors (20%) are the most frequent in cat (**Figure 2b**). Malignant tumors represent 78% of the total cases collected in cat (N = 269) and 54% of the total cases collect-

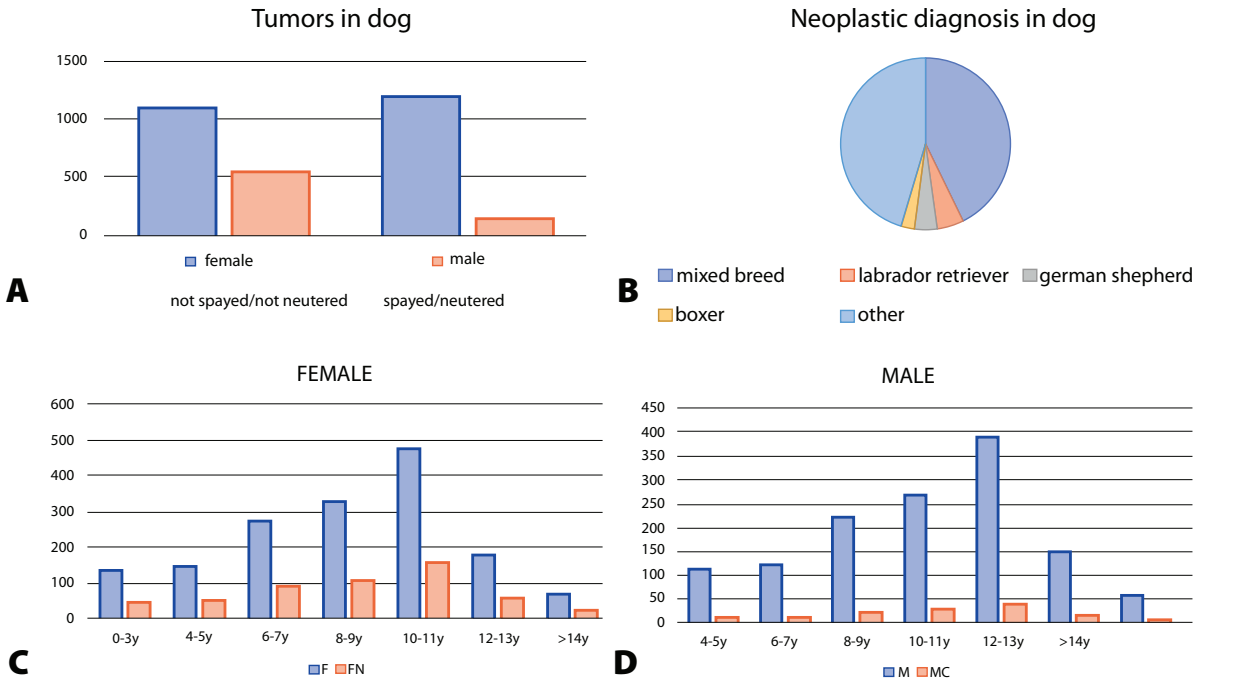

Figure 1. *Distribution of tumor diagnosis according to data collected by Piattaforma myClinical in the years 2020-2022. (**a**) Distribution of tumor diagnosis in dogs based on sex and spayed/neutered status; (**b**) distribution of tumor diagnosis in dog based on breed; (**c**) and (**d**) distribution of tumor diagnosis tumor diagnosis in dogs based on sex and age range.*

Table I. *Mean age at first diagnosis by sex, neutering status, and tumour behaviour.*

SEX AND NEUTERING STATUS	MEAN AGE AT DIAGNOSIS (YEARS) - BENIGN	MEAN AGE AT DIAGNOSIS (YEARS) - MALIGNANT
Female, spayed	8.2	9.5
Female, not spayed	8.3	9.2
Male, neutered	8.8	9.8
Male, not neutered	9	9.5

ed in dog (n = 1588). In dogs most of the malignant tumors occur in female (58%). Related to the total canine population, female dogs develop a malignant tumor in 31% of cases compared with 23% of male dogs. Spayed female dog develop a malignant tumor in 20% of cases compared with not spayed female dog that develop malignant tumors in 39% of cases. The same is observed in male dogs where neutered animals develop malignant tumors in 13% of cases compared with not neutered male dogs that develop malignant tumors in 38% of cases. Skin and soft tissue (40%) and mammary gland (30%) are the most frequent location of malignant tumors in the canine population followed by alimentary tract (12%) and hemopoietic system (7%) in male dog, whereas mammary gland (30%) and skin (36%) are the most frequent location of malignant tumors in female dogs (**Figure 2c**). Considering both malignant and benign neoplasms the most involved organ system is represented by skin and soft tissue, followed by mammary gland, genital tract, alimentary and hemopoietic systems (**Figure 2d**).

CONCLUSIONS

Cancer is one of the leading causes of death and illness in human population and it is considered as the most significant global public health issue of the 21st century. In recent years, it has been documented a concerning increase of cancer patients also in companion animals with over 4.2 million dogs diagnosed with cancer annually in USA, 15 to 30% of which die (35). A better quality of veterinary medical care and the improvement of diagnostic tools in veterinary medicine may have partially in-

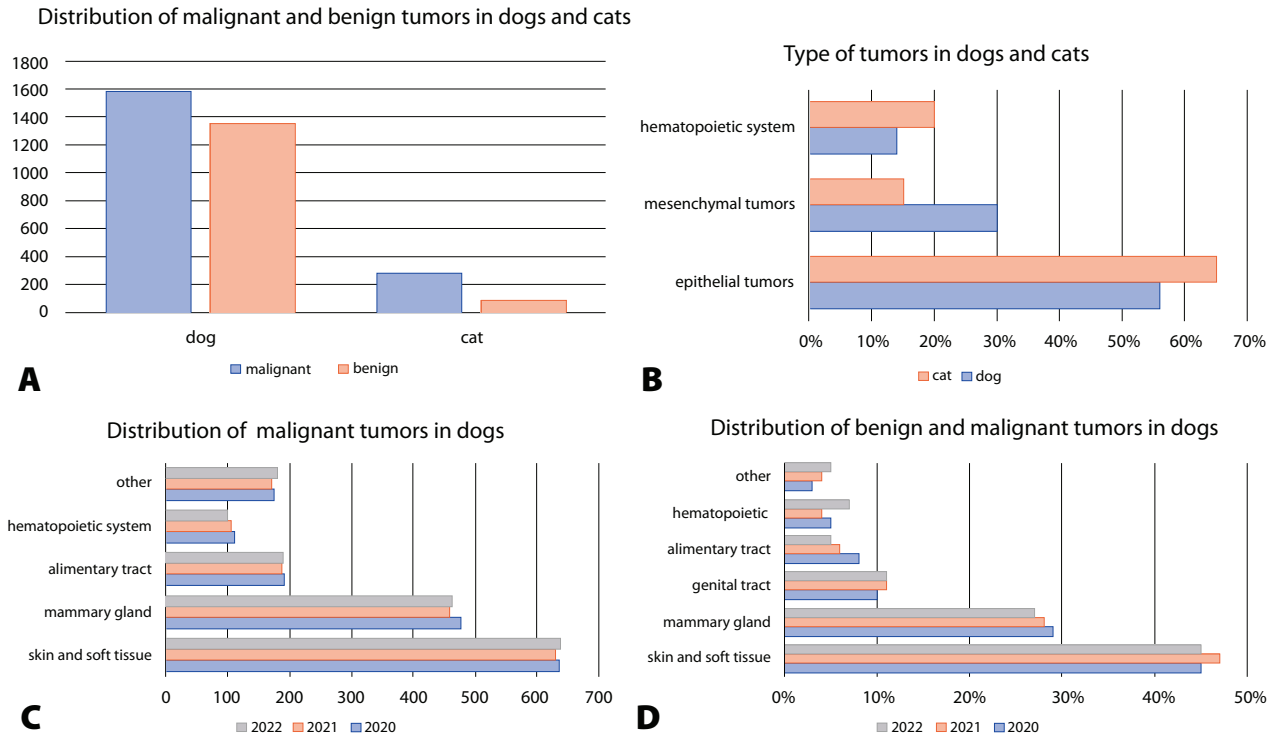

Figure 2. *Distribution of benign and malignant tumor according to data collected by Piattaforma myClinical in the years 2020-2022. (**a**) Number of benign and malignant tumor diagnosis in dogs and cats; (**b**) distribution of tumors in dogs and cats according to histological type; (**c**) distribution of malignant tumors in dogs according to topography; (**d**) distribution of benign and malignant tumors in dogs according to topography.*

fluenced these increasing numbers, but the role of environmental conditions to animal health is not to be underestimated. It is nowadays widely recognized that animals can play an important role of sentinel towards environmental risk factors both for the shorter biological cycle and for the lower "dose" required; moreover, veterinary epidemiologic studies have several advantages such as lower costs and greater ease of obtaining tissue and necropsy data (2, 7).

The data presented in this work represent a preliminary description of data collected in a short time but in a period of revamped interest for cancer registry and data collection, as evidenced by an increased number of publications in this field. Incompleteness or inaccuracy of some data compromise a correct interpretation and attempts of speculation about possible correlations. Among the measures of validity, there is the proportion of missing values for significant variables such as age at diagnosis, sex, or site of the tumors (36). In our institution the most frequently missing data are those related to municipality of residence of the animals that hampers any possible consideration about geographical distribution of tumors. Other data that are often incomplete are those related to sexual condition and age. Samples with missing data are most often those from private laboratories while when samples are sent by practitioners, data are usually complete. It is understandable that some information is lost during the intermediate step especially if it is carried out by a structure that has no direct relationship with animals and owners. Moreover, until two years ago the sample registration procedure has always been manual with samples accompanied by paper case-report format. Since this makes easy to lose information, in 2020 a web-based system has been developed and introduced. In this system the presence of mandatory fields facilitates a more complete data collection. Currently sample registration process is based on a hybrid system where some laboratories or veterinary practitioners have access to the web-based tumor registration system while others still use the paper format, and this explains why many data are often lacking. Another limitation of this system is that topographical code is assigned at the time of registration, based on information provided in case history. This introduces a bias in collecting data from involved anatomical sites increasing the number of topography codes related to ill-defined sites. This happens because morphological diagnosis accurately defines the site of origin of the neoplasm in most cases; hence, topography code should be assigned based on what pathologist states in morphological diagnosis as indicated for human cancer registries. Despite these limitations this system has many resources. The constant education and the well-coordinated work among practitioners, pathologists and computer scientists will improve data collection and uniformity to allow feasible data sharing and will actively contribute to build a true animal PBCR. As already mentioned above, validity, completeness, and comparability of data collected in cancer registry are a pivotal issue. Therefore, initiatives aimed to create a consensus and to promote establishment of standardized methods for animal cancer reporting and registration should be supported worldwide. However, data obtained for veterinary epidemiological studies must be wisely and accurately collected and standardized to create an integrated system of permanent epidemiological cancer surveillance in animals and subsequently in humans. Based on these premises, Animal Cancer Registries are a fundamental and necessary instrument for the detection of neoplastic incidence in an animal population, to evaluate neoplasm trend assessments, to plan interventions and to compare data with those obtained from human studies in a One Health approach.

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Availability of data and materials

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Authors' contributions

All the Authors contributed equally to conception, data collection, analysis and writing of this paper.

Ethical approval

Human studies and subjects

N/A.

Animal studies

N/A.

Publication ethics

Plagiarism

This is a review article and all original studies are cited as appropriate.

Data falsification and fabrication

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

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CASE REPORT

A CASE REPORT OF HPV NEGATIVE SMALL CELL NEUROENDOCRINE CARCINOMA AND SQUAMOUS CELL CARCINOMA OF THE CERVIX: A RARE BUT FATAL MIX

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ABSTRACT: Malignant neoplasms that show divergent differentiation, like squamous cell carcinoma (SCC) and small cell neuroendocrine carcinoma (SNEC), occur very rarely in the cervix. Neuroendocrine tumors of the female genital tract tend to occur in combination with other types of tumors although they have been also described to occur as solitary neoplasms. Here, we present a case of a 51-year-old woman with a one-month history of vaginal bleeding and one week history of persistent lower abdominal pain. On vaginal examination a large irregular fixed cylindrical mass in mid-vagina, extending to the cervix, was felt. A computerized tomographic scan showed a uterine mass with retroperitoneal and pelvic lymphadenopathy together with multiple bilateral lung metastases. Cytological analysis via a cervical pap smear reported a high-grade intraepithelial lesion and atypical glandular cells of undetermined significance. Histological analysis of the cervical biopsies showed a necrotic biphasic neoplasm. The morphological and immunohistochemical findings were those of a poorly differentiated carcinoma with squamous and high grade neuroendocrine (small cell) differentiation. Polymerase chain reaction analysis for Human Papilloma Virus (HPV) performed on shavings from the paraffin-embedded tissue showed no evidence of HPV DNA. The patient was planned to receive primary chemotherapy but passed away within 3 weeks of her diagnosis. In conclusion, tumors showing SNEC differentiation, together with rare cases of primary cervical SNEC exhibit a different disease profile when compared with pure cervical SCC, in that the former are highly aggressive and has a greater propensity for nodal and distant organ metastasis. These tumors are associated with a poor prognosis.

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Impact statement: This paper presents a case report of an HPV negative primary cervical SCC with divergent differentiation into SNEC. Such a combination is highly aggressive and has a greater propensity for nodal and distant organ metastasis, leading to a poor prognosis.

Key words: *small cell neuroendocrine cancer; squamous cell cancer; cervical cancer; prognosis; case report.*

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INTRODUCTION

Carcinomas of the cervix with divergent differentiation have been only sparsely reported and remain a very rare occurrence (1). This is particularly the case when considering the combination of squamous cell carcinoma (SCC) and small cell neuroendocrine carcinoma (SNEC) (2, 3). Neuroendocrine tumors of the female genital tract tend to occur in combination with other types of tumors although they have been also described to occur as solitary neoplasms. SNEC of the cervix exhibits a different disease profile when compared with SCC in that the former is much more aggressive and has a higher tendency for nodal and distant organ metastasis. These tumors are associated with a poor prognosis, with survival rates ranging from 17% to 67% depending on the stage at presentation. The clinical presentation of both SNEC and SCC include vaginal bleeding and abdominal pain. The treatment modalities available to treat these neoplasms include surgical interventions, chemotherapy and/or radiotherapy (4).

CASE PRESENTATION

A 51-year-old female, mother of two healthy children, presented to the Accident and Emergency Department with a one-month history of vaginal bleeding and one week history of persistent lower abdominal pain. She was obese and gave a history of hypertension, dyslipidemia, hypothyroidism and generalized anxiety disorder, all of which were controlled by medications. Although she was invited for cervical cancer screening, she never attended.

On abdominal examination, no masses were felt and no inguinofemoral lymphadenopathy was palpable. On vaginal examination a large irregular fixed mass in mid-vagina, extending to the cervix, was felt. The adnexae were not palpable. No family history of gynecological cancers was reported. Blood investigations including a full blood count, a renal profile and liver function tests were within normal range. Serum level of cancer-antigen 125 (CA125) were three times the upper limit of normal at 98.2 U/mL (range 0-30.2 U/ml) whilst the carcinoembryonic antigen (CEA) levels registered at more than twenty times the upper limit of normal at 52.7ng/mL (0-2.5). Similarly, cancer antigen 19.9 (CA19.9) was markedly elevated at 120 U/mL (0-33 U/ml) as was lactate dehydrogenase (LDH) (431 U/L;135-220 U/L). A computerized tomographic scan (CT) of the thorax, abdomen and pelvis was carried out which showed a uterine mass with retroperitoneal and pelvic lymphadenopathy together with multiple bilateral lung metastases (**Figure 1a** and **b**).

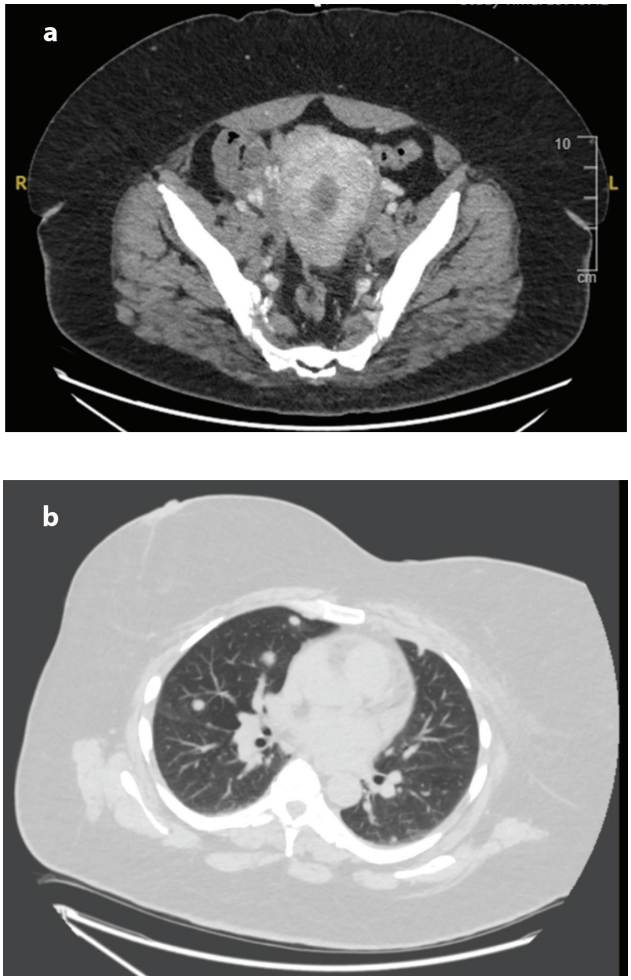

Figure 1. *(**a**) CT scan at the level of the pelvis showing the cervical mass (shown in green arrow) with pelvic lymphadenopathy (shown with yellow arrow); (**b**) at the thorax in lung window show metastatic deposits in the right lung marked with red arrows.*

A multiplanar magnetic resonance imaging (MRI) of the pelvis and cervix was carried out to better characterize the uterine tumor (**Figure 2**).

This showed a large, heterogenous mass replacing most of the anterior uterine wall and all the cervix. It measured 11 cm in maximum craniocaudal dimension and invaded the upper third of the vagina, filling the posterior fornix. Early parametrial invasion was noted at the 10 o'clock and 2 o'clock positions. The tumor showed heterogenous post-contrast enhancement and restricted diffusion. Additionally,

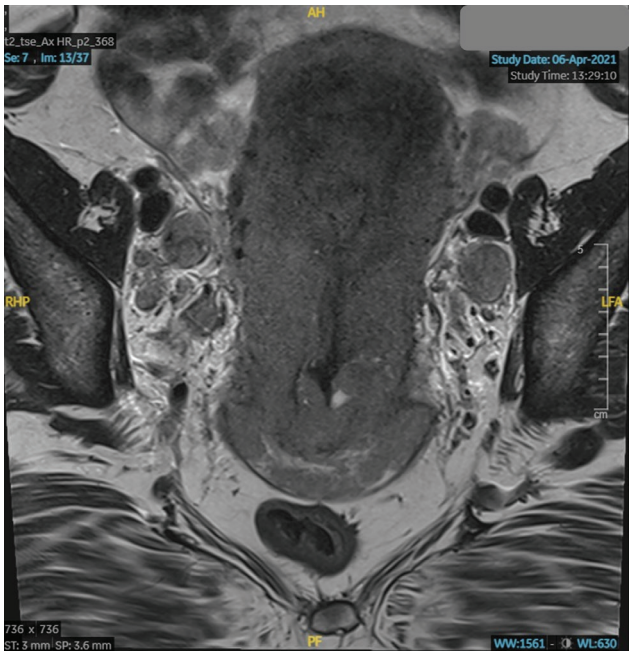

Figure 2. *MRI showing the cervical mass (shown with black arrows) with lymphadenopathy (shown with white arrows).*

there was extensive bilateral pelvic sidewall lymphadenopathy with the largest lymph node measuring more than 2 cm in maximum dimension. A 1.1 cm mesorectal lymph node was also noted and deemed to be suspicious for metastasis. On coronal sequences, there were retroperitoneal lymphadenopathy extending to the renal vessels. There was no evidence of obstructive uropathy and no evidence of upper abdominal organ metastasis. No ascites was present with no omental disease suspected. These findings staged the tumor to FIGO Stage IVB (5).

A cervical biopsy and endometrial pipelle biopsy were taken. Histological analysis of the cervical biopsies showed a necrotic biphasic neoplasm. The tumor was comprised of nests of relatively well-differentiated neoplastic squamous cells which abruptly transitioned to infiltrative sheets and clusters of undifferentiated tumor cells with scant cytoplasm, hyperchromatic nuclei and nuclear moulding (**Figure 3a**). The squamous component of the tumor showed expression of cytokeratin 5/6 (**Figure 3b**) and p63 (data not shown), while the more poorly differentiated component showed a neuroendocrine phenotype, expressing INSM1 (**Figure 3c**) and, more focally, synaptophysin (data not shown). The Ki67 proliferation fraction was 90% in the neuroendocrine component of the tumor (data not shown). The p16 expression was restricted to rare tumor cells (**Figure 3d**).

The morphological and immunohistochemical findings were those of a poorly differentiated carcinoma with squamous and high grade neuroendocrine (small cell) differentiation. The endometrial Pipelle biopsy showed fragments of tumor with an identical histomorphology to that described for the cervix. Polymerase chain reaction analysis for Human Papilloma Virus (HPV) performed on shavings from the paraffin-embedded tissue showed no evidence of HPV DNA.

After discussion during the multidisciplinary meeting, she was not deemed to be a surgical candidate and was offered primary chemotherapy. Regrettably she passed away within 3 weeks of her diagnosis and did not have the opportunity to benefit from chemotherapy. The cause of death was deemed to be due to the rapid progression of her metastatic disease. A post-mortem was not carried out for this patient.

DISCUSSION

This case adds to the very limited body of knowledge on patients with SCC exhibiting divergent SNEC differentiation. Cancer of the uterine cervix is the fourth most common malignancy in the female population worldwide and is also the fourth most common cause of cancer-associated mortality. Squamous cell carcinoma of the uterine cervix is the most common subtype of cervical cancer and carries a relatively favorable prognosis if detected early. Around 95% of cervical SCC are associated with the presence of one or more subtypes of human papilloma virus (HPV) (6). Combined tumor subtypes are rare, with adenosquamous carcinoma being the commonest tumor combination observed (3). Primary SNEC of the cervix is a vanishingly rare tumor, accounting for 1-2% of all cervical cancers, with cases of SCC exhibiting divergent differentiation being even rarer (1). The combination of SCC with SNEC has clinical repercussions both in the treatment modalities and prognostic outcomes.

Whilst the management of SCC according to the stage involves radical surgery, chemoradiotherapy with brachytherapy or combination chemotherapy, the presence of the SNEC component changes the strategy for treatment. Whilst there are no standardized modalities of treatment for SNEC given that these tumors are rare, most teams use a multimodal approach with chemo-radiotherapy often taking precedence on surgical excision. This

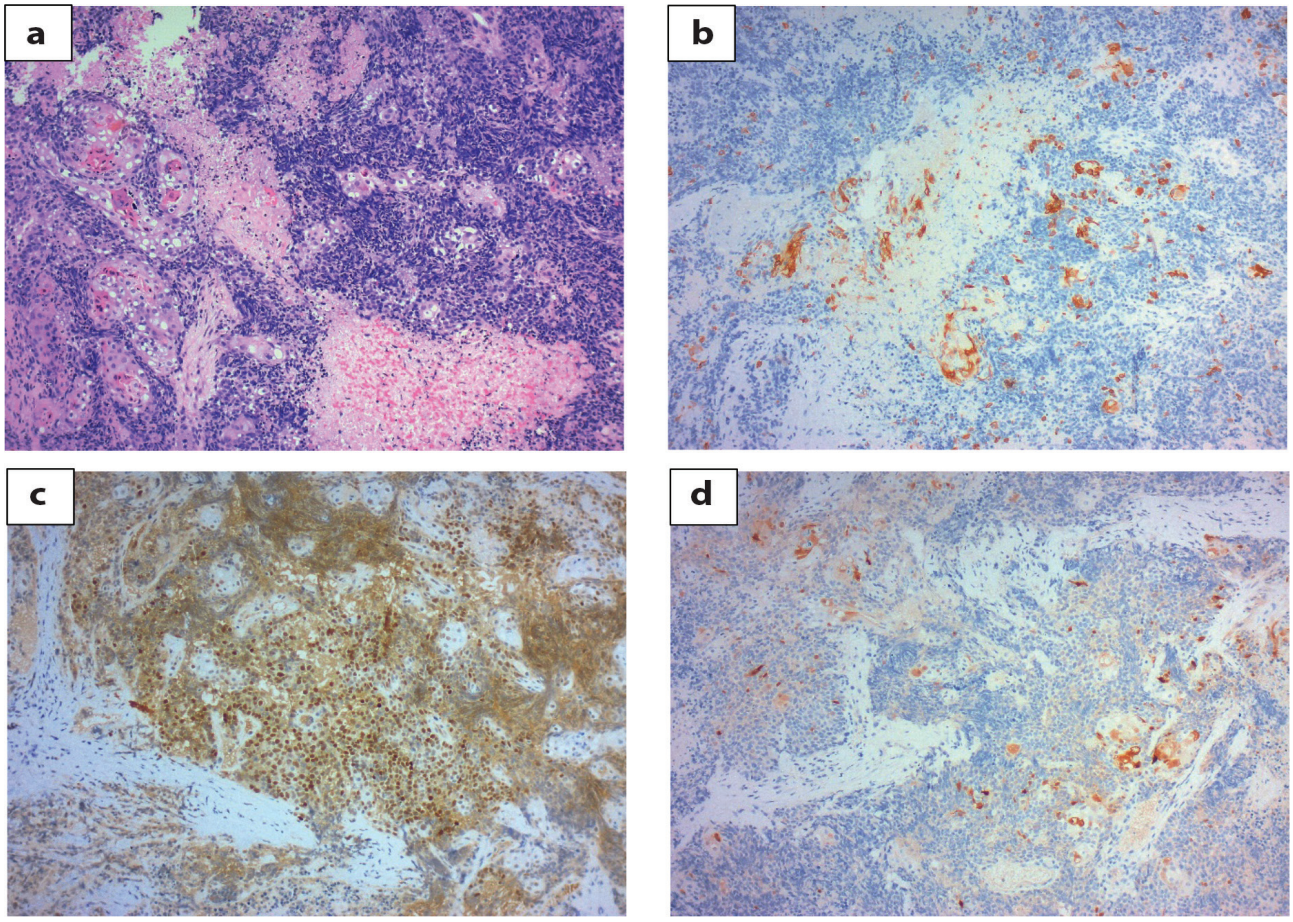

Figure 3. *(**a**) Histological analysis showing a distinctly biphasic neoplasm exhibiting both squamous differentiation (left), with nests of neoplastieosinophilic cells exhibiting central keratinization, and small cell neuroendocrine differentiation (right), with sheets of infiltrative, basaloid neoplastic cells. (H&E x 100). Immunohistochemistry showing expression of: (**b**) CK5/6 in the squamous component of the tumor, (**c**) INSM1 in the small cell neuroendocrine component and (**d**) focal p16 expression (IHC, x100).*

is usually a consequence of the advanced stage of the disease at presentation when these tumors are present. The combination of chemoradiotherapy has been shown to offer better results in the presence of SNEC. In terms of chemotherapy options, the regimen of choice typically mirrors that used for small cell neuroendocrine tumors of the lung and pancreas, which include a combination of etoposide and cisplatin or carboplatin (7). This was the modality of choice for the lady in the case we presented due to the advanced stage of disease at presentation. Unfortunately, this patient passed away before she could start treatment. Patients who either present at an early stage or who exhibit good response to chemo-radiotherapy may be candidates for radical resection. Novel treatment modalities that are more targeted and which address immune-checkpoint inhibitors are being developed in an attempt to improve outcomes particularly in patients with recurrent SNEC (8). Although these drugs are promising, there is a paucity of data to substantiate their routine use.

Both primary cervical SNEC and tumors showing SNEC differentiation carry an overall poor prognosis, with tumor behavior being primary dictated by the SNEC component. This is particularly the case given that patients are typically older, have evidence of lymph node spread, and present at an advanced stage. There is also some evidence to suggest that treatment with primary radiotherapy as opposed to multimodal treatment offers a worse prognosis. Overall, the mean survival of these patients is around 3 years. Patients who present with early stages have better prognosis (9) with a 5-year survival rate of 32% whilst patients who present late having the dismal outcome of 0% 5-year survival rate (10).

The relationship between HPV infection and SNEC is still controversial, unlike in SCC. Evidence has so far shown that the majority of patients with SNEC

have evidence of HPV infection (85%) particularly of subtypes 16 and 18 (11). The presence of HPV proliferation has also been shown to be linked to strong immunohistochemical expression of p16 (which is a cyclin-dependant kinase inhibitor). The latter together with Ki67 are considered sensitive markers for SNEC (12). Whilst Ki67 was abundant in the SNEC portion of this tumor, p16 was negative in this case. In addition, HPV PCR performed on this tumor also failed to reveal the presence of HPV. The link between SNEC and other viral pathogens such as Merkel cell polyomavirus were not proven in recent evaluations however larger studies are recommended to explore this hypothesis (13). Given this, it is possible that SCC showing SNEC differentiation represents a rare distinct tumor subtype whose pathogenesis is not HPV-driven.

CONCLUSIONS

Patients with a SCC of the uterine cervix exhibiting divergent SNEC differentiation present similar to other cervical tumors but have a poor prognostic outcome. Accurate histological assessment is imperative in order to counsel the patient appropriately and choose the right treatment modalities.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interests

The Authors have declared no conflicts of interests.

Availability of data and materials

The data presented in this study are available on request from the Corresponding Author.

Code availability

N/A.

Authors' contributions

AA and JCA: conceptualization; AA, CC and DP: writing; AA, CC, DP, RDF, AV, JDG, DV, NNM and JCA: writing-review and editing; JCA: funding acquisition. All Authors have read and agreed to the published version of the manuscript.

Ethical approval

Human studies and subjects

Informed consent has been obtained in writing from the patient's next of kin who is representing the patient, given that the patient is deceased. This case report contains clinical data from the patient's medical records.

Animal studies

N/A.

Publications ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

All the data correspond to the real.

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OPINION PAPER

THE NEW SHAPE OF THE ITALIAN ETHICS COMMITTEES

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ABSTRACT: 2023 promises to be a turning point in the history of Italian ethics committees, both for factors related to the technical and scientific progress typical of recent years and for the regulatory and procedural revolution introduced by the new European legislation. The publication, on February 8th, of four decrees implementing Law 3/2018 certainly clarified many aspects that had been pending for years however many gray areas still remain.

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Impact statement: Provide an overview of the future reorganization of Ethics Committees in Italy.

Key words: *Ethical Committees; decrees; Regulation 536/2014; observational studies; ethics.*

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INTRODUCTION

2023 promises to be a turning point in the history of Italian ethics committees (EC), both for factors related to the technical and scientific progress typical of recent years and for the regulatory and procedural revolution introduced by the new European legislation (1). Since the first formal institution, in 1998, of the Ethics Committees such as "independent bodies, created within a medical or scientific research institution according to interdisciplinary criteria" (2), different organizational models have followed one another.

An important step towards the evolution, also in terms of competences of the ECs, was represented by the Decree of February 8th, 2013, criteria governing the composition and functioning of ethics committees (3). In Article 1 of that Decree ("Definitions and functions of ethics committees"), paragraph 1 states that *"Ethics Committees (...) shall be independent bodies (...) that have the responsibility of guaranteeing the protection of the rights, safety and welfare of persons involved in trials and of providing public assurance of that protection*". In Paragraph 2 it is specified that "*where these are not already attributed to specific bodies, ethics committees may also perform consultative functions in relation to ethical questions associated with scientific and healthcare activities, in order to protect and promote the dignity of the individual. Ethics committees can also propose initiatives for training of healthcare professionals in relation to the sphere of bioethics*". The natural consequence of this new regulatory act was the drastic reduction in the number of ECs in the area: from 243 in 2012 to 91 in 2014 and 90 in 2019 (4) (**Figure 1**). The new evaluation procedure for clinical trials introduced by Regulation 536/2014 has imposed a new profound reorganization. The old procedure, that included the issuing of a single opinion by a coordinating committee and a subsequent formal acceptance/rejection of this opinion by all the other ECs involved, would not have allowed to meet the tight deadlines of the new legislation. Furthermore, the old process was characterized by a reiteration of processes that would go against the Regulation's inspiring principles.

The first step towards the reorganization was ratified with Law 3/2018 (5), which established 43 ethics committees to be maintained in Italy, 3 national

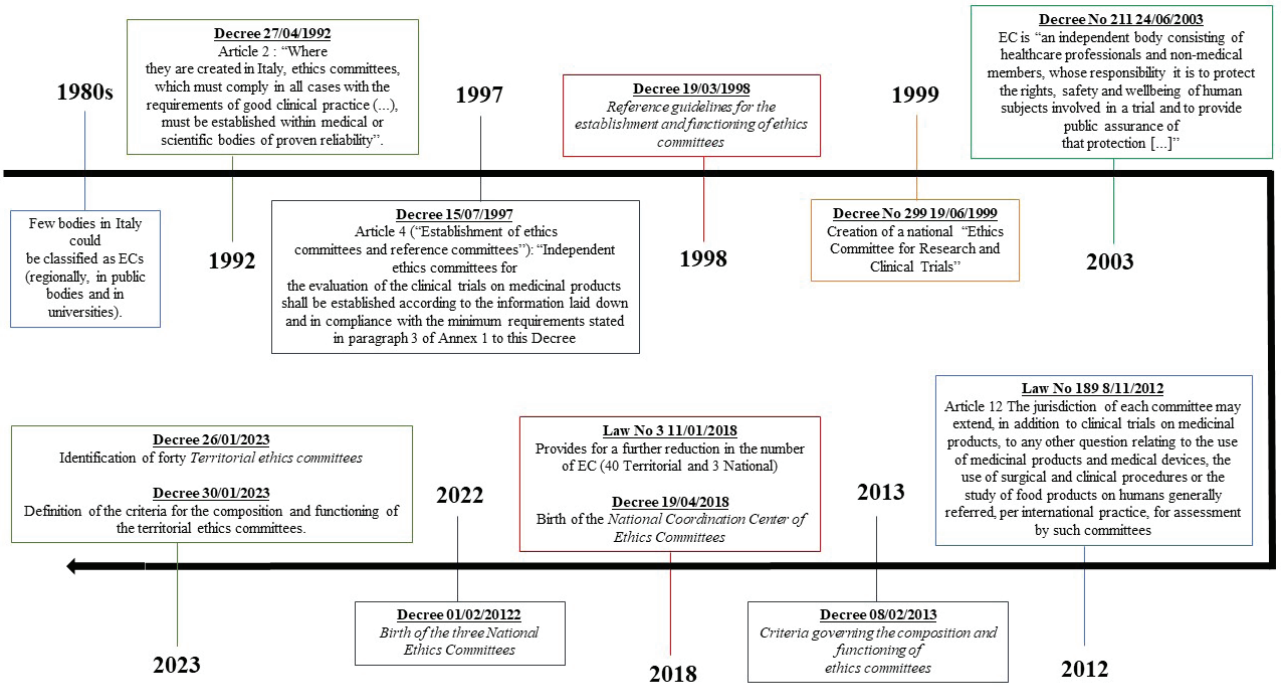

Figure 1. *History if Italian Ethics Committees.*

and 40 territorial. A few months later, a special decree of the Ministry of Health enshrined the birth of the National Coordination Center of Ethics Committees (6), a body with coordination, guidance, and monitoring functions that for a long time has been "a king without a kingdom", as to this day territorial CE is not yet active.

It was necessary to wait another couple of years for the constitution of CEs with a national value (7); one, based at the Istituto Superiore di Sanità (ISS) and immediately operating, has the task to evaluate requests from public research institutes, such as the CNR-Council National Research. The other two, based at Agenzia Italiana del Farmaco (AIFA) and formally active only since the end of January 2023, will evaluate requests relating to studies in the pediatric field and with advanced therapies, respectively.

Despite the formalization of the national EC, many questions remained unanswered for a long time. For example: what should these committees evaluate? Only interventional studies with drug and device or even observational ones? What would have been the limits of jurisdiction between national and territorial EC? Who would evaluate studies other than interventional with drug and device and pharmacological observational, given that only these seemed to be the areas of competence of the territorial EC? What about the assessment of end-of-life requests?

Months went by without having any answers (8) until February 7th 2023, when the long-awaited decrees implementing law 3/2018 have been published (9-12).

IDENTIFICATION, COMPOSITION AND FUNCTIONING OF THE TERRITORIAL EC

Two (9, 10) of the four decrees have clarified tasks and functioning of the territorial EC and have established – after long and debated analyzes with respect to past performance – what would have been the 40 "survived" committees. The idea of also evaluating end-of-life requests, that required special expertise, was dropped due to the sensitivity of the subject the need of new professional figures.

The territorial committees have been entrusted with the evaluation of three types of studies: interventional with drug, interventional with medical device e pharmacological observations. Obviously without going to flow into the areas of competence of the national EC; this means that the territorial committees will never evaluate studies in the pediatric field or contemplating the use of advanced therapy. The promoter will have to choose a single territorial ethics committee that expresses an opinion valid at national level, regardless of the

number and location of the centers involved; the choice, which in the case of interventional studies with drugs can be delegate to AIFA, must be performed ensuring the independence of EC from all the centers that will participate in the study.
Again, with a view to guaranteeing high transparency, the appointment of the members will no longer be up to the General Managers but to the Regions, who will have 120 days (starting from the entry into force of the decree) to proceed with the appointments and set up the committees. Few news, compared to the past, regarding the minimum figures that must be present in these EC and the operating procedures. The components, who will receive a fee equal to 300 euros per session plus any reimbursement of expenses (12), cannot be part of more than one CE at the same time. Still valid is the possibility, in special cases, of also relying on the advice of external professionals; in this case the evaluation must be carried out free of charge and the consultant it should be chosen from special lists/registered maintained by the regions.

EVALUATION OF OTHER TYPES OF STUDIES

Giving the new organizational model, who will be responsible for the assessment of other types of studies that go beyond the competences of national and territorial EC (for example non-pharmacological observational, requests for compassionate use, biological studies)? Also in this case, the decision-making power belongs to the Regions, free to decide whether to entrust these competences of the territorial committees or whether to maintain ancillary committees, defined as "local" (**Figure 2**). The regulatory process, therefore, could slightly differ between different regions.

GRAY AREAS

Despite the relevance of the new regulatory acts, not all doubts have been resolved. The first concerns retrospective pharmacological observational studies that, unlike the prospective ones, will not be able to benefit of a single opinion at national level (13). In addition to the questionability of this choice, it remains unclear who will take charge of the evaluation of such studies, especially if they should fall within the areas of competence of the two national EC under the guidance of AIFA. The same perplexity regarding studies that are not pharmacological observational and non interventional with drug or medical device; also, in this case it seems that an evaluation by multiples committees will be maintained, and it could be a mix between territorial and local EC. Without forgetting the urgent need to open a dialogue with the Italian privacy guarantor, to resolve the delicate problem of retrospective studies on deceased subjects (14).

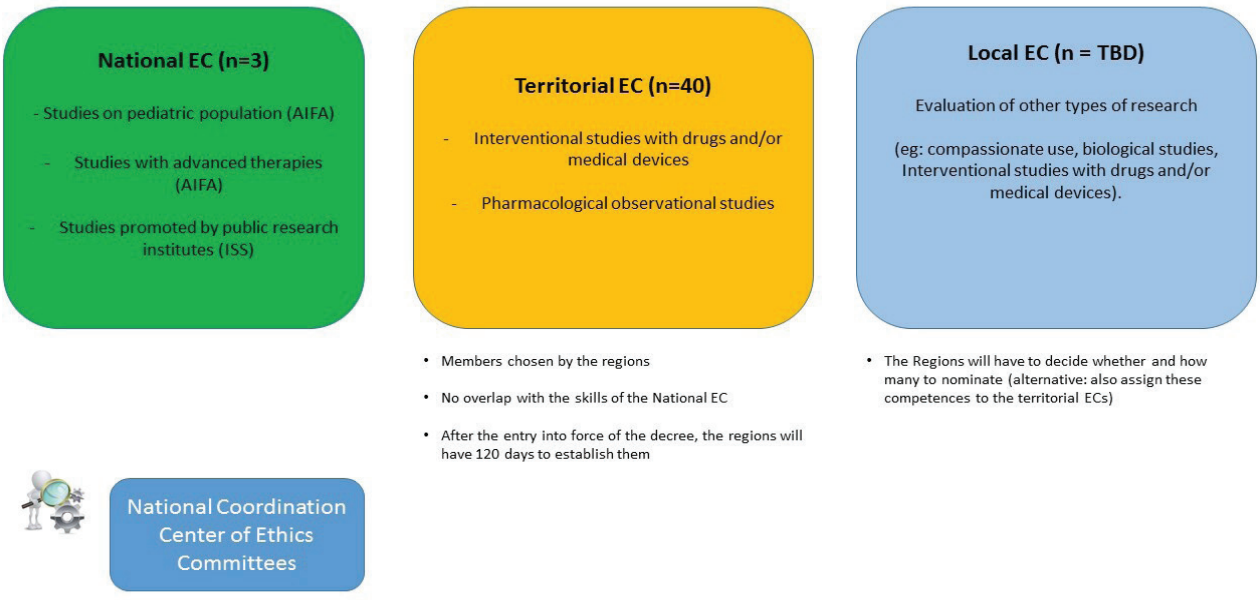

Figure 2. *Future organization of Italian Ethics Committees.*

The transition phase will also be delicate; any substantial amendments to studies still in progress according to Directive 2001/20/EC will be able to benefit from a single national opinion that will be expressed by the old coordinator EC or from one of the newborn territorial EC depending on the progress of reorganization (11).

Big questions also remain about the financial sustainability of the system. Despite the evaluation fees, standard at national level for interventional drug trials are increased compared to the past, the evaluation of a single committee for each trial it will certainly not guarantee a constant and heterogeneous incoming flow throughout the territory. Problems even greater for the regions that will decide to also maintain local EC, that probably will almost exclusively evaluate non-profit studies, therefore without evaluation fees.

The swift and successful completion of the reorganization will depend on three key factors: the adaptability of existing ethics committees; the competence of the regions in correctly managing the transition phase and the appointments of the components; the authority of the national coordination center of the ethics committees in carrying out its monitoring and control functions towards the territorial ethics committees.

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Authors' contributions

All the Authors equally contributed to the final manuscript.

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The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

All the data correspond to the real.

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MEETING REPORT

BRIDGING THE GAP BETWEEN RESEARCH AND CURE IN RARE GYNECOLOGICAL CANCERS: WHERE DO WE STAND? REPORT FROM THE GYNOCARE CONFERENCE IN NAPLES (17TH-18TH FEBRUARY 2023)

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The **European Network for Gynecological Rare Cancer Research**: **from Concept to Cure** (**GYNOCARE COST Action CA18117**) organized a two-day hybrid conference entitled: "*Bridging the gap between Research and Cure in Rare Gynecological Cancers: where do we stand*?". This conference was held in the historic Aula Botazzi at the Luigi Vanvitelli University of Campania in Naples, Italy.

The aim of this conference was to showcase new approaches to improve the diagnosis and treatment of rare gynecological cancers. There were 24 invited talks by high-profile international speakers organized over 3 sessions, followed by a final roundtable. The current state of the art, and even beyond the state of the art, in the field of basic and translational research in rare gynecological cancers have been presented. Ongoing and future innovative clinical trials for rare gynecological cancers have been also discussed with the aim of designing personalized cure. The basis of and advances in pathological diagnosis of rare gynecological cancers have been outlined. The ultimate aim was to bridge the gap between the pharmaceutical industry and biotechnology companies and translational research in rare gynecological cancers, to overcome challenges in this area. The conference focused on the complex interplay of factors that contribute to susceptibility, prevention, and management of gynecological cancers, providing an excellent discussion forum on the key challenges and the latest advancements that mostly promise to propel this field forward.

Gynecological oncology has a paradoxical high prevalence of rare cancers, especially in ovarian tumors, characterized by small number of cases, long trial accrual times, few interested investigators, low funding/pharma priority, fewer patient advocates and lack of trial designs. **Gian Franco Zannoni**, from the Department of Pathology, Fondazione Policlinico Gemelli, Università Cattolica del Sacro Cuore, Rome, Italy explained how clear cell, low grade serous, mucinous, undifferentiated carcinoma, carcinosarcoma, small cell carcinoma hypercalcemic type and mesonephric-like adenocarcinoma can all be considered rare epithelial types of ovarian cancer, distinct for morphology and molecular background. Advances in understanding their heterogeneity should improve pathologic diagnostic criteria, molecular characterization, and hypothesis-generating clinical studies. Gynecological neuroendocrine neoplasms (NENs) are also rare (NET/NEC). Sex cord-stromal tumors represent a heterogeneous group, mostly indolent, with wide range in incidence age, often associated with endocrine manifestations. Peculiar genetic mutation has been revealed. A clinical-histo-molecular

approach: FATWO (no recurrent alterations) STK11 adnexal tumors (STK11), sex cord-stromal tumors (FOXL2, DICER1), is necessary for a better definition and highly diagnostic reproducibility. Germ cell tumors are another type of rare ovarian tumors, occurring in young age. Second opinion, multidisciplinary staff and biology research are fundamental in management of these rare ovarian cancers.

The role of antibody-drug conjugate (ADC)-based treatments in epithelial ovarian cancer was described by **Neil Conlon** from the National Institute for Cellular Biotechnology, Dublin City University, Ireland. His research team showed that a TROP2-directed ADC displayed potent *in vitro* activity against epithelial ovarian cancer cell lines and demonstrated synergy when combined with other approved DNA damaging drugs. Analysis of publicly available data showed that TROP2 gene expression was correlated with poorer progression-free and overall survival, especially in advanced disease and in those treated with carboplatin/paclitaxel or topotecan.

Therefore, these drug combinations warrant further investigation. In support of this, **Francesco Legge**, from the Obstetric and Gynecology Unit, F. Miulli General Regional Hospital, Bari, Italy, explained how personalized treatment algorithms, including debulking surgery, chemotherapy and maintenance therapy, for advanced ovarian cancer should take into account the specific molecular fingerprint of the disease as well as the individual risk of complications.

Endometrial cancer is overall common in postmenopausal women; however, a small proportion of women are diagnosed with endometrial cancer under the age of 40 years (5%). Immunohistochemical predictive markers of response to conservative treatment of endometrial hyperplasia and early endometrial cancer were outlined by **Antonio Raffone** from the University of Naples Federico II, Naples, Italy. In endometrial cancer, research carried out based on clinical-morphological and molecular-genetic studies revealed new pathogenetic mechanisms and predictors of the recurrence, such as the presence of the c.389G > A (p.R130Q) PTEN gene, promoter methylation MLH1, the proliferation marker Ki-67.

This led to the development of a mathematical model for predicting the evolution of the endometrial cancer in patients from different risk groups which was presented by **Irina Tripac** from the Department of Gynecology, Institute of Oncology of Moldova. Standard surgical treatment of women with endometrial cancer precludes further fertility, therefore if fertility sparing treatment is desired, especially in the rarer cases of endometrial cancer in younger women, an attempt for medical treatment is done.

The state of the art and future perspectives in fertility sparing management in patients with endometrial cancer was presented by **Valeria Masciullo**, Department of Gynecology, Fondazione Policlinico Gemelli, Università Cattolica del Sacro Cuore, Rome, Italy.

This was followed by further insights into translational science in fertility sparing procedures for endometrial cancer by **Monika Sobocan** from the Division for Gynecology and Perinatology, University Medical Center Maribor, Maribor, Slovenia. Candidates for fertility sparing treatment are selected based on histological and clinical criteria. The integration of molecular classifiers into risk assessment has led to new venues of clinical assessment also in fertility sparing treatment. Molecular classifiers, which classify endometrial cancer into subgroups of POLE mutated, mismatch repair deficient (MMRd), p53 abnormal (p53 abn) and of no specific mutational profile (NSMP) can be used to additionally provide therapy individualization. In fertility sparing treatment therefore first data show, that women with MMRd endometrial cancer have shown worse response to fertility sparing treatment. However, studies focusing on fertility sparing treatment and molecular classifiers are lacking. Initial data also point towards lower complete response rates at 6 months and overall complete response in MMRd tumors than NSMP tumors. Yet most young women still classify into the NSMP subgroup of endometrial cancer requesting additional characterization and more robust data. Studies suggest also the need for integration of additional refinement and analysis of carcinoma in women with endometrial cancer using markers such as PI3K/Akt/mTOR markers and other interconnected signaling pathways.

In addition, while medical assisted reproductive therapies may pose risk of gynecological cancer, such treatment also needs to be accessible for patients who experienced gynecological cancer.
This was stressed by **Vera Dimitrievska** from the University of American College, North Macedonia and **Gligor Tofoski** from the Gynecology and Obstetrics Department at Ss. Cyril and Methodius University in Skopje, North Macedonia.

As early detection is crucial for successful treatment outcomes, there is a need for effective screening methods. However, currently, there is no mass screening available for endometrial cancer in asymptomatic individuals. In a study carried out by **Jacopo Troisi** from the Scuola Medica Salernitana, University of Salerno, Italy, the diagnostic accuracy of an ensemble machine learning algorithm that uses serum metabolomics signatures for screening of endometrial cancer was evaluated. The results showed that the proposed metabolomics signature was not affected by the stage and grading of endometrial cancer or the presence of other comorbidities. However, it was influenced by different endometrial cancer histotypes, providing additional substratification. These findings suggest that metabolomics could be a promising and novel screening tool for endometrial cancer.

Multi-omics, which is the integration of various omics technologies, including genomics, transcriptomics, proteomics, and metabolomics provides a more comprehensive understanding of the molecular mechanisms underlying diseases, including rare gynecological cancers. In the context of studying rare gynecological cancers, multi-omics can help identify key molecular pathways and potential biomarkers associated with the development and progression of these cancers. By analyzing multiple omics data sets, researchers can gain a more comprehensive view of the genomic, epigenomic, transcriptomic, proteomic, and metabolic alterations associated with rare gynecological cancers and gain insight into the functional consequences of these molecular alterations. As outlined by **Yashwanth Subbannayya**, from the School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK; and **Sureyya Ozcan Kabasaka** from the Department of Chemistry, Middle East Technical University, Ankara, Turkey, integrated approaches such as proteogenomics can help identify variants such as mutant peptides, fusion proteins, and alternative splicing events and provide evidence for the translation of pseudogenes, long non-coding RNAs, and micropeptides. These, in turn, can serve as biomarkers for diagnosis, prognosis, or aid as potential therapeutic targets. Overall, multi-omics approaches can provide a more comprehensive understanding of the molecular mechanisms underlying rare gynecological cancers, which can help identify potential therapeutic targets and biomarkers for early detection and personalized treatment.

In cervical cancer, Human Papillomavirus (HPV) 16 and 18 genotypes cause 70% of cases. A new method was described by **Anna De Filippis** from the Department of Experimental Medicine, L. Vanvitelli University of Campania, Naples, Italy, long DNA regions modified with biotinylated oligos, called "Long Regions of DNA Pull-down" (LDP) were generated. Approximately 4350 proteins have been identified by the mass spectrometric analysis, of which 310 interact with a single oligo. Gene Ontology studies were conducted to analyze the biological processes, molecular functions, and cellular components in which these proteins are involved. Most of them play a role in DNA replication, transcription, and translation. Some interactors were already known and implicated in HPV-associated diseases, while others had never been described. These data may pave the way for further studies to find new potential and effective therapies to combat HPV16 infection, progression, and pathogenicity. Cervical screening is a way of preventing cancer by detecting high risk HPV can cause cervical cells to become abnormal.

Cristina Clare Gallego de Largy from Marqués Valdecilla University Hospital, Santander, Spain described how a cervical cancer screening project began in March 2022, using digital colposcopy with guidance from Spain to Uganda. The incidence of cervical cancer in Uganda is one of the highest in the whole world, with a relative risk of 4.4 in this country.

Aljoša Mandić from the Oncology Institute of Vojvodina, University of Novi Sad, Serbia gave an overview on the rare neuroendocrine cervical cancer, which accounts for 1.4% of all invasive cervical cancers. Even in this rare cervical tumor, HPV has an important role. Despite a small number of studies that had examined mutational hotspots in these neoplasms and attempted to characterize them from a molecular/somatic mutation standpoint the most frequently altered genes were PIK3CA (19.6% of cohort), MYC (15.5%), TP53 (15.5%), and PTEN (14.4%). The most common primary treatment was radical surgery combined with chemotherapy. There was no standard chemotherapy regimen but platinum and etoposide was the most commonly used treatment. Radiotherapy-based treatment schemes were also commonly utilized in the upfront setting for early-stage disease. Multimodal therapy for all stages of neuroendocrine tumors of the cervix is recommended, and the majority of patients receive a combination of surgery, radiation, and chemotherapy.

For early stage disease (tumors ≤4 cm) and negative nodes on imaging, radical hysterectomy and pelvic lymphadenectomy followed by chemotherapy with platinum and etoposide is the primary management recommended with consideration for additional radiotherapy. Large studies in patients with uncommon or rare tumors are difficult with limited prospective data with which to guide decisions.

To date, the best chemotherapy regimen in cervical cancer has a response rate of 48% with an overall survival of 17 months, and limited options for second-line chemotherapy. Immunotherapy can induce a strong immune response in cervical cancer due to retained viral antigens. Although the response rates to programmed cell death 1 (PD-1) inhibition alone have been modest, the landmark survival reported in the recently published trials suggests that immunotherapy will represent a paradigm shift in the treatment of advanced and recurrent cervical cancer.

Domenica Lorusso from the Department for Obstetrics and Gynecology, Catholic University of Sacred Heart of Rome, Italy demonstrated how, in the Empower Cervical 1 trial, the anti PD1 inhibitor *cemiplimab* provides better outcome in comparison to single agent chemotherapy in second and third line setting of advanced disease. The KEYNOTE-158 trial has led to the approval of pembrolizumab in recurrent programmed cell death ligand 1 (PD-L1) positive cervical cancer based on a significant increase in overall and progression free survival when combined to platinum-based chemotherapy plus or minus bevacizumab. Combinations of programmed cell death 1 and anticytotoxic T-lymphocyte-associated protein 4 inhibitors (CTLA4) inhibitors have shown promising and durable activity. There is active research with new combinations of checkpoint inhibitors, as well as combinations of these drugs with chemotherapy and radiation, and other novel approaches. Responses to immunotherapy can be dramatic and durable. Continued work to find the optimal combination and setting for immunotherapy is ongoing and all these aspects were discussed during the COST conference.

Vulvar cancer is a rare disease that requires multimodal treatments including surgery, radiotherapy, and chemotherapy. The disease often relapses, and treatment options are limited, especially for elderly women with comorbidities. **Anna Myriam Perrone** from the Division of Gynecologic Oncology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, University of Bologna, Italy explained how electrochemotherapy with bleomycin has proven to be a valid alternative, and our studies have demonstrated an efficacy of around 80% in local control. These results were validated in a large scale with a multicenter study called Elechtra, which confirmed the findings of the first studies with low toxicity and minimal side effects. The quality of life of these patients was evaluated and those who responded to the therapy experienced a significant reduction in pain, particularly for smaller lesions located in the posterior vulva. A new study is currently ongoing, using bleomycin associated with platinum-based chemotherapy.

The holistic care of patients with rare gynae cancer was highlighted by **Francesco Pegreffi**, from the Department for Life Quality Studies, University of Bologna, Italy in his lecture on the Impact of Sarcopenia and Exercise Training on Skeletal Muscles in Patients with Rare Cancer. In cancer patients, low muscle mass and function due to sarcopenia has been identified as negative prognostic factor predisposing to falls and fractures. Furthermore, muscle wasting, and weakness caused by cancer-related inflammation, significantly affect quality of life in everyday living activities. Because the pathophysiology is not yet fully understood, therapeutic options remain limited and physical activity remains a viable modality to enhance muscle function in older sarcopenic adults. Lifestyle interventions, especially exercise and nutritional supplementation, prevail as mainstays of treatment, concerning rarer forms of gynecological tumors. However, not enough studies have currently been conducted to estimate the prevalence of sarcopenia and how it can affect clinical and functional outcomes in everyday clinical practice and life. COST is an excellent starting point for future research studies and interdisciplinary collaborations.

Identification of early stage disease markers and a fast detection of markers accompanying disease development play a crucial role in modern personalized medicine. Novel biotechnology, such as antibody-modified nanomembrane as specific ultrasensitive detectors for clinical application was presented by **Evzen Amler** from the Department of Biophysics, Charles University, Prague, Czech Republic. Preparation of nanofiber-based detectors functionalized on nanofiber surface with relevant antibodies, aptamers or other bioactive substances are the key steps towards highly sensitive and specific bionanosensors and also towards smart targeted and controlled drug delivery

systems. Nanofibers are unique due to their extremely large ratio between the surface and volume. Thus, the probability of the disease marker interaction with the nanofiber is also very large. Consequently, the application of the smart gynecological tampon based on nanofibers functionalized on the surface with a specific antibody against the disease marker could lead to the specific interaction of the specifically modified nanofiber surface with the disease markers even at their very low concentration and, thus, to identification and possible even treatment of the disease at its early stage.

Smart clinical trials, especially those intended to design cures for rare gynecological cancers, are crucial in order to allow advances to be made. The European Union enacted a new Clinical Trials Regulation in 2014, replacing the previous Clinical Trials Directive of 2001, in a bid to create a level playing field across EU member states, whilst simplifying the application process for researchers and providing more visibility and more transparency to the general public on clinical trials. As explained by **Neville Calleja** from the Department of Public Health, University of Malta, Malta, the European Medicines Agency has introduced the Clinical Trials Information System (CTIS), an IT infrastructure through which all clinical trial applications are to be processed, by applicants and regulatory authorities, and which also includes a public interface for the general public to be informed of what research is happening, together with documentation pertaining to such clinical trials.

Characteristics of clinical trials investigating pharmacological treatments for rare gynecological cancers were described by **Klejda Harasani** from the Department of Pharmacy, Faculty of Medicine, University of Medicine Tirana, Albania. There was only little variation in the design of completed clinical trials, where most were open-label (no masking) with single group assignment and allocation was not applicable as they were single-arm trials. Only one completed clinical trial had a calculated Jadad score higher than 2 (Jadad score = 3) which indicates higher quality. Results were unpublished in 65 % of the completed clinical trials and 45 % of them had neither publications, nor had posted any results. These findings highlight the need for higher-quality clinical trials and better reporting of data from the current ones.

As discussed by **Riccardo Audisio**, from the University of Göteborg, Sweden, although modern clinical decision-making rests on evidence-based medicine, where Randomized Controlled Trials (RCTs) and metanalyses are considered unequivocal, such undisputable truth is often equivocal. Young and fit patients are more frequently enrolled, while older and frail ones are excluded as well as those on polypharmacy, deprived and dependent ones. Individuals with poor literacy are preferred to unlearned patients. Negative trials are frequently unpublished, therefore meta-analytic investigations present biased results as they rest on skewed data. Moreover, RCTs are extremely expensive.

Now that real-time, good quality data can safely be harvested, the scientific community should look forward to relying more consistently on Cancer Registry data. There are numerous examples of their effectiveness and their clinical impact. Consecutive and real-life information provides extremely reliable and sound results. It is very important to collect robust data to steer clinical research. As explained by **Gemma Gatta**, former director of the Evaluative Epidemiology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, different objectives characterize clinical and population-based studies. For instance, in survival studies, clinical study evaluates the effectiveness of treatment and indicates the highest achievable survival in selected patient groups. By contrast population-based study evaluates the effectiveness of the health care systems and estimates the average survival actually achieved in the general population. Clinical studies require an ad hoc design and data collection while in population-based studies, data are currently collected by disease (cancer) registries (PBCR). The definition of the disease needs to follow international classification, the variables collected are few and of high quality to permit the comparison between populations. In high income countries PBCR collect more clinical variables, which follow strict rules given by the IARC. PBCR with their data on incidence are able to provide estimation on number of new diagnoses per year or incidence rates by country then make available those for rare gynecological tumors. Also, PBCR indicates time trends then to know if there is an increment/reduction of diagnosis or if incidence remains stable. The correct pathological diagnosis, which for rare cancers needs sometimes of a second opinion, have to be acquired and correctly codified by registry. To provide survival by type of tumour, PBCR have to access to reliable and complete sources to know the life status of cases included in the registry. For the majority of rare gynecological tumors, this contribution will show in-

cidence and survival differences by type of tumors, country, time period, hospital.

While RCTs are the gold standard in novel therapy development, in rare diseases the low patient numbers as well as the cost involved in interventional clinical trials represent a significant barrier. As such, a more efficient research approach through real world evidence (RWE) and pragmatic (combining interventional and real-world arms) studies would leverage the abundant historical patient data in patient journals and databases and bypass the need for large numbers of real-time patients and high financial burden. **Stephanie Darmanin** and **Ylva Kaiser**, working for private pharma companies in Sweden, gave their perspective from the industry experience. Identifying relevant key opinion leaders, establishing relationships at a local, national, and global level and engaging in international collaborations around RWE to create bigger data collections is critical. When approaching pharmaceutical company medical affairs teams and patient organizations for industry-sponsored research grants and joint projects, researchers should have a clear, clinically relevant idea, with a connection to patient data/samples, a correlation to parameters of disease/clinical phenotypes, and outcomes that can eventually be applied in the clinic, or at least be of clear relevance to clinicians and patients. The opportunity presented by investigator-sponsored studies (ISS), where the investigator is the instigator of the study proposal as well as the principal owner of the data, but resources are provided by a partner within the pharmaceutical industry, should be further explored. A collaborative approach involving clinicians is preferable in order to gain company interest and create a platform for future investment in the disease area. Innovative protocols for personalized one-patient clinical trials, designed around the individual patient's genomic, proteomic and histological profile are an appealing option, which will however require consideration of regulatory and ethical implications.

Gynecological cancer is a leading cause of morbidity and mortality worldwide. Considering the huge diversities in etiological factors, the complexity of cancer progression and the accessibility of treatments only well organized and massive translational and clinical research can improve global outcomes. **Mariela Vasileva-Slaveva** from the Bulgarian Breast and other gynecological Cancers Association, Sofia, Bulgaria; and Medical University Pleven, Pleven, Bulgaria presented data supporting the fact that access to education and training as well as access to appropriate networking are key limiting factors in developing clinical research by healthcare professionals.

In conclusion, rare gynecological tumors are not so rare, characterized by various clinical presentations and different natural histories. Further collaborative efforts will implement new trials design, and care pathway organization at all levels, in order to better molecular classification, improve prognosis and define possible new molecular targets. The GYNOCARE Conference in Naples has been an outstanding experience, with over 140 participants. It has favoured several new collaborations with experienced and committed colleagues working in several disciplines in a lot of countries within and outside Europe. Despite the intense schedule of inspiring talks, the conference provided many opportunities for discussions and interactions with leading scientists from around the globe in a very pleasant atmosphere. The next GYNOCARE conference will be held in Sofia, Bulgaria in July 2023.

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APPENDIX - MEETING PROCEEDINGS

Bridging the gap between research and cure in rare gynecological cancers: where do we stand? Report from the GYNOCARE Conference in Naples (17th-18th February 2023)

LOCAL ORGANIZERS:

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- Michele **Caraglia**
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All the data correspond to the real. The **European network for Gynecological Rare Cancer Research**: **from Concept to Cure** (**GYNOCARE COST Action CA18117**) organized a two-day hybrid conference entitled: 'Bridging the gap between Research and Cure in Rare Gynecological Cancers: Where do we stand?'. This conference was held in the historic Aula Botazzi at the Luigi Vanvitelli University of Campania in Naples, Italy.

The aim of this conference was to showcase new approaches to improve the diagnosis and treatment of rare gynecological cancers (RGCs). The current state of the art, and even beyond the state of the art, in the field of basic and translational research in RGCs, have been presented. Ongoing and future innovative clinical trials for RGCs have been also discussed with the aim of designing personalized cure. The basis of and advances in pathological diagnosis of RGCs have been outlined. The aim was to bridge the gap between industry and biotechnology companies and translational research in RGCs, to overcome challenges in this area. The conference focused on the complex interplay of factors that contribute to RGC susceptibility, prevention, and management, providing an excellent discussion forum on the key challenges and the latest advancements that mostly promise to propel this field forward, hosting high-profile speakers.

SESSION 1.

BASIC AND TRANSLATIONAL RESEARCH: PRESENT AND FUTURE

Presentation 1.1. Investigation of antibody-drug conjugate-based treatments in epithelial ovarian cancer

- Neil **Conlon**
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Antibody drug conjugates (ADCs) allow for the targeted delivery of cytotoxic agents to increase anti-cancer efficacy and minimize toxicities. ADCs are biotherapeutics composed of an antibody specific to an extracellular antigen, a linker molecule that conjugates the toxic payload that is then trafficked into the target cell. Mirvetuximab soravtansine, a folate receptor alpha-targeting ADC, gained FDA approval for folate receptor alpha-positive advanced epithelial ovarian cancer in 2022. In addition, several other ADCs are in clinical testing for the treatment of ovarian cancer. This work focused on the potential of ADCs with DNA-damaging payloads. We showed that a TROP2-directed ADC displayed potent *in vitro* activity against epithelial ovarian cancer cell lines and demonstrated synergy when combined with other approved DNA damaging drugs. Analysis of publicly available data showed that TROP2 gene expression was correlated with poorer progression-free and overall survival, especially in advanced disease and in those treated with carboplatin/paclitaxel or topotecan. We believe that these drug combinations warrant further investigation.

Presentation 1.2. Proteomics: A road map towards a clinical test

Sureyya Ozcan **Kabasaka**
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Proteomics is comprehensive research of proteins that facilitates development of novel biomarkers for diagnostic applications and exploration of prospective therapeutic targets. Changes in protein profile reflect biological changes. Thus, protein-based biomarkers can be used for both diagnostic and prognostic purposes. A roadmap towards a protein-based biomarker clinical test starts with well-designed clinical study. Candidate markers obtained from the study should then be analyzed in an independent clinical set to verify the finding and subsequently validated. A robust, reliable, and accurate clinical test requires comprehensive workflows that need are further evaluated by regulatory bodies.

Presentation 1.3. Integrated omics analysis to identify biological mechanisms of gynecological cancers

Yashwanth **Subbannayya**
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Multi-omics refers to the integration of various omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, to provide a more comprehensive understanding of the molecular mechanisms underlying diseases, including rare gynecological cancers. In the context of studying rare gynecological cancers, multi-omics can help identify key molecular pathways and potential biomarkers associated with the development and progression of these cancers. By analyzing multiple omics data sets, researchers can gain a more comprehensive view of the genomic, epigenomic, transcriptomic, proteomic, and metabolic alterations associated with rare gynecological cancers and gain insight into the functional consequences of these molecular alterations. Integrated approaches such as proteogenomics can help identify variants such as mutant peptides, fusion proteins, and alternative splicing events and provide evidence for the translation of pseudogenes, long non-coding RNAs, and micropeptides. These, in turn, can serve as biomarkers for diagnosis, prognosis, or aid as potential therapeutic targets. Overall, multi-omics approaches can provide a more comprehensive understanding of the molecular mechanisms underlying rare gynecological cancers, which can help identify potential therapeutic targets and biomarkers for early detection and personalized treatment.

Presentation 1.4. Personalized clinical management of ovarian cancer

Francesco **Legge**
Mother and Child Department, Obstetric and Gynecology Unit, F. Miulli General Regional Hospital, Acquaviva delle Fonti, Bari, Italy
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Ovarian cancer (OC) represents the most challenging gynecological cancer, with approximately 70% of patients presenting advanced stage of disease and among those with peritoneal disease about 70% relapsing within 3 years from the end of first line therapy. The survival benefit of maximal primary cytoreductive surgery must be balanced with the risk of serious complications delaying chemotherapy initiation. OC is a heterogeneous disease with specific molecular alterations characterizing clear cell (*e.g.*, PTEN, PIK-3CA, ARID1A), mucinous (*e.g.*, KRAS), low grade serous (*e.g.*, BRAF, KRAS, ER, PR) and high grade serous (*e.g.*, p53, wt-1, BRCA1/2) histotypes influencing sensitivity to specific drugs as well as the surgical management. In particular, half of high-grade serous OC exhibit a high degree of genomic instability due to deficiencies in homologous recombination including somatic and germline BRCA mutation, which are associated to sensitivity to platinum-based chemotherapy and PARP-inhibitors. Personalized treatment algorithms, including debulking surgery, chemotherapy, and maintenance therapy, for advanced OC should consider the specific molecular fingerprint of the disease as well as the individual risk of complications.

Presentation 1.5. Personalized clinical management of endometrial cancer using molecular approach

Irina **Tripac**
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Endometrial cancer (EC) is the sixth most common cancer in females. Roughly, 382,000 new cases are

diagnosed each year and EC accounts for 90,000 deaths worldwide. The estimated five-year survival rate in developed countries is about 80% with primary treatment. The median age at uterine cancer diagnosis 62 years. The estimated number of new endometrial carcinoma cases in Europe in 2021 was 134,578 with 41,638 deaths, and the incidence has been rising with aging and increased obesity of the population. The adequacy of risk stratification systems in EC have recently been compared and challenged. There are 5 major risk stratification systems in EC, of which the modified European Society of Medical Oncologists (ESMO) classification was demonstrated to best discriminate for recurrence and nodal metastases in early-stage disease. Considering the high number of possible markers, only a few have been included in internationally recommended guidelines for risk stratification. However, none of the existing schemes was deemed highly accurate. In addition, all current systems stratify women based on pathologic data obtained after surgical staging (stage is a component of risk assignment). There is great need to obtain earlier and more biologically informative data from EC tumors that could assist in planning the optimal course of treatment for the individual.

The results of the complex study performed on a group of 269 patients with EC in stages I-II revealed clinical-morphological predictors of the recurrences: rare uterine cancers such as clear cell carcinoma, mucinous carcinoma, serous carcinoma; invasion of more than 50% in the myometrium; the depth of tumor invasion in the myometrium >1 cm and in the stroma >0.5 cm; the presence of lymphovascular invasion and the presence of necrosis in the tumor. The level of the Ki-67 expression estimated in the immunohistochemical examination in 50 patients with EC in stages I-II, was imposed by its predictive value on survival, so that with its increase, the survival rate decreases. In patients with a Ki-67 level close to a proliferative activity of 0-33%, the survival rate was 80.8 + 3.3%, and with a proliferative activity greater than 34%, the survival rate decreased to 57.9 during the 3-year follow-up period.

DNA testing in 50 patients with EC in stages I-II detected mutations in the MLH1 gene in 10 (20%) cases and is to be used to determine the risk of disease recurrence. The analysis of the obtained results showed that in EC patients the presence of the MLH1 epimutation significantly affects overall survival regardless of the risk group stratification. The mean time to EC progression in patients with the MLH1 epimutation was 14.6 months.

The mutation c.389G > A (p.R130Q) of the PTEN gene was detected in 12 (24%) patients. The 3-year follow-up of patients (n = 12) carrying the mutation in PTEN gene, regardless of the risk group, revealed a poor prognosis of the disease, which was occurred either by the recurrence of endometrial adenocarcinoma (n = 8), or by the appearance of metastases (n = 4). Research based on clinical-morphological and molecular-genetic studies revealed new pathogenetic mechanisms and predictors of EC recurrence, such as the presence of the c.389G > A (p.R130Q) PTEN gene, promoter methylation MLH1, the proliferation marker Ki-67, which allowed the development of a mathematical model for predicting the evolution of the disease in EC patients from different risk groups over a 3-year period.

Presentation 1.6. Fertility sparing in endometrial cancer: state of the art and future perspectives

Valeria **Masciullo**
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Endometrial cancer is the most common gynecologic malignancy in developed countries and approximately 10% of the women with endometrial cancer (EC) are below the age of 45. Management of endometrial cancer in young women who desire to maintain fertility presents a unique set of challenges since the standard surgical treatment based on hysterectomy and salpingo-oophorectomy is often not compatible with the patient's goals. A fertility sparing approach can be considered in selected patients with early stage and low-grade endometrial cancer or atypical hyperplasia (EAH). Oral therapy with megestrol acetate is recommended based on extensive experience, although without consensus on dosages and treatment length. However, Levonorgestrel intrauterine device appears an alternative treatment, particularly in patients who are not planning to conceive soon or do not tolerate oral therapy. The pooled complete response rate, recurrence rate, and pregnancy rate of EC were 76.3%, 30.7% and 52.1%, respectively. In a randomized controlled trial, megestrol acetate plus metformin guaranteed an earlier complete response rate than megestrol acetate alone for endometrial hyperplasia, however some other studies failed to show the same efficiency. Hysteroscopic lesion resection increases efficiency of megestrol acetate and prognostic outcome in patients with EAH and EC who wish to preserve their fertility.

Treatment of grade 2 cancers seems feasible however it should only be allowed as part of clinical trials. Molecular characterization of EC could probably model a tailored treatment in the future. However, fertility preservation is not the standard approach for staging and treatment, potentially worsening oncologic outcomes and hysterectomy should always be performed once the conceiving plan is completed.

Presentation 1.7. Translational science in fertility sparing procedures for endometrial cancer

Monika **Sobocan**
Division for Gynecology and Perinatology, University Medical Center Maribor, Maribor, Slovenia; Faculty of Medicine, University of Maribor, Maribor, Slovenia (Monika.sobocan@ukc-mb.si; monika.sobocan3@um.si)

Endometrial cancer (EC) is a common gynecological cancer with peak incidence at the age of 60 years. Although most women with endometrial cancer are postmenopausal a small proportion of women are diagnosed with EC under the age of 40 years (5%). Standard surgical treatment of women with EC precludes further fertility, therefore if fertility sparing treatment is desired an attempt for medical treatment is done. Candidates for fertility sparing treatment are selected based on histological and clinical criteria. The integration of molecular classifiers into risk assessment has enabled us new venues of clinical assessment also in fertility sparing treatment. Molecular classifiers, which classify EC into subgroups of POLE mutated, mismatch repair deficient (MMRd), p53 abnormal (p53 abn) and of no specific mutational profile (NSMP) can be used to additionally provide therapy individualization. In fertility sparing treatment therefore first data show, that women with MMRd endometrial cancer have shown worse response to fertility sparing treatment. However, studies focusing on fertility sparing treatment and molecular classifiers are lacking. Initial data also point towards lower complete response rates at 6 months and overall complete response in MMRd tumors than NSMP tumors. Yet most young women still classify into the NSMP subgroup of EC requesting additional characterization and more robust data. Studies suggest also the need for integration of additional refinement and analysis of carcinoma in women with endometrial cancer using markers such as PI3K/Akt/mTOR markers and other interconnected signaling pathways.

Presentation 1.8. Gynecological cancer risk associated with ART-technologies – prevention and management strategies

Presented by Gligor **Tofoski**
Department of Gynecology and Obstetrics, Ss. Cyril and Methodius University in Skopje, North Macedonia (gligor.tofoski@gmail.com)

There is concern that assisted reproductive technology (ART) may increase gynecological cancer risk, such as ovarian cancer. However, to date most studies are inconclusive, due to multiple confounding factors. Such factors include infertility itself and older age. Observational studies overall found that women treated with fertility medications may have no greater risk of ovarian cancer than untreated infertile women. While steps have been taken to minimize bias, the conclusions are limited because they are based on observational data and do not account for confounding factors such as treatment regimen, parity and specific infertility diagnosis. Many prospective and retrospective studies were carried out on this topic, but no relevant randomized clinical trials. Fertility-sparing treatments with safe oncological outcomes are feasible in endometrial, cervical, and ovarian cancer cases. After fertility-preserving treatment for gynecological cancers, ART can enable pregnancy to be achieved with apparent oncological safety. The success of such procedures should directly impact clinical practice and management of those patients who require fertility-sparing treatment.

Presentation 1.9. Medical Assisted Reproduction (MAR) for patients who experienced gynecological cancer - how accessible is it?

Presented by Vera **Dimitrievska**
University of American College, North Macedonia (vdimitrievska@gmail.com)

In 2015, the World Health Organization (WHO) estimated that cancer is a main cause of death worldwide and a significant cause of morbidity, loss to productivity, and rising cost of healthcare. According to the Centers for Disease Control and Prevention (CDC), in 2016, 14 million new cases of cancer were diagnosed worldwide with 8.2 million deaths attributed to cancer.
Gynecological cancers have a prevalence of 15%-20% of the total neoplasms involving women.

About 20% of gynecological cancer affects women under 40 years of age, who often have not completed parity or are before their first pregnancy. Infertility is a global health issue affecting around 48 million couples and 186 million individuals worldwide. There are significant social inequalities in the prevalence, diagnosis, and treatment of infertility, as well as health risks for women, men and their offspring associated with these treatments.
As part of the European B²-InF project, with the main aim to explore young adults' knowledge, perceptions and concerns about infertility and Assisted Reproduction Technology (ART), and to contrast it with the information provided by ART providers in 8 countries (Albania, Belgium, Spain, Italy, Kosovo, Northern Macedonia, Slovenia, Switzerland), we examined 33 medical centers (3 to 5 centers per country). The transcripts of the clinics' information collected were analyzed following qualitative approaches from a socio-cultural and gender perspective. The objective is to describe and analyze, from a gender perspective, the social representations that these different centers make visible on their websites, through the texts and images posted.
Regarding the information provided by clinics, the explored websites present a large amount of information with an excess of technical and scientific terms without plain language interpretations, thus hindering its understanding by the general population. In addition, information about risk and success rates is not always available or it is presented in an unspecific or unclear way. Furthermore, information about infertility treatment services, and the marketing resources used to present this information, are primarily directed towards white and heterosexual couples, and skewed towards women, which may contribute to the unequal social burden of female infertility.
To better inform European citizens about infertility and MAR treatments, clinics must align the information they provide with the concerns and expectations of the public, by all means meeting the legal standards for truthful advertising.

SESSION 2.

CLINICAL TRIALS: DESIGNING CURES 2.0

Presentation 2.1. The Clinical Trials Regulation in a Nutshell

Neville **Calleja**
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The European Union enacted a new Clinical Trials Regulation in 2014, replacing the previous Clinical Trials Directive of 2001, in a bid to create a level playing field across EU member states, whilst simplifying the application process for researchers and providing more visibility and more transparency to the general public on clinical trials. Indeed, following the publication of this regulation, applicants need only to apply to one competent authority in one of the involved member states, as opposed to two competent authorities (scientific and ethical) in each member state where the clinical trial is expected to occur. In addition, the European Medicines Agency has introduced the Clinical Trials Information System (CTIS), an IT infrastructure through which all clinical trial applications are to be processed, by applicants and regulatory authorities, and which also includes a public interface for the general public to be informed of what research is happening, together with documentation pertaining to such clinical trials.

Presentation 2.2. New treatments for advanced metastatic cervical cancer

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Novel therapies are needed for the treatment of recurrent cervical cancer. The best chemotherapy regimen to date has a response rate of 48% with an overall survival of 17 months, with limited options for second-line chemotherapy. Immunotherapy can induce a strong immune response in cervical cancer due to retained viral antigens and is reviewed in this article. Although the response rates to programmed cell death 1 (PD-1) inhibition alone have been modest, the landmark survival reported in the recently published trials suggests that immunotherapy will represent a paradigm shift in the treatment of advanced and recurrent cervical cancer. The Empower Cervical 1 trial has demonstrated that anti PD1 inhibitor Ceimplimab provide increased outcome in comparison to single agent chemotherapy in sec-

ond and third line setting of advanced disease. The KEYNOTE-158 trial has led to the approval of pembrolizumab in recurrent programmed cell death ligand 1 (PD-L1) positive cervical cancer based on a significant increase in overall and progression free survival when combined to platinum-based chemotherapy plus or minus bevacizumab. Combinations of programmed cell death 1 and anticytotoxic T-lymphocyte-associated protein 4 inhibitors (CTLA4) inhibitors have shown promising and durable activity. There is active research with new combinations of checkpoint inhibitors, as well as combinations of these drugs with chemotherapy and radiation, and other novel approaches. Responses to immunotherapy can be dramatic and durable. Continued work to find the optimal combination and setting for immunotherapy is ongoing and all these aspects were discussed during the COST meeting that represents an excellent network for future research collaborations.

Presentation 2.3. Neuroendocrine cervical cancer: do we made step forward in treatment?

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Neuroendocrine cervical cancer first was described by Albores-Saavedra in 1972, these tumors account for 1.4% of all invasive cervical cancers. The term neuroendocrine refers to the fact that the tumor cells originate from the embryonic neuroectoderm and display an immunohistochemical profile consistent with endocrine glandular cells. The small-cell neuroendocrine tumor is the most common (80%), than large-cell neuroendocrine carcinoma (12%), and other histologic types such as undifferentiated neuroendocrine tumors (8%). NECC are aggressive tumors with a high tendency for nodal involvement and distant metastases. Independent prognostic factors are age, lymph node metastases, smoking, pure small-cell histology, tumor size. Overall, the 5-year survival rate is 36% and the median overall survival ranges between 22 and 25 months and 2–15% for patients with Stage III-IV disease. In 2017–18, the WHO and International Agency for Research on Cancer (IARC) convened introduce new terminology incorporated into the 5th edition of WHO classification of tumor. Low-grade cervical NETs are very rare and encompass Grade 1 (typical carcinoid) and Grade 2 (atypical carcinoid). Using the 5th edition WHO terminology, the poorly differentiated (high grade) include small and large cell variants. The difference in grading of cervical NETs is based on mitotic index and Ki-67. Even in this rare cervical tumor HPV has an important role. In Castle P et al meta-analysis in SCNC, 85% were HPV positive, 78% were HPV16 and/or HPV18 positive, 51% were singly HPV18 positive, and 10% were singly HPV16 positive. In a subset of 5 SCNC studies (75 cases), 93% were positive for p16INK4a by immunohistochemistry and 100% were HPV positive. For LCNC, 88% were HPV positive, 86% were positive for HPV16 or HPV18, 30% were singly HPV18 positive and 29% were singly HPV16 positive. Despite a small number of studies that had examined mutational hotspots in these neoplasms and attempted to characterize them from a molecular/somatic mutation standpoint the most frequently altered genes were PIK3CA (19.6% of cohort), MYC (15.5%), TP53 (15.5%), and PTEN (14.4%). Also comparing with another neuroendocrine tumors, it was identified PI3-kinase or MAPK pathway activating mutations in 67% of NECC. When compared to NECC, lung and bladder small cell carcinomas exhibited a statistically significant higher rate of coding mutations ($P < .001$ for lung; $P = .001$ for bladder). Mutation of TP53 was uncommon in NECC (13%) and was more frequent in both lung (103 of 110 tumors [94%], $P < .001$) and bladder (18 of 19 tumors [95%], $P < .001$) small cell carcinoma. These comparative genomics data suggest that NECC may be genetically more similar to common cervical cancer subtypes than to extra-cervical small cell neuroendocrine carcinomas of the lung and bladder. Comparing HGNECC with small cell lung cancer (SCLC), significant differences in TMB, microsatellite instability, HPV-positive status, and in PIK3CA, MYC, PTEN, TP53, ARID1A, and RB1 alteration rates were found. These results may have implications for the selection of cytotoxic and targeted therapy regimens for this rare disease. The most common primary treatment was radical surgery combined with chemotherapy. There was no standard chemotherapy regimen, but platinum and etoposide were the most commonly used treatment. Radiotherapy-based treatment schemes were also commonly utilized in the upfront setting for early-stage disease. Both the SGO and the Gynecologic Cancer Intergroup (GCIG) recommend multimodal therapy for all stages of neuroendocrine tumors of the cervix, and the majority of patients receive some combination of surgery, radiation, and chemotherapy. For early-stage disease (tumors ≤4 cm) and negative nodes on imaging, radical hysterectomy and pelvic lymphadenectomy followed by chemotherapy with platinum and

etoposide is the primary management recommended with consideration for additional radiotherapy. Frumovitz and colleagues presented the results from a retrospective NeCTuR registry study of topotecan, paclitaxel, and bevacizumab (TPB) in patients with neuroendocrine cervical cancer. This analysis included 62 women who had received TPB and 56 who had nonTPB-based treatments, with the caveat that some patients had received those drugs individually. Results showed that the median PFS with TPB was 8.7 months *vs*. 3.7 months without TPB (HR: 0.27), and at 1 year, 26% of patients remained on TPB regimens *vs*. 9% with non-TPB regimens. However, no significant OS difference was seen between the TPB *vs*. non-TPB groups (median OS: ~15 months; HR: 0.87). Nevertheless, the authors concluded that TPB should be considered an option for recurrent, high-grade neuroendocrine cervical cancer. In multiple resistance immune checkpoint inhibitors and targeted therapies may be beneficial however, the literature is limited. In Caroll M et al study the checkpoint inhibitors, was with no responders. high-grade neuroendocrine cervical tumors are almost always PD-L1-negative and microsatellite-stable so checkpoint inhibitors should be used with caution. Large studies in patients with uncommon or rare tumors are difficult with limited prospective data with which to guide decisions. For women with small- and large-cell neuroendocrine cervical carcinoma there currently exists a rich network on social media sites, making recruitment to protocols much more feasible. In 2013, The University of Texas MD Anderson Cancer established a Neuroendocrine Cervical Tumor Registry (NeCTuR) where data are prospectively entered.

Presentation 2.4. Cancer registries and clinical practice

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Modern clinical decision-making rests on evidence-based medicine, where Randomized Controlled Trials (RCTs) and metanalyses are considered unequivocal. Such undisputable truth is often equivocal: young and fit patients are more frequently enrolled, while older and frail ones are excluded as well as those on polypharmacy, deprived and dependent ones. Individuals with poor literacy are preferred to unlearned patients. Negative trials are frequently unpublished, therefore meta-analytic investigations present biased results as they rest on skewed data. More on, RCTs are extremely expensive, with phase III RCTs costing several tenth of millions of euros. At a time when real-time, good quality data can safely be harvested, the scientific community should look forward to relying more consistently on Cancer Registry data. There are numerous examples of their effectiveness and their clinical impact. Consecutive and real-life information provides extremely reliable and sound results.

Presentation 2.5. The importance of robust data collection to steer clinical research

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Different objectives characterize clinical and population-based studies. For instance, in survival studies, clinical study evaluates the effectiveness of treatment and indicates the highest achievable survival in selected patient groups. By contrast population-based study evaluates the effectiveness of the health care systems and estimates the average survival actually achieved in the general population. Clinical studies require an ad hoc design and data collection while in population-based studies, data are currently collected by disease (cancer) registries (PBCR). The definition of the disease needs to follow international classification, the variables collected are few and of high quality to permit the comparison between populations. In high income countries PBCR collect more clinical variables. However, the additional variables follow strict rules given by the IARC. PBCR with their data on incidence are able to provide estimation on number of new diagnoses per year or incidence rates by country then make available those for rare gynecological tumors. Also, PBCR indicates time trends then to know if there is an increment/reduction of diagnosis or if incidence remains stable. The correct pathological diagnosis. which for rare cancers needs sometimes of a second opinion, have to be acquired and correctly codified by registry. To provide survival by type of tumor, PBCR have to access to reliable and complete sources to know the life status of cases included in the registry. For the majority of rare gynecological tumors, this contribution will show incidence and survival differences by type of tumors, country, time period, hospital.

Presentation 2.6. Electrochemotherapy in vulvar cancer

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Vulvar cancer is a rare disease that requires multimodal treatments including surgery, radiotherapy, and chemotherapy. Unfortunately, the disease often relapses, and treatment options are limited, especially for elderly women with comorbidities. Therefore, we aimed to find a low-impact and effective therapy for these patients. Electrochemotherapy with bleomycin has proven to be a valid alternative, and our studies have demonstrated an efficacy of around 80% in local control. The first study was conducted in 2012, evaluating only eight cases. Subsequent studies in 2014 with 25 cases confirmed the initial findings and showed that the therapy could be repeated, resulting in further response. In 2019, we validated these results on a large scale with a multicenter study called Elechtra, which confirmed the findings of the first studies with low toxicity and minimal side effects. In 2021, we evaluated the quality of life of these patients and found that those who responded to the therapy experienced a significant reduction in pain, particularly for smaller lesions located in the posterior vulva. In Bologna, we are conducting a new study that uses Bleomycin associated with platinum-based chemotherapy. After ten years, the European Society of Gynecological Oncology guidelines have recognized electrochemotherapy as an alternative in palliative treatments of vulvar tumors.

Presentation 2.7. The Impact of Sarcopenia and Exercise Training on Skeletal Muscles in patients with rare cancer

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Sarcopenia is frequently encountered in the elderly, and more frequently in subjects with cancer. In these patients, low muscle mass and function due to sarcopenia has been identified as negative prognostic factor predisposing to falls and fractures. Furthermore, muscle wasting, and weakness caused by cancer-related inflammation, significantly affect quality of life in everyday living activities. Because the pathophysiology is not yet fully understood, therapeutic options remain limited and physical activity remains a viable modality to enhance muscle function in older sarcopenic adults. Despite lifestyle interventions, especially exercise and nutritional supplementation, prevail as mainstays of treatment, concerning rarer forms of gynecological tumors, not enough studies have currently been conducted to estimate the prevalence of sarcopenia and how it can affect clinical and functional outcomes in everyday clinical practice and life. COST is an excellent starting point for future research studies and interdisciplinary collaborations.

Presentation 2.8. The rehabilitation of women with incontinence

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Rehabilitation has been defined by the WHO as a set of interventions designed to optimize functioning and reduce disability in individuals with health conditions in interaction with their environment. Urinary incontinence affects 25% to 45% of women worldwide. The most common forms are: 1. Mixed urinary incontinence that is the involuntary loss or urine associated with both stress and urgency; 2. Stress urinary incontinence that is the involuntary loss of urine through physical exertion or effort, coughing or sneezing; and 3. Urgency urinary incontinence that is the involuntary loss of urine associated with a sudden and compelling desire (urgency) to urinate that is difficult to delay. The person with urinary incontinence should undergo a multidisciplinary treatment including rehabilitation. There are several treatments that might be proposed, including: pelvic floor muscle training with or without biofeedback, electrical stimulation, magnetic stimulation, vaginal cones, bladder training, prompted voiding, anti-incontinence devices, lifestyle interventions such as weight reduction, and complementary alternative techniques such as acupuncture. Evidence on the effectiveness of the management of urinary incontinence are still scarce in quality and quantity. Larger, well conducted trials, using CONSORT guidelines for data reporting, and addressing important clinical outcomes should be performed.

SESSION 3.

THE DIFFICULT DIAGNOSIS: FROM CLASSIC METHOD TO DIGITAL PATHOLOGY

Presentation 3.1: Rare ovarian tumors: a pathological point of view

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Gynecological oncology has a paradoxical high prevalence of rare cancers, especially in ovarian tumors, characterized by small cases number, long trial accrual times, few interested investigators, low funding/Pharma priority, fewer patient advocates and lack of trial designs. Clear Cell, Low Grade Serous, Mucinous, Undifferentiated carcinoma, Carcinosarcoma, Small cell carcinoma hypercalcemic type and Mesonephric-like adenocarcinoma can be considered rare epithelial entities, distinct for morphology and molecular background. Advances in understanding their heterogeneity should improve pathologic diagnostic criteria, molecular characterization, and hypothesis-generating clinical studies.
Gynecological Neuroendocrine neoplasms (NENs) are rare (NET/NEC) and more frequent in mixed forms (NECs more frequent than NETs).
Sex cord-stromal tumors represent a heterogeneous group, mostly indolent, with wide range in incidence age, often associated with endocrine manifestations. Peculiar genetic mutation has been revealed. Sex cord like architectural features can be frequently observed in other NO SEX-CORD tumors. A clinical-histo-molecular approach: FATWO (no recurrent alterations) STK11 adnexal tumors (STK11), Sex cord-stromal tumors (FOXL2, DICER1) is necessary for a better definition and highly diagnostic reproducibility.
Germ cell tumors are rare ovarian tumors, occurring in young age. Since introduction of modern chemotherapy, cure rate is 95%. Second opinion, multidisciplinary staff and biology research are fundamental. Questions open for the medical treatment.
Finally, rare gynecological tumors are not so rare, characterized by various clinical presentations and different natural histories. Further collaborative efforts will implement new trials design, and care pathway organization at all levels, in order to better molecular classification, improve prognosis and define possible new molecular targets.

Presentation 3.2. From colposcopy to digital colposcopy with guidance from Spain to Uganda

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Cervical screening is a way of preventing cancer. It tests for a virus called high risk human papillomavirus (HPV). High risk HPV can cause cervical cells to become abnormal. This virus is present in 90% of young women since the first sexual intercourse. However, the immune system is able to wash away the virus in the majority of cases under 25 years, this is why the screening begins at this age approximately in first world countries. For some unknown reason, the incidence of cervical cancer in Uganda Is one of the highest in the whole world, with a relative risk of 4,4 in this Country. In 2020 the World Health Organization (WHO) said the incidence of cervical cancer is 97,5% with a mortality for this cause of 66%. The cervical cancer screening project began in March 2022 and will go on every six months by the Association Idiwaka. It consists of giving out information and engaging with the community. For this, regular meetings with women in the area are held in order to say who Idiwaka is and what the aim is of the project in order to get their approval.
Once the patients are at the hospital, the screening begins with more information about cervical cancer and the importance of prevention. They then fill in and sign the consents and HIV and HPV tests are performed. If HPV tests positive, they then are performed a diagnostic colposcope as a triage test according to (WHO) guidelines. The colposcope is a stereoscopic low-power microscope used to detect an abnormality, to determine the extent of the lesion and the severity of the lesion, and to guide biopsy from the most appropriate area. Excisional treatment of cervical precancerous lesions is performed under colposcopic visualization.
A way to improve the guidance from Spain to Uganda and to continue doing training from the distance is the digital colposcopy. It is an Internet-enabled portable colposcope designed to simplify the workflow during and after a colposcopy

procedure. User-friendly configuration for easy adoption by novice and experienced clinicians. Video of the procedure in real time with an Iiwaka cervical expert is possible and so the colposcopy can be remotely monitored in real time, directing the clinician to the point of care throughout the entire consultation. Moreover, pictures to store in the patient's clinical record to revise in future consults. This way, apart from having regular training campaigns in Uganda, online guidance can improve their practices around the rest of the year with full support from the team.

Presentation 3.3. Development and validation of a serum metabolomic signature for endometrial cancer screening in postmenopausal women

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Endometrial cancer (EC) is a type of cancer that develops in the glandular lining of the uterus and is the most prevalent gynecological cancer globally. It accounts for about 50% of all female urogenital tumors and is the sixth most commonly diagnosed cancer in women. As early detection is crucial for successful treatment outcomes, there is a need for effective screening methods. However, currently, there is no mass screening available for asymptomatic individuals. We conducted a study to evaluate the diagnostic accuracy of an ensemble machine learning algorithm that uses serum metabolomics signatures for EC screening. The algorithm was trained using metabolomics profiles from 88 EC patients and 80 controls and validated on 1430 women with unknown EC status. The study revealed that the algorithm accurately identified 16 out of 18 true-positives, with only two false-positives and no false-negatives. We further validated the model on a cohort of 871 subjects undergoing hysterectomy, of which 126 were diagnosed with EC. The results showed that the proposed metabolomics signature was not affected by the stage and grading of EC or the presence of other comorbidities. However, it was influenced by different EC histotypes, providing additional EC-linked substratification. These findings suggest that metabolomics could be a promising and novel screening tool for EC.

Presentation 3.4. Antibody-modified nanomembrane as specific ultrasensitive detectors for clinical application

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Identification of early-stage disease markers and a fast detection of markers accompanying disease development play a crucial role in modern personalized medicine. Preparation of nanofiber-based detectors functionalized on nanofiber surface with relevant antibodies, aptamers or other bioactive substances belong among the key steps towards highly sensitive and specific bionanosensors and also towards smart targeted and controlled drug delivery systems. Nanofibers are unique due to their extremely large ratio between the surface and volume. Thus, the probability of the disease marker interaction with the nanofiber is also very large. Consequently, the application of the smart gynecological tampon based on nanofibers functionalized on the surface with a specific antibody against the disease marker could lead to the specific interaction of the specifically modified nanofiber surface with the disease markers even at their very low concentration and, thus, to identification of the disease at its early stage. Such a layer could, in addition, serve also as the active part of the bionanosensors in microchips or, alternatively, can be applied for nanotheranostic application.

Presentation 3.5. Immunohistochemical predictive markers of response to conservative treatment of endometrial hyperplasia and early endometrial cancer

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Progestogens are widely used for the conservative treatment of endometrial hyperplasia and early

endometrial cancer. However, they do not always achieve the regression in all cases. Although several immunohistochemical markers have been assessed to predict the response to treatment, their usefulness is still unclear. We carried out a systematic review to analyze the usefulness of each immunohistochemical marker studied in predicting the response to progestogens in endometrial hyperplasia and early endometrial cancer. After reviewing 27 studies with a total of 1360 women; 43 immunohistochemical markers were identified and assessed. The most studied predictive markers in the pretreatment phase were progesterone and estrogen receptors. Progesterone receptor B appeared more promising. Further studies are needed to confirm the usefulness of mismatch repair proteins, Dusp6, GRP78 and PTEN combined with other molecules such as phospho-AKT or phospho-mTOR. In the follow-up phase, Nrf2 and survivin showed the stronger evidence; a role may also be played by Bcl2 and Ki67. Further studies are necessary for Fas, NCoR, AKR1C1, HE4, PAX2 and SPAG9. In conclusion, several immunohistochemical markers might be helpful in predicting the response to conservative treatment of endometrial hyperplasia and early endometrial cancer on pretreatment and follow-up specimens. Further studies are needed to confirm their usefulness and possibly integrate them in a predictive immunohistochemical panel.

Presentation 3.6. **Identification of the first map describing the human and the HPV-16 viral genome interaction**

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Human Papillomavirus (HPV) infects actively proliferating cells such as epithelial cells, and its replication cycle is associated with host cell differentiation. Therefore, it can cause asymptomatic infections or clinical manifestations, ranging from slight to severe diseases. HPV16 and 18 genotypes cause 70% of cervical cancers worldwide. The aim of this work is the identification of human proteins that interact with the HPV16 genome in infected cells, to search for new potential therapies through an innovative approach. A new method was developed to generate long DNA regions modified with biotinylated oligos, called "Long Regions of DNA Pull-down" (LDP). Hybrid oligos were designed using Primer3 software and the whole genome of the HPV16 sequence. Primers contain a specific part for each sequence and the other part corresponding to unique sequences do not present in the human and viral genome. The pHPV-16 *Escherichia coli* plasmid (ATCC® 45113™) was amplified, extracted, and used as a template for the first amplification step by pull-down PCR. The 500 bp amplifications were used as templates in the second round to obtain fragments with biotinylated 5' ends. SiHa cells, containing integrated copies of HPV16, were grown, and nuclear extraction was performed. Subsequently, DNA pull-down was carried. The biotinylated dsDNA fragment interacted with the streptavidin beads; then, the SiHa nuclear extract was added to this complex and incubated to promote interaction. In this way, it was possible to isolate specific interacting proteins that was processed by mass spectrometry. Approximately 4350 proteins have been identified by the mass spectrometric analysis, of which 310 interact with a single oligo. Gene Ontology (GO) studies were conducted to analyze the biological processes, molecular functions, and cellular components in which these proteins are involved. Most of them play a role in DNA replication, transcription, and translation. Some interactors were already known and implicated in HPV-associated diseases, while others had never been described. For example, chromodomain-helicase-DNA binding protein 4 (CHD4) is a component of the NuRD complex that participates in chromatin remodeling by deacetylating histones. These data may pave the way for further studies to find new potential and effective therapies to combat HPV16 infection, progression, and pathogenicity.

SESSION 4:

ROUND TABLE. PHARMA-BIOTECH: CHALLENGES IN THE 21ST CENTURY

PANEL MEMBERS:

- Francesco **Cosentino**
- Danica **Cujic**
- Massimiliano **Galdiero**
- Carlo **Ronsini**
- Vanda **Salutari**
- Sergio **Sandrucci**
- Klejda **Harasani**

Presentation 4.1. Characteristics of clinical trials investigating pharmacological treatments for rare gynecological cancers

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Members from Working Group 5 "*Coordination of interactions between clinical trials, translational research, and basic research*" have focused on developing a clinical trial reference depository and systematically reviewing clinical trials for rare gynecological malignancies. Master protocols are classified into basket trials, umbrella trials, and platform trials.

The PRISMA 2020 guidelines for systematic reviews and meta-analysis were followed and two different databases were searched. The WHO's International Clinical Trials Registry Platform (ICTRP) and the clinicaltrials.gov databases were consulted, as well as the articles published in PubMed/MEDLINE reporting on results of the included clinical trials on rare gynecological cancers. A total of 212 records, covering trials recorded during the period 1993-2022, were included in the final review.

The number of clinical trials investigating therapies for rare gynecological cancers (RGC) has steadily increased over the years, reaching 79 clinical trials initiated in the period from 2017 to 2022. Moreover, the most evident finding is the increment in trials involving targeted therapies. Indeed, 35 new clinical trials on targeted therapies started in the last five years compared to just 26 from 2000 to 2016. Furthermore, during the last five years (2017-2022) there has been an increase in clinical trials investigating a combination of therapies (targeted therapies plus chemotherapy) and a decrease in clinical trials focused on chemotherapeutic agents only. Our review revealed that overall, the majority of clinical trials included predominantly chemotherapy (41.98%), followed by targeted therapy (30.19%) and a combination of therapies (23.11%). A paradigm shifts in oncology drug expenditure from chemotherapy and hormonal therapy to targeted approaches has occurred over the past 25 years.

More than half of the trials were phase II trials (110; 51.89%), followed by phase I (53; 25.00%), and phase III (32; 15.09%). The status of recruiting was mainly completed (80; 37.74%), recruiting (59; 27.83%), and active, not recruiting (25; 11.79%). There were 26 (12.26%) terminated or withdrawn clinical trials due to various reasons such as low accrual (reported in 8 clinical trials), lack of approval by the ethics committee, sponsor decision to withdraw funding, principal investigator departure from institution or due to toxicity. Funding was public in 101 clinical trials (47.64%), private in 66 (31.13 %), private, non-profit in 40 (18.87%). Most of the trials were international and multi-center.

Basket trial: combination immunotherapy with the anti-programmed cell death protein 1 (anti-PD-1) antibody nivolumab and the anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) antibody ipilimumab has demonstrated significant clinical efficacy across a range of common malignancies, justifying evaluation of this combination in rare gynecological cancers.

There was only little variation in the design of completed clinical trials, where most were open-label (no masking) with single group assignment and allocation was not applicable as they were single-arm trials. Only one completed clinical trial had a calculated Jadad score higher than 2 (Jadad score = 3) which indicates higher quality. This phase III clinical trial of carboplatin and paclitaxel with or without bevacizumab in women with stage III or IV epithelial ovarian, primary peritoneal or Fallopian tube cancer was randomized, double blind and included a description of withdrawals and dropouts. Results were unpublished in 65% of the completed clinical trials and 45% of them had neither publications, nor had posted any results. These findings highlight the need for higher-quality clinical trials and better reporting of data from the current ones.

Presentation 4.2. MCCR alumni club survey on tackling hurdles in front of young clinical investigators in oncology

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Cancer is a leading cause of morbidity and mortality worldwide. Considering the huge diversities in etiological factors, the complexity of cancer progression and the accessibility of treatments only well organized and massive translational and clinical research can improve global outcomes. Methods in clinical cancer research Alumni Club (MAC) created a survey to identify the main challenges in front of young clinical investigators in oncology.

An anonymized survey was spread in social media between April and November 2022. Target population were health-care professionals involved in any aspect of oncology – physicians, nurses, and researchers. We divided participants to young - below the age of 40, and more experienced – ≥40 years; and according to country of practice to Europeans and non- Europeans. Results: we received 121 responses from 36 different countries. 89 participants were from European countries and 32 were from non-European. 87 (72% of the participants) were below 40 years. Older, more experienced professionals and Europeans are more likely to be involved in clinical trials in every aspect. The main source of funding – independently of geographic location – were Pharma grants. Investigators out of Europe have less participation in international grants. Over 50% of participants dedicate time for clinical research from their personal time and are not paid for it. Almost 50% of investigators don't have access to an experienced mentor in conducting clinical trials in their institution.

In conclusion, despite the limited number of participants, our data support the fact that access to education and training as well as access to appropriate networking are key limiting factors in developing clinical research by healthcare professionals.

Presentation 4.3. Pharma-Biotech collaboration in biomarker discovery

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Laboratory testing is quite economical compared to other medical procedures: up to 60% of medical decisions is based on laboratory results, which account for 5 to 10% of medical expenses. There are several interested parties, who might benefit from reliable clinical biomarker: 1) patient who expect as little invasive and unpleasant medical tests as possible, 2) clinicians who need reliable disease indicator, 3) scientists who are focused on focused on discovering disease mechanisms and identifying a sensitive and specific disease markers, 4) government that aims to reduce the costs of diagnostics and treatment and 5) industry that is seeking for new and innovative diagnostic products.

Biomarker discovery is complex, long-lasting, and expensive process. There is substantial difference between biomarker research in science laboratory and their clinical use in medical labs or even next to the patient's bed. Biomarker discovery usually requires expensive equipment, procedures that might be not always easy to perform or are based on custom made protocol, but not always appropriate for large scale testing and high throughput analysis. On the other hand, biomarker is suitable for clinical use if it is: reproducible, easy to perform, with affordable price, provides result in a short time, method is convenient for automatization.

Clinicians are an indispensable party in the biomarker discovery pipeline. While during initial processes of disease mechanism revealing and biomarker identification, tight collaboration between doctors and scientist is essential, later during validation, doctors are included in clinical studies, mainly supported by industry.

The research excellence is based not just on researchers' idea, but also greatly depends on available funding. Nowadays, the collaboration between SROs and industry, particularly life science, clinical diagnostics, and IT industry, is very important. Scientific institutions usually do not have potential to launch their invention on the market but could collaborate with industry in different ways: outsourcing R&D sectors from industry to SRO, submitting joint project application (*e.g.*, Horizon Europe 2021-2027), technology

transfer from science to industry through licensing or spin-off companies. This collaboration is not always smooth and there are few obstacles that could hinder this cooperation: researchers' fear of leaving basic research, administrative procedures reject industry to collaborate, regulation of IP rights and profit share. In the last few years, significant efforts are made to improve the research infrastructure in Serbia. The Serbia Accelerating Innovation and Growth Entrepreneurship Project (SAIGE Project), supported by the World Bank, aims to improve excellence of scientific research and to promote innovative and entrepreneurial thinking among researchers. The establishment of BIO4 campus tends to unite institutes and faculties involved in biomedicine, biotechnology, bioinformatics, and biodiversity and stimulate exchange of knowledge and expertise. Beside the most up-to-date research infrastructure equipment organized, through several thematic facilities that will push forward basic research, the idea of the campus is also to stimulate innovative research and bring together research and industry knowledge and capacities.

Presentation 4.4: Perspective from industry experience

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Ylva **Kaiser**, independent contributor
Medical Advisor and Medical Affairs, Sanofi, Sweden

Disclaimer: this content was prepared by Stephanie Darmanin and Ylva Kaiser in their personal capacity, with a genuine interest to support the efforts being made to improve research outcomes and cure of rare gynecological cancers. The views, information and opinions expressed are solely those of the authors and do not reflect the view of their employers or anyone else.

While randomized controlled trials are the gold standard in novel therapy development, in rare diseases the low patient numbers as well as the cost involved in interventional clinical trials represent a significant barrier. As such, a more efficient research approach through real world evidence (RWE) and pragmatic (combining interventional and real-world arms) studies would leverage the abundant historical patient data in patient journals and databases and bypass the need for large numbers of real-time patients and high financial burden.

Identifying relevant key opinion leaders, establishing relationships at a local, national, and global level and engaging in international collaborations around RWE to create bigger data collections is critical. When approaching pharmaceutical company medical affairs teams and patient organizations for industry-sponsored research grants and joint projects, researchers should have a clear, clinically relevant idea - with a connection to patient data/samples, a correlation to parameters of disease/clinical phenotypes, and outcomes that can eventually be applied in the clinic, or at least be of clear relevance to clinicians and patients. The opportunity presented by investigator-sponsored studies (ISS), where the investigator is the instigator of the study proposal as well as the principal owner of the data, but resources are provided by a partner within the pharmaceutical industry, should be further explored. A collaborative approach involving clinicians is preferable in order to gain company interest and create a platform for future investment in the disease area.

Innovative protocols for personalized one-patient clinical trials, designed around the individual patient's genomic, proteomic, and histological profile are an appealing option, which will however require consideration of regulatory and ethical implications.

AWARD CEREMONY

Prof. Jean Calleja-Agius
GYNOCARE COST Action Chair, has been presented with the **Giovan Giacomo Giordano NIAF Lifetime Achievement Award for Ethics and Professionalism in Medical Research** (https://www.ilmattino.it/napoli/cronaca/premio_giordano_riconoscimento_a_due_ricercatori_della_federico_ii-7246479.html).

This award was established in 2010 in honor of the late Prof. Giovan Giacomo Giordano, renowned pathologist and former chair of the Department of Pathology, National Cancer Institute of Naples and Professor at the Federico II University of Naples, who dedicated more than sixty years of his life to the study of cancer and the role of environmental factors in the onset of this disease. Prof. Giovan Giacomo Giordano was also a major advocate against corruption in the Italian medical community and the driving force for the establishment of medical ethical standards among his colleagues.
The award was presented to Prof. Jean Calleja-Agius by Prof Antonio Giordano, son of the late Prof. Giovan Giacomo Giordano, and who himself is a Professor of Molecular Biology at Temple University in Philadelphia and a 'Chiara Fama' Professor in the Department of Pathology and Oncology at the University of Siena, in Siena, Italy. Prof. Antonio Giordano is also the Director of the Sbarro Institute for Cancer Research and Molecular Medicine and the Center for Biotechnology at Temple's College of Science & Technology in Philadelphia, USA. This was the first time that this award was ever awarded to a non-Italian or Italian-American professional. The other two recipients of the award this year are: Prof. Orlando Paciello and Prof. Roberto Ciarcia from the Faculty of Veterinary Medicine at the Federico II University of Naples, Naples, Italy.
Prof. Orlando Paciello, Professor of Veterinary Pathological Anatomy of the Federico II University of Naples has a PhD in "Environmental Pathology". He has been involved in the study of animal pathologies tumors related to environmental pollution and the possible role of animals as sentinels of the state of health of the environment from the point of view of One Health.
Professor Ciarcia is an Associate Professor in Veterinary Pharmacology and Toxicology, also at the Federico II University of Naples. From 2016 to 2021, he was the coordinator responsible for the international agreement between the Sbarro Health Research Organization (S.H.R.O.) of Philadelphia and the Federico II University of Naples. This award recognizes the merit of having studied the role of various bioactive substances for the treatment of oncological and chronic degenerative diseases, as well as the study of the potential role of natural compounds included in animal diets in mitigating the toxicity of food contaminants, such as mycotoxins, which represent a danger and a threat to human health and to various animal species. The studies of environmental contaminants, conducted by Professor Ciarcia, have become part of the so-called "One Health" approach, including food safety, the control of zoonoses and the fight against antibiotic resistance.

CONCLUSIONS

The GYNOCARE Conference in Naples has been an outstanding experience. It has favored several new collaborations with experienced and committed colleagues working in several disciplines in a lot of countries within and outside Europe. Despite the intense schedule of inspiring talks, the meetings provided many opportunities for discussions and interactions with leading scientists from around the globe in a very pleasant atmosphere. The next GYNOCARE conference will be held in Sofia, Bulgaria in July 2023. More information can be found on the GYNOCARE website: www.gynocare.net.