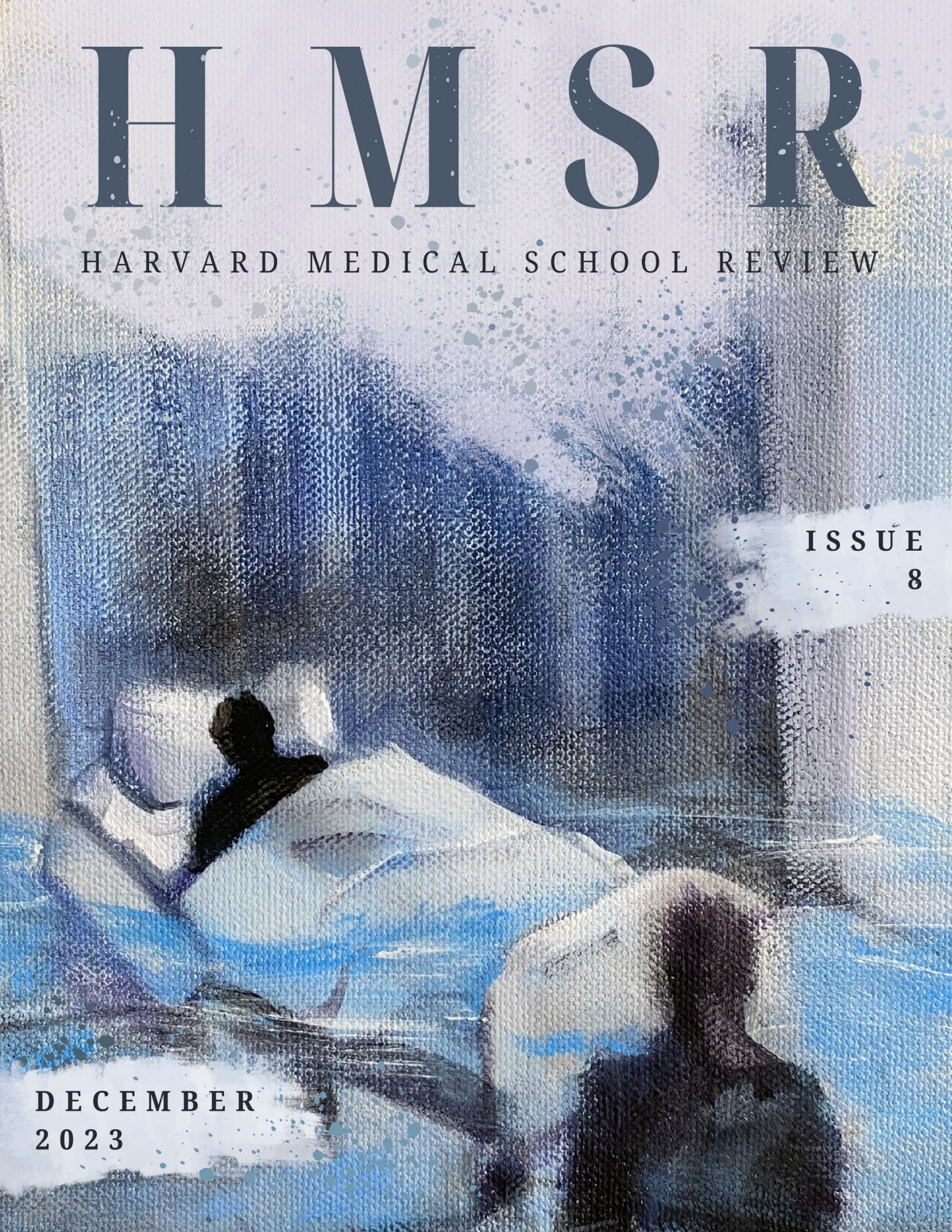
HMSR

HARVARD MEDICAL SCHOOL REVIEW



ISSUE
8

DECEMBER
2023

HMSR

December 2023

Dear Readers,

On behalf of the editorial board, I am proud to present the 8th edition of the *Harvard Medical School Review*. Towards our mission of promoting the inclusion of trainee voices in academic discourse, we present here the innovative work of medical students and resident physicians across the globe, reflecting the issues in medicine which are front of mind for the next generation of physician leaders.

From the insightful explorations of emerging treatment modalities to critical discussions on the ethical dilemmas in healthcare, our authors have approached their subjects with diligence and passion. We also showcase the artistic lens of medical trainees through original poetry and visual art. The blend of diverse intellectual inspirations with cutting-edge medicine will no doubt lead to never-before imagined advancements in patient care; we are grateful to our authors for offering an enticing glimpse into what the next decades may entail for our field, and to all who submitted to *HMSR* for contributing to this discourse.

This publication would not be possible without the outstanding efforts of our editorial board, consisting entirely of Harvard Medical School students. They have demonstrated unwavering commitment to enriching this community of budding physicians through thoughtful peer review and advocacy. I would also like to thank our esteemed Faculty Advisory Board for continuing to encourage and amplify trainee voices.

As we anticipate the future of medicine, it is our hope that this publication serves as a source of inspiration and knowledge. We encourage our readers to engage with the content, share their insights, and continue the discussions sparked by the articles presented in this edition. Congratulations to our authors and thank you to our readers!

Sincerely,

Arya Rao
Editor in Chief
Harvard Medical School Review

HMSR

Executive Board

Arts Editors

Associate Editors

About HMSR

The Harvard Medical School Review (HMSR) is student-founded, student-managed, and student-administered under the guidance of faculty and staff. Its mission is to provide a platform for students to contribute to important issues facing health and medicine through a variety of formats, including scholarly articles, editorials, and original artwork. Contributions are invited from the Harvard medical, dental, and public health schools, the rest of Harvard University, and other medical schools.

The works herein represent the views and opinions of the original authors and do not necessarily represent the views or opinions of the Harvard Medical School Review or Harvard Medical School.

HMSR

Table of Contents

December 2023: Issue 8

"Ships in a Gale" by Willem van de Velde. Courtesy National Gallery of Art, Washington.

On Dying

Qiang Zhang[1]

[1]David Geffen School of Medicine at University of California Los Angeles
Correspondence: QiangZhang@mednet.ucla.edu

is she dying? Is she,
going to die?
her words laced with a depth more profound than death,
i could not soothe -
both our hearts.

"but I saw her move",
she said, pleadingly,
"i held her hand, and i felt,
her tighten her hand around mine,
it was real. it was real."

i wonder if God exists. in this hospital bed,
surrounding these white walls, God in the dim light of day,
in the IVs,
    the ventilator,
        in the swimming sea of us - lost.

i sit with her. she plays me a song that,
she danced to at her wedding;
i sing it with her -
again, and again, we both drown together.

"The Death of Socrates". Courtesy Metropolitan Museum of Art, New York.

How to Define Death: Variation in Donation After Circulatory Death Policies

Devon E. Cassidy[1], Meredith Barrett[2], Michael J. Englesbe[2], Valeria S. M. Valbuena[2,3,4]

[1]University of Michigan Medical School, Ann Arbor, Michigan
[2]University of Michigan Department of Transplantation Surgery, Ann Arbor, Michigan
[3]University of Michigan Centers for Outcomes and Policy, Ann Arbor, Michigan
[4]National Clinician Scholars Program
Correspondence: CDevon@med.umich.edu

Organ transplantation utilizes a shared and scarce resource. In order to best utilize this resource, a network of organ procurement organizations, hospitals and individuals must work together. National societies make recommendations for policies that govern organ transplantation recoveries, however at each tier of the network there is room for variability. Donation after circulatory death (DCD) policies are one example of organ transplantation policies that are not standardized. The American Society of Transplantation Surgeons defines death in DCD recoveries as "irreversible cessation of cardiac and respiratory function." However, many individual hospitals have policies that may differ from this practice of observing pulseless electrical activity (PEA) for the ASTS recommended wait time of 2 minutes. In this study, we examined the DCD protocols of 50 adult hospitals representing a single OPO within Michigan. We hypothesized there would be institutional variance in the definition of death, the provider who can declare death and maximum wait time for the donor to expire after extubation until organ recovery is no longer pursued. We found that there was substantial variation in how each

hospital defined death, with the most common definition being asystole. Most hospitals require a physician to declare death in DCD and the minutes to expire range from 60 to 120 minutes. Given that the difference between PEA and asystole may result in time lost and organs to become nonviable, we recommend that standard policies are created and there is increased education to physicians and designees that declare death in DCD recoveries.

INTRODUCTION

Although hospitals have policies outlining their protocol for Donation after Circulatory Death (DCD) organ donation, the rules and regulations around withdrawal practices vary significantly (1). National societies including the American Society of Transplant Surgeons (ASTS) make recommendations for DCD recoveries, and organ procurement organizations provide guidelines to hospitals within their region, but these recommendations are open to institutional interpretation (2, 3). Given the importance of timely recovery of organs for transplantation, the variation in hospital DCD policies should be analyzed to limit organ discard.

There are many aspects of DCD protocols which can be scrutinized, but one specific area of interest is how hospital policies define death to allow for organ recovery. Death in DCD recoveries is defined by the ASTS as "irreversible cessation of cardiac and respiratory function" which in practice has come to mean observing pulseless electrical activity for a predetermined wait time to insure autoresuscitation does not occur (2). However, the power to write DCD policies lies within individual hospitals, and little is known about how closely these policies follow society guidelines.

METHODS

Within this context, we sought to explore the variation in how hospital policies define death to allow for organ recovery. Additionally, we sought to investigate variation in the providers who declare death and the maximum time between extubation and death.

Using content analysis, we examined 50 Michigan adult hospitals DCD policies, all serviced by a single organ procurement organization. We hypothesized there would be substantial variation in the definition of death within Michigan hospitals.

Hospital DCD policies were identified through Gift of Life Michigan. The study was submitted to the university Institutional Review Board and met the criteria for exemption from further IRB oversight. Written policy documents were accessed using iTransplant database in August, 2021 utilizing the most up to date policy that was provided. A sample representing hospitals with diversity in location, size, and type (transplant center non-transplant center) was used. Content analysis was conducted for the hospital policies (4). The protocols were manually searched and coded for the presence or absence of a definition of death. Policies were coded by a single investigator (D.C.) and analyzed for observation of ASTS guidelines.

RESULTS

There was substantial variation in how each policy defined death, with the most common definition being asystole. **Figure 1** demonstrates the different definitions of death encountered in the hospital DCD policies and the number of hospital policies that had these definitions. Of note, policies often contained more than one definition of death in their DCD protocol. Although ASTS recommends waiting for the onset of pulseless electrical activity, this was only found in 46% of policies. Eight policies did not clearly define the declaration of death. **Figure 2** illustrates the process of DCD

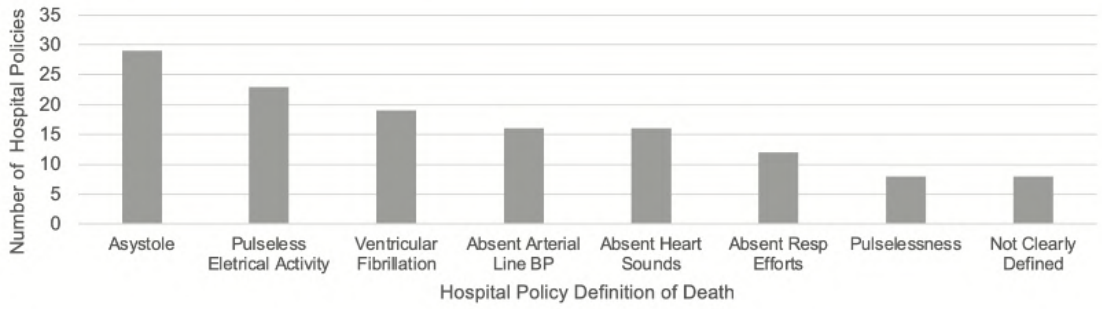


Figure 1: *Michigan hospital policy definitions of death.*

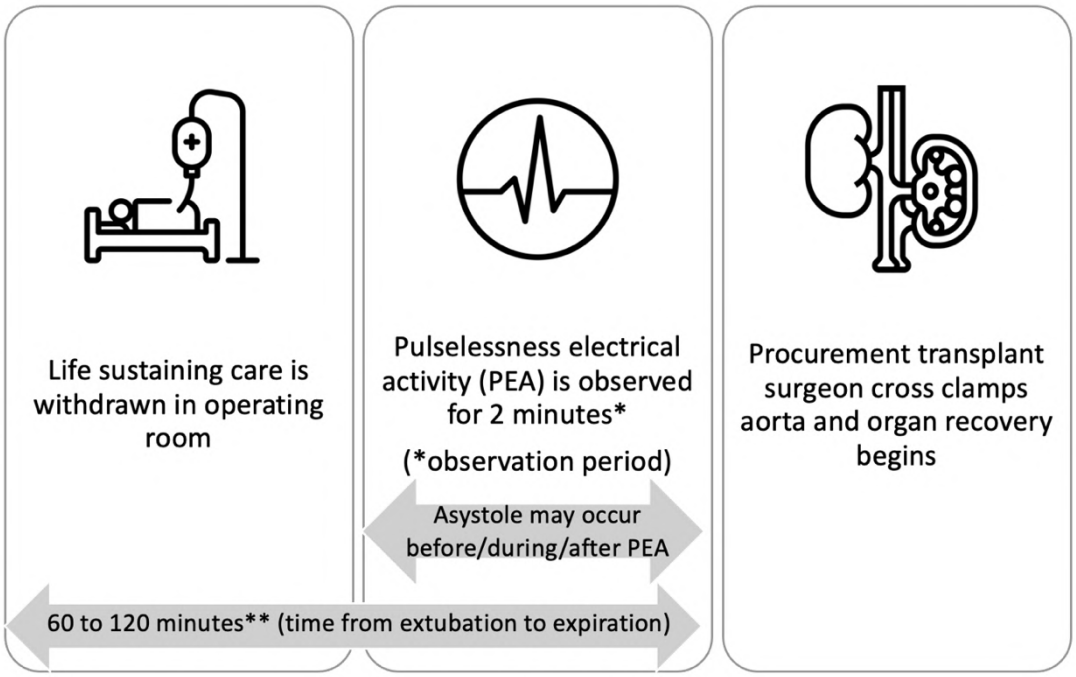


Figure 2: *Donation after circulatory death timeline from withdrawal procurement*

recoveries and how the maximum time from extubation to expiration ranged from 60 to 120 minutes in this study. Of note, 14% of hospitals allowed the recovery team to set the maximum time to expire and 14% did not provide a time within their policy. Additionally, we found that 96% of hospitals required a physician to declare death in DCD, in contrast to a nurse or advanced practice provider.

DISCUSSION

There is broad variation in the definition of death across hospitals in Michigan. This is just one aspect of many components of DCD donation that can result in delays in declaration and recovery. Given this variation in policy—or lack of documented policy at all—there are likely donors which are not declared dead until asystole (flatline of the ECG) is noted, which may jeopardize the donation intentions of the family.

This study has several limitations, including that only written DCD hospital policies were reviewed. These policies may not accurately reflect actual performance,

may be outdated and practices at the selected hospitals may not reflect hospital policy. Additionally, this study utilized a single coder and changes may have been made to polices since the data abstraction period. Finally, a diverse selection of the hospitals in Michigan was used, so conclusions about statewide policy variation should be made with caution.

In order to optimize organ utilization, a stakeholder-driven standardization of DCD policies is necessary. Beginning at an institutional level, it will be key to examine and maintain hospital DCD policies that align with current national consensus practice guidelines. Including stakeholders such as donor families, organ procurement organizations, hospital ethics representatives, hospital leadership, transplant centers, nurses, and anesthesia providers must be engaged in this work. The ASTS and the Association of Organ Procurement Organizations have put forward best practices for DCD liver recovery that serve as a model (5). There is a shared goal amongst stakeholders to maximize the gift of organ donation and a broad adaptation to a shared policy will allow for improved outcomes for donors and recipients alike.

DISCLOSURES

Funding: Not applicable.
Conflicts of interest: None.
Availability of data and material: Not applicable.
Code availability: Not applicable.
Ethics approval: Not applicable.
Consent to participate: Not applicable.

REFERENCES

1. Rhee, J.Y., Ruthazer, R., O'Connor, K., et al. (2011). The impact of variation in donation after cardiac death policies among donor hospitals: a regional analysis. American Journal of Transplantation, 11(8), 1719-1726. doi:10.1111/j.1600-6143.2011.03634.x
2. Reich, D.J., Mulligan, D.C., Abt, P.L., et al. (2009). ASTS recommended practice guidelines for controlled donation after cardiac death organ procurement and transplantation. American Journal of Transplantation, 9(9), 2004-2011. doi:10.1111/j.1600-6143.2009.02739.x
3. Choubey, A.P., Siskind, E.J., Ortiz, A.C., et al. (2020). Disparities in DCD organ procurement policy from a national OPO survey: A call for standardization. Clinical Transplantation, 34(4). doi:10.1111/ctr.13826
4. Bengtsson, M. (2016). How to plan and perform a qualitative study using content analysis. NursingPlus Open, 2, 8-14. doi:10.1016/j.npls.2016.01.001
5. Hobeika, M.J., Glazner, R., Foley, D.P., et al. (2020). A Step toward Standardization: Results of two National Surveys of Best Practices in Donation after Circulatory Death Liver Recovery and Recommendations from The American Society of Transplant Surgeons and Association of Organ Procurement Organizations. Clinical Transplantation, 34(10), e14035. doi:10.1111/ctr.14035

"Both Members of This Club", social commentary on Jim Crow by George Bellows. Courtesy National Gallery of Art, Washington.

Racism in Medicine Conference: A Student-Led Health Professional Event Cultivating Anti-Racism Education through Safe Spaces

Simran Kripalani[1], H-W. Banks[1], A. Sivendra[1], C. Rugama[1], I. DiBartolo[1,2]

[1]Cooper Medical School of Rowan University, Camden, New Jersey 08103
[2]Cooper University Hospital, Camden, New Jersey 08103
Correspondence: Kripal36@rowan.edu

Medical schools around the US recognize the importance of reform in medical education, as well as the pervasive role of racism and health disparities in medicine. The 2019 Racism in Medicine Conference (RiMC) hosted by Cooper Medical School of Rowan University (CMSRU) is an example of a medical student-led initiative centered on education and advocacy through the implementation of safe spaces. Pre- and post- survey responses from conference participants were collected to determine if RiMC influenced attendees' comfort addressing racism and knowledge on the subject. Specifically, qualitative comments in the post-conference survey showed the confidence and encouragement that attendees attained during the event, namely, to speak about racial issues in medicine and healthcare amongst colleagues. The conference served as a platform for future healthcare professionals to strengthen their foundational knowledge on the topics by studying and implementing a safe space model. This

construct was followed to encourage education and thereby a commitment to elimination of fear of retaliation and retribution when participating in anti-racism work.

INTRODUCTION

Despite the medical community's longstanding efforts to address health disparities, gaps in care for marginalized people still remain (1). The causes of these disparities are multifactorial and include a long-standing history of structural racism and systemic bias. This structural racism is pervasive in society as well as in medicine (2). Recently, there has been an institutional push to revamp medical education to not only focus on recognizing individual implicit biases and its relation to health inequities, but to also critically analyze the nuanced social contexts that perpetuate racism and maintain health disparities (3). Additionally, AAMC Medical Education Senior Leaders have released a document to provide resources for immediate action and goal setting in order to address and dismantle racism in educational programs (4).

While several medical schools around the US began revamping their curricula, medical students at several institutions began to recognize the importance of reform in medical education (5). Many of these students then took the initiative to bridge perceived gaps in health equity education in the interim by creating anti-racism task forces, social justice committees, and other avenues to address and engage in discussions around the topic (5-7). Medical students collaborated across medical schools to engage in initiatives to bring awareness to issues of racism and to create platforms for continued collaboration (6, 8).

The Racism in Medicine Conference (RiMC) is another example of medical student led activism centered on anti-racism. In 2015, an anti-racism group at Perelman School of Medicine at the University of Pennsylvania collaborated with interested medical schools in the Philadelphia area to host a conference to discuss the challenges and impacts of racism in healthcare. Attendees consisted of health professional students, as well as healthcare professionals in the community. Since then, RiMC has become an annual conference hosted by multiple sponsoring medical schools in the Greater Philadelphia and Delaware Valley area. The Conference's themes and objectives evolve every year at the hosting medical school's discretion, but always encompass social injustices occurring in the current socio-political climate and must abide by the joint memorandum of understanding drafted and modified by the schools involved in the conference (9).

In 2019, several student-run diversity organizations at Cooper Medical School of Rowan University (CMSRU) hosted the 5th annual RiMC conference. Leaders of the conference implemented learning objectives that required discussions that promote evaluation of racism's role in medicine in the past, present, and possible future. In addition, organizers aimed to widen discussions around systemic racism's role in medicine by including how it impacts our colleagues, other health care professionals, medical institutions, and patients we serve. Therefore, the conference had explicit objectives that included to:

1. Understand the historical roots of racism in medicine,
2. Recognize racism and its impact in health care settings, and
3. Empower students, health care professionals, and community leaders to utilize tools learned from the conference to address racism in their own institutions.

The organizers of RiMC 2019 understood the necessity of active discussions around racism but were also aware of its challenges, which

included the:

1. Challenge of putting yourself in others' shoes to learn from each other's experiences (1, 5, 10),
2. Fear of expression and open sharing due to potential retribution (11, 12),
3. Hierarchies present in medicine and healthcare (10), and
4. Trauma of sharing past experiences (12).

Recognizing the difficulties stated above, the RiMC 2019 organizers sought to create "safe spaces" within the conference, so all participants can have meaningful discussions around anti-racism and anti-marginalization. A safe space is a concept that emerged during feminist, queer, and anti-racist movements in the late 20th century (13). There are specific components of safe spaces that we sought to include in our conference (**Table 1**).

After integrating the above principles and establishing a memorandum of understanding with neighboring institutions to address all needs of workshop hosts and participants, we made sure to integrate trained volunteers to each room, and to provide extra spaces for therapy, debriefing, and praying.

Table 1

Conference Attendee Demographics	***Pre-Survey Respondents***	
	N = 82	**(%)**
Age	26.2 +/- 5.2	
Gender		
Male	18	(22)
Female	62	(76)
Non-Binary	2	(2)
Race		
Non-White	64	(78)
White	18	(22)
Occupation		
Student	77	(94)
*Other**	5	(6)
Ethnicity	**N = 64**	
Black or African American	23	(28)
Asian	21	(26)
Hispanic	10	(12)
Middle Eastern	3	(4)
Mixed	5	(6)
Other	2	(2)

**Staff or Clinical Faculty*

METHODS

The 2019 RiMC took place at Cooper Medical School of Rowan University (CMSRU) in Camden, NJ on November 19, 2019, and followed the standard format of past conferences. Attendees were given the opportunity to learn from and participate in small group discussions concerning a number of issues affecting marginalized groups. The purpose of these discussions was to empower participants to address racism in their personal and professional environments. The conference featured two keynote speakers who addressed all attendees in a large lecture format. The opening keynote speaker provided an introduction to the difficult conversations participants will be having via a general didactic talk about the history of racism in medicine. Student leaders then provided an overview of the conference and itinerary. This introduction was essential to set the tone for the conference and ensure

participants explicitly understood that this conference was meant for them to openly explore and engage in learning and discussion. It also contributed to adding to the participants' knowledge base to help aid in facilitating these open discussions during workshops. These small-group workshops were formatted as a round-table discussion. The subcommittee ensured that the breakout session leaders adhered to strict learning objectives to ensure standardization in format. Workshop leaders were expected to provide time for open discussion, tangible tools, resources, and strategies for participants to use personally or at their own institutions to combat health inequity and racism in medicine. During the workshops, a RiMC volunteer was present to address any issues that arose during the session, thus maximizing time for open discussions and minimizing technical difficulties and any other potential interruptions.

For the closing keynote speaker, we invited an individual that would empower and provide tools for immediate action in regard to addressing racism in medicine. He encouraged participants to learn more about pathways in academic medicine and medical education, as well as the need to increase diversity in academic medicine. He emphasized ways for attendees to become involved in publishing and academic scholarship. This talk was framed in such a way where representation would pave the way for the creation of safe spaces in academia by providing underrepresented minority (URM) medical students with an in-depth understanding as well as avenues for immediate action. Through representation and academia, URM students could have their voices heard and respected in medical education spaces.

A new addition to the 2019 conference was the curation of safe spaces that provided an open, comforting environment with ample space to physically and emotionally process these difficult conversations and then have opportunities to make actionable changes. It was the committee's top priority to provide designated zones in the form of healing corners on each floor utilized for breakout sessions so that participants can decompress, share experiences, express concerns, and/or have the capacity to receive and internalize information. For any possible emotional distress experienced, emotional support systems were also put in these spaces via recruitment of licensed social workers and therapists on site for attendees who needed to utilize their immediate professional expertise. There were also multiple physical "quiet zones" created for attendees to have time to themselves for decompression and recharging in a more private setting. In addition, there were numerous peer student volunteers available to talk to participants on demand if desired.

Creation of physical spaces was not the only accommodation made. It was important to the committee to take the concept of safe spaces and extend it to all aspects of the conference. Intentional planning of conference branding, food planning, community service, and social media promotion were all avenues taken to further the theme of fostering open communication. Conference subcommittees were formed to specifically work on the individual aspects. Food planning focused on providing sustenance with an emphasis on heritage and cultural diversity. By committing to supporting catering from local, small businesses of immigrant background, the conference exemplified a tangible example of "actionable change" and further created a welcoming and open space through representation. The community service aspect created a real time opportunity for attendees to participate in change themselves through winter clothing donations and a canned food drive held on site throughout the

conference. Finally, the social media component served as a way to promote the conference and its speakers through use of multiple platforms ensuring an increase in public visibility and awareness. Conference attendees were also able to facilitate conversations and interact on these platforms, thus creating a physical archive of the conference and the dialogue shared.

A major goal of the conference was to evaluate its impact and any changes in attendee perception on topics of racial discrimination in medicine. The aim was to collect data (quantitative and qualitative) for each attendee both before attending the conference and after. Pre- and post-surveys were provided to participants in the form of QR codes in the folders they picked up during registration. Surveys were IRB approved and were created using past survey questions, which were not previously operationalized and were conceived for the conference itself. The survey was condensed compared to the previous questionnaires used in order to account for participant compliance. Pre-surveys were found in attendee folders (in the form of a QR code and link), which were provided to participants upon registration before the commencement of the conference. Attendees received a unique identification number which was the same for both pre- and post-surveys. Researchers did not have access to any personal identifiers linked to the numbers, as folders were not pre-assigned to participants. At the end of the conference, the post conference survey was provided to individuals and time was allotted at the end of the conference to ensure completion. Responses were collected using Qualtrics and analyzed using qualitative and quantitative statistical analyses, such as Fisher's exact test. Open ended responses were used to determine common themes among participants. This qualitative analysis on identifying common themes was done by two authors (SK and HWB), who each went through the responses separately and came up with common themes. The authors then came together and compared extracted themes. If there were any discrepancies between the themes, a third author reviewed the theme and served as a tiebreaker (AS) on whether or not to include the theme. No tiebreakers had to be conducted in this case.

RESULTS

Of the 82 participants who responded to the pre-test survey, 94% (77) identified as students. 76% (62) of identified as female, 22% (18) identified as male, and 2% (2) identified as non-binary (Table 1). 78% (64) of participants self-identified as non-White; of those, 28% (23) identified as Black or African-American, 26% (21) identified as Asian, 12% (10) identified as Hispanic, 4% (3) identified as Middle Eastern, 6% (5) identified as mixed, and 2% (2) identified as other. The remaining 22% (18) of participants identified as white (**Table 1**). There were 32 participants who responded to the post-test survey.

When asked what participants wanted to gain from this conference, qualitative analysis was done to determine common themes stated through open-ended responses (**Table 2**). Themes that were noted in these responses largely stated that participants wanted knowledge and guidance on how to integrate anti-racist practice into residency training and serve as an advocate for patients of color. Others stated that they wanted to gain more insight on how to become an ally and learn about current initiatives that medical students and institutions are implementing to combat racism in medicine and bridge the gap in existing healthcare. Additional themes included to have the proper tools to address racism and discrimination in medicine, the education foundation and exposure to identify and prevent disparities, and the practical advice on how to deal with racially discriminatory

Table 2. Extracted Qualitative Themes on What Participants Wish to Gain from the Conference
Gain knowledge and guidance on how to integrate anti-racist practices into residency
How to serve as an advocate for patients of color
Gain insight on how to be an ally
Learn about current initiatives medical students and institutions are implementing to combat racism in medicine
Understand how to bridge the gap in healthcare disparities
Identify proper tools to address racism and discrimination in medicine
Ascertain practical advice on how to deal with racially discriminatory experiences
Understand the education foundation and exposure to identify and prevent disparities
Network with motivated medical students with similar goals

experiences. Another theme included being able to network with motivated medical students with similar goals. Some of these responses, as well as those we received from post-conference participants, are displayed together as a qualitative way to assess the participants' attitudes and perspectives (**Table 3**). Many respondents stated that knowledge and understanding was crucial in their experience, and echoed that, in one way or another, education and awareness is needed in order to facilitate change and advocate for anti-racism in this space. Post-conference participants showed that, through their responses, this event was crucial in reinvigorating and rejuvenating their being and energy.

Notably, when participants were asked about their level of comfort in confronting an administrator who may be saying or doing something racist, there was a significant difference between pre-conference and post-conference responses ($p=0.0371$). The statement specifically read that "I am comfortable confronting an administrator who I see making a discriminatory or racist remark or discriminatory action," and post-conference responses indicated that participants felt more comfortable confronting an administrator who made such remarks. Additional pre- and post-conference survey responses regarding comfortability were collected using a Likert scale (strongly agree to strongly disagree), and statistical analyses were performed using Fisher's exact tests (**Table 4**).

DISCUSSION

For the pre-conference comments and themes that participants identified and wished to be addressed during the course of the event, all expectations mentioned one common component: a safe space that was not only accepting, but also one that was challenging. Our conference was conducted to foster these open discussions and create spaces that allowed open learning on historical and structural racism and how to address these disparities in care. Specifically, qualitative comments in the post-conference survey showed the confidence and encouragement that attendees attained during the event, to speak about racial issues in medicine and healthcare amongst colleagues. Because this space was provided to attendees and was openly identified as a supportive and nurturing environment, it served as a platform for future healthcare professionals to strengthen their foundational knowledge on the topics so that they are able to change the "face and culture of healthcare."

Many participants stated that a large reason that contributed to this increased drive for change was the speaker sessions, as these individuals empowered students to "embrace the tasks of fixing these issues through practical efforts" and provided them with the

Table 3. Attitudes and Perspectives on what participants hoped to gain and/or gained from RiMC

Pre-Conference	Post-Conference
"I hope to learn how to feel comfortable confronting situations where I witness racism and discrimination against minorities and how to do it in an efficient way."	"A lot of hope and inspiration. I felt empowered that maybe things aren't great now--the room is full of students--but the face and culture of healthcare employment WILL change in the next 10-15 years."
"Innovating ways of handling racism in the medical field personally and learning ways to implement change as I move forward in my career. Also, just learning more about other people's perspectives and experiences."	"I had very meaningful conversations that have inspired me to work hard to be an advocate."
"A more comprehensive understanding of how to address racism-related health disparities."	"I gained the confidence to speak about such issues amongst my colleagues. I also learned the extent to which systemic racism has been woven into clinical practice and academic curriculum in medical schools. I was empowered by the speakers to embrace the tasks of fixing these issues through practical efforts. I was inspired to pursue change via leaderships and academia (something I had never considered before). More importantly, I felt I was equipped with the tools to do so."
"Gain insight on the various perspectives on the topic of racism in the medical field. Learn tips for navigating through racism in the field of study I am currently in."	
"I hope to learn from students from other schools and physicians with various experiences and specialties regarding what they're doing to address racism in medicine and explore what I can do to explore this in my training."	"A sense of community and I saw people who are the type of physician I would like to be when I complete medical school. I also was encouraged that there are platforms for people to talk about their experiences and space in the medical field for inclusion. I saw a room full of people who care about the issue of racism, and even some of my classmates who I didn't think cared about the importance of minorities in medicine, whether at the patient or physician level. Maybe they don't care, but the fact that these topics were covered and […] present made a difference to me."
"I hope to gain knowledge on the effects of racism in our healthcare today and what are the changes that are being done to help move towards ending it."	
"Better understanding of the historical and structural violence that shapes POC's experiences in medicine."	"I took home that genetically, all people are the SAME and [simply have] have different phenotypes, and that is really important when it comes to thinking about [which] races are 'pre-disposed' to certain health problems--it's very rarely based [solely] on genetics... [Being] African American [is often associated with having] diabetes or high blood pressure, [and] that notion can make us more apathetic [when] treating them[as providers] because well, the patient is Black, so of course they have [these diseases].
"Understand the disparity facing underrepresented communities and new innovative ideas to combat it."	
"Understanding of current social issues related to racism or discrimination in practice and how they are currently being or could be addressed."	
"Understand the nuances and importance of bias, race, Intersectionality, and other key factors in medicine and patient care"	
"Advance my awareness in areas I lack experience to better help me serve marginalized communities."	
"A better understanding of how to deal with implicit and explicit racism in the medical field. Especially when it comes to patient interactions and providing care."	
"A real-life perspective of the way racism affects healthcare for both patients of color and healthcare professionals of color."	

tools to do so. By providing this student body of future healthcare professionals in the Greater Philadelphia and surrounding region an annual conference on racism and inequity in medicine, we were able to provide trust and support to people who will develop into confident leaders in healthcare.

The change in participant comfort level pre and post conference for confronting an administrator who may be saying or doing something racist was statistically significant. Post-conference responses showed that the participants were more comfortable bringing this up to the administration, likely pointing to the racism in medicine conference as the catalyst for change. The 2019-20 report for the Collective Action for Safe Spaces (CASS) organization reported that, by hosting bystander intervention training and other safe action initiatives, participants were able to develop strategies to respond directly to harassment and violence (18). This organization as well as our conference reported similar data showing that safe spaces, when organized in the right way, had the ability to provide tools and confidence for actionable change. Safe spaces are often misconceived as "echo chambers" and ways to cushion reality and uncomfortable topics. In reality, they do the opposite by providing an atmosphere where people can share differing experiences and perspectives

Table 4. Pre-Survey vs Post-Survey Responses

*Indicates statistical significance

Survey Question	***Pre-Survey*** N **(%)**	***Post-Survey*** N **(%)**	*p-value*
How big of a role do you believe discrimination plays in the medical field?			
Extremely Important and/or Very Important	*74 (91.4)*	*31 (93.9)*	*0.53*
I am comfortable confronting a coworker in my professional environment who I witness making a discriminatory or racist remark.			
Strongly Agree, and/or Agree	36 (44.4)	19 (59.4)	*0.26*
Strongly Disagree, and/or Disagree	2 (2.5)	0 (0.0)	
I am comfortable discussing the discriminatory or racist behaviors of a colleague with administration.			
Strongly Agree, and/or Agree	32 (40)	22 (68.8)	*0.23*
Strongly Disagree, and/or Disagree	5 (6.3)	0 (0.0)	
I am comfortable confronting an administrator who I see making a discriminatory or racist remark or discriminatory action.			
Strongly Agree, and/or Agree	17 (21.3)	17 (53.1)	*0.04**
Strongly Disagree, and/or Disagree	9 (17.5)	2 (6.25)	

received with an open-minded and the absence of scrutiny and hostility.

Qualitative responses suggest that the creation of the Racism in Medicine conference as a safe space and annual platform was a large reason for the difference of participants stating that they were more comfortable confronting and addressing administration if they heard or saw a racist remark or action. These spaces are groups or communities that allow for "license to speak and act freely, form collective strength, and generate strategies for resistance," a model used by the RiMC 2019 organization committee (11). By providing attendees with this platform where they are able to feel a sense of belonging and validation for their viewpoints, the conference was already a place where people felt that they would not be dismissed and would be heard.

Having a collective group conscience through symbolic convergence theory (SCT) enabled participants to envision a future where healthcare professionals are knowledgeable enough to dismiss false claims about race in medicine, confident enough to address and confront situations where racism is the motive, and work together to make racism and inequality in medicine a thing of the past (15). Talking about these important topics to higher professionals and administration require places to cultivate this strength.

Student cohesiveness, meaningful real-time feedback, and an amalgamation of thoughts and ideas are but some of the ways in which a safe space can develop (19). By providing an annual space for such conversations and allocating space and time for medical students to strengthen the foundation of knowledge on relevant subjects, we are positively influencing medical professionals and helping them mold into developed and confident leaders in healthcare.

One limitation in our data collection includes a smaller response rate, which decreased especially in the post-conference responses. While we initially thought that the conference would inspire people to answer the survey at the end of the conference, especially because time was allotted to do so, participants may have been emotionally and mentally drained after a day of deep and heavy conversation. Because the participants of this conference were possibly more invested in this specific topic to attend a weekend conference, another potential factor that could have contributed to the overwhelmingly positive responses could have been potential bias in terms of feedback, as the attendees may have naturally gained more due to their vested interest in the material and application. Because many survey questions were utilized from past conferences to possibly compare data, we relied more on qualitative and open-ended responses to focus on how the conference served as a successful safe space. We do believe that open-ended responses to questions on serious topics would provide a greater space and voice for true feelings and may indirectly promote feelings of understanding and security.

Furthermore, based on gathered attendee feedback, future sessions should include topics centered on how to specifically navigate conversations that may be uncomfortable in relation to micro- and macro- aggressions in professional settings. Attendees also recommended including more conversations about Asian-Americans and Native Americans, as they are often forgotten in discussions around race. In future studies, we wish to emphasize the importance of additional quantitative data. We believe that matched pre- and post-conference responses can add a strong element that further validates the utility and importance of this conference through paired statistical analyses.

CONCLUSIONS

The reality of limited avenues available for students and faculty members in healthcare to learn about and openly discuss issues of race and marginalization, especially in the context of medicine, has been at the forefront of modern medical education. The RiMC series was created to address this deficit and provide one possible solution in the form of creating a designated forum for these complicated conversations as well as creating an environment that makes addressing these issues in a professional setting as comfortable as possible. This allowed the creation of a safe space for participants to have a platform to increase their knowledge and participate in transformative conversations about racism in medicine. For allies and individuals who want to contribute to promoting changes within medicine, safe spaces provide an environment for uninhibited dialogue and avenues for action. This initiative was considered successful as feedback shows most attendees felt heard and cared for which enabled even more raw and impactful discussions. While this initiative was overall a success, there is still room for immense improvement as well as keeping in mind that this conference format should only be an interim solution while medical institutions revamp their internal curricula. However, for allies who want to implement this kind of format within their own educational efforts, the RiMC organization committee strongly endorses the provision of safe spaces and thereby a commitment to elimination of fear of retaliation, retribution, and ostracization when participating in anti-racism work.

ACKNOWLEDGEMENTS

The authors of this paper would like to acknowledge the work of and sincerely thank all those who participated and made the 2019 Racism in Medicine Conference a reality. We extend our gratitude to: Cooper Medical School of Rowan University, Dean Annette Reboli, Dean Mitchell-Williams, and Director of Service, Susan Liu, for providing a venue for the conference and assisting in all parts of planning and execution. Ms. Susan Cavanaugh for being a resourceful librarian. Roberto Merino Gonzalez for the creation of many tables utilized in this paper. The entire medical student conference planning committee for their commitment and hard work in bringing the conference to fruition. The Keynote Speakers, workshop leaders, community panelists, student volunteers, catering services of Old San Juan of Camden, NJ, and CMSRU Security for all of their innumerable contributions. The partnering healthcare programs and medical institutions for their co-sponsorship and monetary support. Finally, we would like to thank the founder of the Racism in Medicine Conference, Dr. Dorothy Charles, MD, who serves as a leader and advocate for patients and people of color. Thank you for all of your hard work; we hope we did justice to your vision.

DISCLOSURES

Funding: This research has not received any funding. This conference event received funding from the host school, Cooper Medical School of Rowan University, as well as partnering institutions in the Greater Philadelphia and Delaware Valley area.
Conflicts of interest: None.
Availability of data and material: Collected on Qualtrics, unidentified.
Code availability: Not applicable.
Authors' contributions: Authors listed in the manuscript have contributed per submission guidelines and standards for authorship.
Ethics approval: Study was IRB approved, exempt.
Consent to participate: Implied consent to participate, participation voluntary, survey completion indicated consent.

REFERENCES

1. Phelan, S. M., Burke, S. E., Cunningham, B. A., Perry, S. P., Hardeman, R. R., Dovidio, J. F.,

Herrin, J., Dyrbye, L. N., White, R. O., Yeazel, M. W., Onyeador, I. N., Wittlin, N. M., Harden, K., & van Ryn, M. (2019). The Effects of Racism in Medical Education on Students' Decisions to Practice in Underserved or Minority Communities. *Academic medicine : journal of the Association of American Medical Colleges*, *94*(8), 1178–1189.

2. Ansell, D. A., & McDonald, E. K. (2015). Bias, black lives, and academic medicine. *The New England journal of medicine*, *372*(12), 1087–1089.
3. Functions and Structure of a Medical School: Standards for Accreditation of Medical Education Programs Leading to the MD Degree. Published online March 2020.
4. Medical Education Senior Leaders. Creating Action to Eliminate Racism in Medical Education: Medical Education Senior Leaders' Rapid Action Team to Combat Racism in Medical Education. Published online 2021.
5. Tsai, J., Ucik, L., Baldwin, N., Hasslinger, C., & George, P. (2016). Race Matters? Examining and Rethinking Race Portrayal in Preclinical Medical Education. *Academic medicine : journal of the Association of American Medical Colleges*, *91*(7), 916–920.
6. Charles, D., Himmelstein, K., Keenan, W., Barcelo, N., & White Coats for Black Lives National Working Group (2015). White Coats for Black Lives: Medical Students Responding to Racism and Police Brutality. *Journal of urban health : bulletin of the New York Academy of Medicine*, *92*(6), 1007–1010.
7. Adelekun, A. A., Beltrán, S., Carney, J., Lett, E., Orji, W. U., Rider-Longmaid, E., Stokes, D. C., Teeple, S., & Aysola, J. (2019). Recognizing Racism in Medicine: A Student-Organized and Community-Engaged Health Professional Conference. *Health equity*, *3*(1), 395–402.
8. Empowering Womxn of Color Conference. Accessed January 31, 2021. https://sua.ucsc.edu/assembly/officers/past/2018-2019/diversity/ewocc/index.html
9. 2018-2019 Cooper Medical School RiMC Planning Committee. Memorandum of Understanding: Delaware Valley Racism in Medicine Conference. Published online July 29, 2019.
10. Karani, R., Varpio, L., May, W., Horsley, T., Chenault, J., Miller, K. H., & O'Brien, B. (2017). Commentary: Racism and Bias in Health Professions Education: How Educators, Faculty Developers, and Researchers Can Make a Difference. *Academic medicine : journal of the Association of American Medical Colleges*, *92*(11S Association of American Medical Colleges Learn Serve Lead: Proceedings of the 56th Annual Research in Medical Education Sessions), S1–S6.
11. Hardeman, R. R., Burgess, D., Murphy, K., Satin, D. J., Nielsen, J., Potter, T. M., Karbeah, J., Zulu-Gillespie, M., Apolinario-Wilcoxon, A., Reif, C., & Cunningham, B. A. (2018). Developing a Medical School Curriculum on Racism: Multidisciplinary, Multiracial Conversations Informed by Public Health Critical Race Praxis (PHCRP). *Ethnicity & disease*, *28*(Suppl 1), 271–278.
12. Acosta, D., & Ackerman-Barger, K. (2017). Breaking the Silence: Time to Talk About Race and Racism. *Academic medicine : journal of the Association of American Medical Colleges*, *92*(3), 285–288.
13. Clark-Parsons R. Building a digital Girl Army: The cultivation of feminist safe spaces online. *New Media & Society*. 2018;20(6):2125-2144. doi:10.1177/1461444817731919
14. Simon A. Safe Spaces: Their Construction and Their Purpose. *Communication Studies Graduate Publications and Presentations*. Published online January 1, 2017:1-31.
15. Bormann EG. Symbolic Convergence Theory: A Communication Formulation. *Journal of Communication*. 1985;35(4):128-138.
16. Ford, C. L., & Airhihenbuwa, C. O. (2010). The public health critical race methodology: praxis for antiracism research. *Social science & medicine (1982)*, *71*(8), 1390–1398.
17. Ford, C. L., & Airhihenbuwa, C. O. (2010). Critical Race Theory, race equity, and public health: toward antiracism praxis. *American journal of public health*, *100 Suppl 1*(Suppl 1), S30–S35.
18. *COLLECTIVE ACTION FOR SAFE SPACES THE EVOLUTION: Our 2020 Annual Report*. Collective Action for Safe Spaces; 2020.
19. Prashanti, E., & Ramnarayan, K. (2020). Ten maxims for creating a safe learning environment. *Advances in physiology education*, *44*(4), 550–553.

"The Vaccine" by Louis Léopold-Boilly. Courtesy National Gallery of Art, Washington.

Beyond Prophylaxis: Could the HPV Vaccine be Repurposed in Skin Cancer Treatment?

Rachel Sally[1]

[1]New York University Grossman School of Medicine, New York
Correspondence: Rachel.Sally@nyulangone.org

Counseling patients—in pediatrics, sometimes their parents—on human papillomavirus (HPV) vaccination frequently includes a turn of phrase believed to be so persuasive that not only is there an entire CDC webpage dedicated to it, but it has practically become a commandment on infographics for patients on cancer prevention—one has only to peruse WebMD for moments to find dozens of examples (1, 2). Medical students across the country are taught to lean in and say, "this is one of two vaccines that can prevent cancer."

Papillomaviridae is a family of mucosal and cutaneous epitheliotropic viruses that cause hyperproliferative lesions. For over four decades, the causative link between high risk (HR) mucosal HPV and malignancies, particularly cervical cancer, has been robustly established. HPV is currently the most common sexually transmitted infection worldwide and represents a tremendous disease burden, with up to 5% of cancer diagnoses attributable to HPV infection (3, 4). Hence the importance of counseling on the vaccines (bivalent, quadrivalent, and nonavalent) that exist against a number of HR HPV types in order to prevent both benign and malignant diseases. The high risk mucosal types of HPV all fall within the alpha genus of papillomaviridae. Their mechanism of mucosal carcinogenesis is

dependent on continuous expression of oncoproteins E6 and E7, often following a DNA integration event (5). During HR HPV chronic infection, these oncoproteins interact with various cellular targets to prevent apoptosis and promote unchecked growth.

Viruses from the beta, gamma, mu, and nu genera (and some alpha HPV types), colonize the epidermis, with hair follicle stem cells representing a natural reservoir of persistent infection (6). Cutaneous HPVs are ubiquitous on human skin and have been increasingly implicated in the development of non-melanoma skin cancer (NMSC), particularly β-HPVs and squamous cell carcinomas (SCC). However, up to 90% of healthy individuals test positive for cutaneous β-HPVs (5). In contrast to mucosal HR HPV carcinogenesis, it appears that E6 and E7 expression is only required at the beginning of skin carcinogenesis. The expression of these oncoproteins facilitates the accumulation of UV-induced mutations by preventing apoptosis in skin cells with high mutation burdens, known as the "hit-and-run" mechanism (5). The high prevalence of HPV in healthy people combined with a lack of evidence for an integration event as in mucosal carcinogenesis and an absence of active virus expression in cutaneous tumor samples support the hypothesis of synergism between UV radiation and HPV in the early pathogenesis but not maintenance of cutaneous SCC (7).

Currently, the FDA-approved HPV vaccines contain virus-like particles (VLP) of L1 major capsid proteins. This protein is not conserved among HPV types, with only 60% sequence homology between α- and β-types, and the resulting antibodies after vaccination are thus theoretically highly type-specific to α-HPV (8, 9). Though these vaccines were developed to prevent mucosal infection, there have been numerous reports of complete resolution of disseminated, treatment-resistant verruca vulgaris after quadrivalent HPV vaccination, even when the HPV type isolated from the lesions did not match the quadrivalent vaccine, which covers types 6, 11, 16, and 18, suggesting the existence of cross-protection against heterologous types (10).

Further, there exist in the literature five recent reports of a novel use of the quadrivalent and nonavalent HPV vaccines in patients with cutaneous squamous cell carcinoma. Nichols et al. offered two patients with SCC and BCC three systemic doses of the quadrivalent vaccine for prophylaxis for skin cancer. Sixteen months after the first dose, the male patient's new cancerous lesion rate had a 62.5% reduction in new SCC (12 to 4.4 per year) and 100% reduction in BCC (2.25 to 0) and at 13 months, the female patient had a 66.5% reduction in SCC (5.5 to 1.84) and 100% reduction in BCC (0.92 to 0) (11).

The third case is the first documented therapeutic use of HPV vaccination and involves a 90-year-old immunocompetent patient with multiple inoperable cutaneous squamous cell carcinomas, biopsy-proven to be basaloid SCC, a rare SCC characterized by invasive growth, high recurrence rate after resection, and a propensity for metastasis. The patient underwent Mohs surgery on the largest tumor but given the severity of the tumor burden and the patient's advanced age, additional surgery and radiation were deemed infeasible; the patient subsequently declined systemic chemotherapy. She was treated with a series of systemic and intratumoral nonavalent HPV vaccines (2 doses intramuscularly, 4 doses intratumorally in the largest 3 tumors over 10 months) to complete resolution of her tumors, including the non-injected ones. At eleven months after the first intratumoral dose, a small papule at the site of a previous large tumor was biopsied and showed no histologic evidence of residual disease. At 24 months after her first

intratumoral dose, the patient remained with a complete response to treatment, with no clinical evidence of SCC (12). The same research team performed the treatment—two intramuscular and two intratumoral injections with the nonavalent vaccine—on an 87-year-old immunosuppressed renal transplant recipient with inoperable SCC in situ who elected to forego radiation. His lesion resolved with biopsy-proven histologic cure (13). The final case currently reported is from Geizhals and Lebwohl, who treated an 84-year-old immunocompetent patient with multiple invasive cutaneous keratoacanthoma-type SCC with 2 intramuscular and 3 intratumoral injections of the nonavalent vaccine. Ten months after the first injection, there was neither clinical nor histologic evidence of residual carcinoma (14).

The current understanding of the cofactor-like hit-and-run relationship between cutaneous β-HPV and early squamous cell carcinogenesis would not suggest cross-protectivity and immunogenicity of highly α-type-specific L1-based vaccines for established SCC. However, the growing body of observational data of the efficacy of the vaccine as a therapeutic treatment for a number of cutaneous HPV-related conditions, from verruca vulgaris to, rather strikingly, aggressive squamous cell carcinomas implies that the reality of immunologic treatment based on specific HPV serotypes is complicated.

As with any case report, there are significant limitations to their conclusions, not least that there are currently only 5 patients with cutaneous carcinomas known to be treated with this modality. A major question stemming from the lack of controls is whether the possible immunogenic effects of the vaccines were due to the VLP contents or the vaccine adjuvants. While regression of non-injected tumors suggests systemic effects beyond local adjuvant augmentation, a broader downstream adjuvant-activated immunologic cascade cannot be ruled out. The lack of HPV testing is also a hindrance to broader conclusions being drawn from these reports, as understanding cross-protectivity is rendered impossible without knowing the serotypes present. Another distinct possibility, particularly given the small sample size, is that the observed regressions were spontaneous.

There are a number of ongoing investigations aimed at creating papillomavirus vaccines based on the more broadly conserved but less immunogenic L2 minor capsid proteins to broaden protection to include heterologous HPV types, including cutaneous (15). However, these anecdotal studies raise the possibility of an already available alternative to the current orthodoxy of physical destruction (resection, ablation, topical/intralesional chemotherapeutics) for treatment of HPV-related cutaneous SCC. Further exploration and controlled experimentation is warranted into whether the commercially available HPV vaccines may be used as therapeutic, not just prophylactic, cancer vaccines. More robust and durable data must be collected and validated, and several questions must be answered, the most important being what the immunologic mechanism of action is; whether there are predictors of outcomes such as HPV vaccination status, serotype, and tumor type; and the safety profile of using the vaccine as a therapeutic agent.

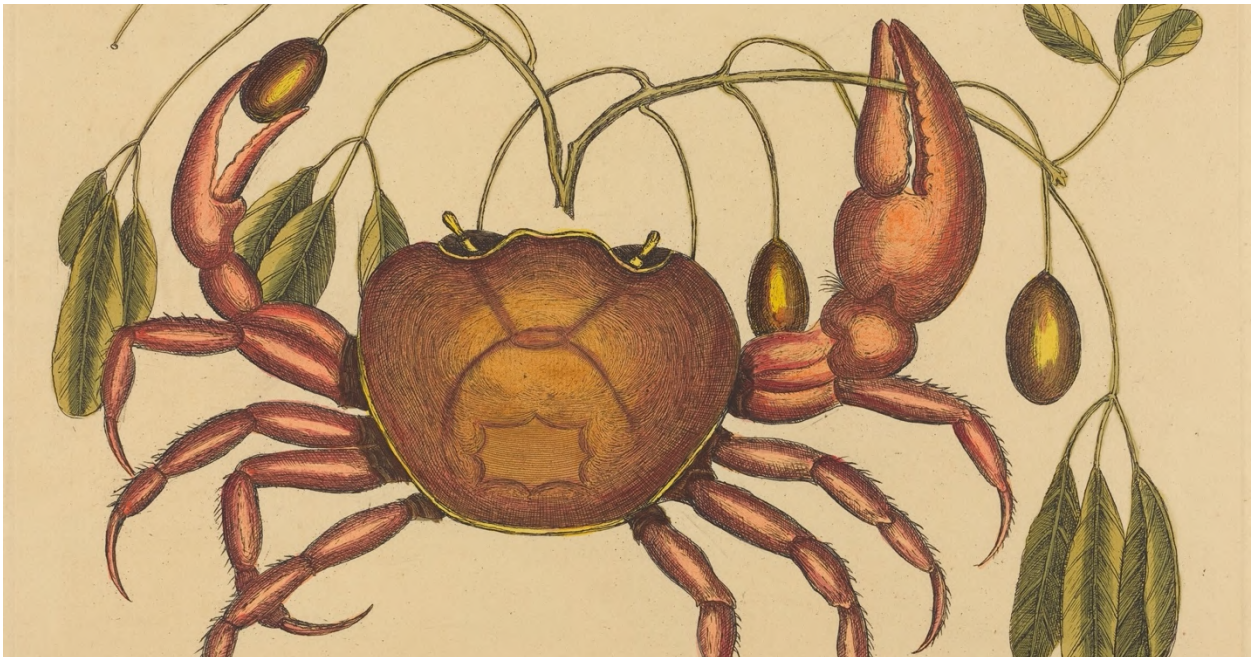
DISCLOSURES

Funding: None.
Conflicts of interest: None.
Availability of data and material: Not applicable.
Code availability: Not applicable.
Authors' contributions: Authors listed in the manuscript have contributed per submission guidelines and standards for authorship.

Ethics approval: Not applicable.
Consent to participate: Not applicable.

REFERENCES

1. Vaccines (Shots) Against Cancer | CDC. Published December 17, 2020. Accessed July 26, 2021. https://www.cdc.gov/cancer/dcpc/prevention/vaccination.htm
2. Can You Prevent Cancer? What Helps Lower Your Risk. Accessed July 26, 2021. https://www.webmd.com/cancer/cancer-prevention-strategy
3. Brianti, P., De Flammineis, E., & Mercuri, S. R. (2017). Review of HPV-related diseases and cancers. The new microbiologica, 40(2), 80–85.
4. Rosalik, K., Tarney, C., & Han, J. (2021). Human Papilloma Virus Vaccination. Viruses, 13(6), 1091.
5. Gheit T. (2019). Mucosal and Cutaneous Human Papillomavirus Infections and Cancer Biology. Frontiers in oncology, 9, 355.
6. Köhler, A., Forschner, T., Meyer, T., Ulrich, C., Gottschling, M., Stockfleth, E., & Nindl, I. (2007). Multifocal distribution of cutaneous human papillomavirus types in hairs from different skin areas. The British journal of dermatology, 156(5), 1078–1080.
7. Arron, S. T., Ruby, J. G., Dybbro, E., Ganem, D., & Derisi, J. L. (2011). Transcriptome sequencing demonstrates that human papillomavirus is not active in cutaneous squamous cell carcinoma. The Journal of investigative dermatology, 131(8), 1745–1753.
8. Yadav, R., Zhai, L., & Tumban, E. (2019). Virus-like Particle-Based L2 Vaccines against HPVs: Where Are We Today?. Viruses, 12(1), 18.
9. de Villiers, E. M., Fauquet, C., Broker, T. R., Bernard, H. U., & zur Hausen, H. (2004). Classification of papillomaviruses. Virology, 324(1), 17–27.
10. Cyrus, N., Blechman, A. B., Leboeuf, M., Belyaeva, E. A., de Koning, M. N., Quint, K. D., & Stern, J. J. (2015). Effect of Quadrivalent Human Papillomavirus Vaccination on Oral Squamous Cell Papillomas. JAMA dermatology, 151(12), 1359–1363.
11. Nichols, A. J., Allen, A. H., Shareef, S., Badiavas, E. V., Kirsner, R. S., & Ioannides, T. (2017). Association of Human Papillomavirus Vaccine With the Development of Keratinocyte Carcinomas. JAMA dermatology, 153(6), 571–574.
12. Nichols, A. J., Gonzalez, A., Clark, E. S., Khan, W. N., Rosen, A. C., Guzman, W., Rabinovitz, H., Badiavas, E. V., Kirsner, R. S., & Ioannides, T. (2018). Combined Systemic and Intratumoral Administration of Human Papillomavirus Vaccine to Treat Multiple Cutaneous Basaloid Squamous Cell Carcinomas. JAMA dermatology, 154(8), 927–930.
13. Nichols, A. J., De Bedout, V., Fayne, R. A., Burke, G. W., Kirsner, R. S., & Ioannides, T. (2020). Systemic and intratumoral 9-valent human papillomavirus vaccine treatment for squamous cell carcinoma in situ in a renal transplant recipient. JAAD case reports, 6(4), 289–291.
14. Geizhals S, Lebwohl MG. (2020). The Successful Treatment of Multiple Cutaneous Malignancies with HPV Vaccination: Case Report. J of Skin, 4(2), 148.
15. Vinzón, S. E., Braspenning-Wesch, I., Müller, M., Geissler, E. K., Nindl, I., Gröne, H. J., Schäfer, K., & Rösl, F. (2014). Protective vaccination against papillomavirus-induced skin tumors under immunocompetent and immunosuppressive conditions: a preclinical study using a natural outbred animal model. PLoS pathogens, 10(2), e1003924.



"The Land-Crab (Cancer ruricola)" by Mark Catesby. Courtesy National Gallery of Art, Washington.

Dendritic Cell Vaccines in Ovarian Cancer: Have We Reached Their Potential?

Jennifer Rowley[1]

[1]Harvard Medical School, Boston, Massachusetts 02115
Correspondence: Jennifer_Rowley@hms.harvard.edu

Ovarian cancer is the most lethal gynecological malignancy, largely driven by high rates of relapse and chemoresistance. Ovarian cancer is thought to be immunogenic, making it amenable to immunotherapy. However, immunotherapies such as PD-L1 inhibitors and T cell transfers have produced modest, if any, survival benefit. One particular immunotherapy of interest is the dendritic cell vaccine, which delivers mature dendritic cells loaded with tumor antigens with the goal of mounting a T cell response against tumor cells. This review will focus on the role that dendritic cells play in the ovarian tumor microenvironment, general approaches to engineering dendritic cell vaccines and assessing their efficacy alone and in combination with other immunotherapies and systemic chemotherapies. Finally, we will discuss important areas of ongoing research in the field, including the development of personalized neoantigen-targeting DC vaccines.

INTRODUCTION

Ovarian cancer is the most lethal gynecological cancer, with an overall 5-year survival of 48% in 2020. Of those that present at an advanced stage, the 5-year survival is just 29% (1). The current standard treatment of epithelial ovarian cancer (EOC) is debulking surgery followed by platinum-

based chemotherapy. Despite good initial responses in most patients, chemoresistance and relapse are common (2, 3). For patients with resistance to platinum-based therapies, their treatment options remain limited.

Ovarian cancer is thought to be immunogenic as it expresses multiple well-known tumor-associated antigens (TAA). Some tumors are infiltrated by lymphocytes, which correlates positively with progression-free survival and overall survival (4, 5). These data suggest that ovarian cancer could be amenable to immunotherapy targeting. However, to date, immunotherapies have shown modest, if any, benefit. For example, PD-1 inhibitors have a response rate of 11.5% in advanced metastatic disease, which is thought in part due to poor T cell infiltration as well as poor antigen presenting function of antigen-presenting cells (APCs) (6, 7).

DENDRITIC CELLS IN OVARIAN CANCER

Dendritic cells in the tumor microenvironment take up and process tumor-associated antigens and present them on MHC I/II molecules to activate CD8+ and CD4+ cells, respectively. In comparison to other APCs such as B cells, mononuclear cells and macrophages, DCs are regarded as the most powerful cell type in its ability to capture, process and present antigens (8). In general, different DCs subtypes can play a multitude of roles in the tumor microenvironment. Conventional DCs (cDC) are the main subtype tasked with activating CD8+ T cells (particularly cDC type 1) and differentiation of CD4+ T cells (cDC type 2) through cytokine production (9). Conversely, plasmacytoid DCs (pDC), which are the main subtype of DCs in ovarian cancer, can exert both anti-tumor and immunosuppressive effects. Whether pDCs skew towards being tumor-protective or tumor-suppressive is largely determined by the signals they receive from their tumor microenvironment (9, 10).

Investigations into ovarian cancer have revealed that dendritic cells (DCs) make essential contributions to the depressed immune function observed in the ovarian tumor microenvironment. While ovarian cancer lesions have a high degree of DC infiltration, these DCs can have low efficacy of antigen presentation due to DC tolerance, which is characterized by downregulated expression of costimulatory molecules on the surface of DC cells and weaker antigen-presenting ability (11). Further, DCs can support the immunosuppressive milieu through their interactions with Tregs. For example, DC expression of indoleamine 2,3-deoxygenase, an essential enzyme in amino acid metabolism, can reduce the amount of tryptophan near Tregs and as a result maintain Tregs in an immunosuppressive state through mTORC-Akt signaling (12). DCs have also been shown to activate immunosuppressive Tregs by expressing ICOS ligand, leading to tumor progression (13).

Vaccines of functional DCs loaded with tumor-associated antigens have held promise for expanding tumor-specific T cell populations by restoring antigen presentation to T cells and bypassing the dysregulated milieu of the tumor microenvironment. There are different types of cancer vaccines, including cell-based vaccines, peptide/protein vaccines, epigenetic vaccines and genetic vaccines (14). This review will focus on the two most common DC vaccine types: cell-based and peptide/protein-based vaccines, which are designed to present T cells with tumor-associated antigens. Specifically, we will review the general approaches to engineering these vaccines as well as assessing their efficacy alone and in combination with other immunotherapies and systemic chemotherapies. Finally, we will discuss important areas of ongoing research

in the field, including the development on personalized neoantigen-targeting DC vaccines.

ENGINEERING DENDRITIC CELL VACCINES

Dendritic cell vaccines have long been an immunotherapy of interest for ovarian cancer, particularly given the demonstrated dysfunction of DCs surrounding ovarian tumors. Further, these vaccines are generally well-tolerated by patients and can induce long-term immunologic memory (15).

Currently, vaccines targeting DCs ex vivo are produced using three general steps. First, apheresis is performed to obtain either immune cells that have the potential to become DCs, such as monocytes, or immature DCs from peripheral blood. Among all cell types, monocyte-derived DCs (MoDCs) are most often used as immature DCs are typically not found in sufficient quantity in peripheral blood to produce a vaccine. Monocytes are subsequently cultured in vitro with a cytokine cocktail of GM-CSF and IL-4 that induces differentiation into immature DCs (16). However, from a functional standpoint, MoDCs have been shown to be inferior to cDCs in inducing long-lasting immune responses through T-cell activation, raising questions of their appropriateness as the DC subtype used in many EOC vaccines (17).

Second, once immature DCs have been obtained, they are loaded with tumor-associated antigens, ranging from specific peptides to proteins to multiple antigens from whole tumor lysates. To date, the most common approach has been to load DCs with one or several peptides known to be expressed on ovarian cancer cells. One example includes Wilms tumor 1 (WT-1), which is overexpressed in ovarian cancer along with many other solid tumors and can be targeted by cytotoxic T cells (CTLs). One group incubated DCs with an MHCI-restricted WT-1 peptide and a streptococcal primer and showed that these DCs elicited a CTL effect (18). This has been repeated with other peptides expressed by ovarian cancer cells, such as Her-2/neu, epithelial mucin 1, and p53. These vaccines reproducibly generated antigen-specific IFN-y secreting T cells (19, 20). However, the success of these single peptide/protein vaccines has been limited, resulting in short-term disease stabilization that ultimately gives way to progression after several months. One possible theory for this is that when a vaccine target is a non-mutated self-antigen or shared antigen that is overexpressed in the tumor, vaccine efficacy can be low because T cell recognition of self-antigens will be limited by central tolerance (21).

More recently, whole tumor cell lysates have been investigated as an antigen source for DC vaccines. In this scheme, DCs are pulsed with lysed ovarian tumor cells. These cells can be derived from ovarian cancer cell lines or even from a patient's own tissue sample (22). This has the benefit over single peptide vaccines in that lysates can elicit responses to more than one neoantigen, thus reducing avenues for tumor escape. Further, in the case of an autologous tumor cell lysate, the patient can produce a more "personalized" tumor-specific T cell pool by targeting their own unique set of tumor-associated antigens (23, 24). Previous studies with DC vaccines loaded with whole tumor lysate have demonstrated clinical benefit for patients with non-Hodgkin's lymphoma and melanoma (25, 26). There are multiple approaches to stimulating cell death to induce antigen release, including repetitive freeze-thaw cycles or exposing cells to hypochlorous acid (HOCl). Chiang et al. found that autologous ovarian tumor cells killed with oxidation and lysed with freeze-thaw cycles were superior to cells killed with irradiation or freeze-thaw lysis in priming T cell responses in vitro (27).

Finally, regardless of the antigen, immature antigen-presenting DCs are then matured in the presence of immunogenic substances like LPS and IFN-y to trigger expression of co-stimulatory molecules on the DC surface. These co-stimulatory molecules are essential for T cell activation upon antigen presentation in the lymph node. Once this step is complete, DCs are typically fractionated into multiple doses to be used as serial vaccines over a defined treatment period. Typically, vaccines are given intranodally, but can also be given with intramuscular or subcutaneous injection (16).

CLINICAL EFFICACY OF DC VACCINES

Since the early 2000s, multiple DC vaccines have been investigated in clinical trials, though most therapies have not progressed past phase II trials and most trials have consisted of small patient cohorts ranging from 3 to 56 patients at different stages of ovarian cancer (16).

Thus far, DC vaccine trials have primarily focused on patients with recurrence after standard therapy. One study investigated DC vaccines pulsed with WT1 peptide given to patients resistant to chemotherapy. Only one of three patients responded to the vaccines and reached stable disease (18). With regard to the efficacy of DC vaccines early in a patient's disease course, one retrospective study evaluated the potential benefit of early DC vaccines against multiple TAAs when given before recurrence as part of maintenance therapy. After 5-7 doses, the mean survival time from diagnosis was 30.4 months and 14.5 months from first vaccination. The authors argued that early DC vaccines could have the potential to elongate progression-free survival, although a larger prospective study is necessary to substantiate this (28).

In 2018, Tanyi et al. reported the results of their phase I trial of an ovarian cancer dendritic cell vaccine using autologous whole tumor cell lysate as an antigen source (29). In this trial of 25 patients with platinum-treated, immunotherapy-naive, recurrent ovarian cancer, intranodal injections of the vaccine was safe and feasible. Patients mounted T cell responses to autologous tumor antigens, and the vaccine was associated with significantly prolonged survival. Patients were randomized to either receive bevacizumab (a VEGF inhibitor) and cyclophosphamide, bevacizumab, cyclophosphamide and their personalized DC vaccine or just bevacizumab and their personalized DC vaccine. In the cohort receiving all three treatments, the median progression free survival was 11.1 months compared with 4.1 months in a historical control cohort. Remarkably, 24-month survival in patients with a confirmed immune response was 100% for vaccine/ cyclophosphamide/ bevacizumab. This was compared to 40% for vaccine/ bevacizumab and 40% for bevacizumab/ cyclophosphamide, indicating that low-dose cyclophosphamide was needed to improve survival. However, for patients without an immune response, no clinical benefit was observed.

Further, additional immunotherapies might act synergistically with DC vaccines. In one study evaluating DC vaccines and T cell transfer, seven recurrent advanced-stage ovarian cancer patients received DC vaccines (30). Of these patients, three had a partial disease response and went on to receive autologous T cell transfer after these T cells were expanded in vitro. This resulted in one complete response, one achieved stable disease and one unfortunately had disease progression after T cell transfer. With regards to immune checkpoint inhibitors, while there is a theoretical benefit to combining these therapies, no clinical trials are currently investigating these therapies in combination.

Of note, some trials have reported an increase in adverse side-effects from DC vaccines when used in combination with chemotherapies, however it is unclear how much is related to the DC vaccines themselves (29).

PERSONALIZED NEOANTIGEN-TARGETING VACCINES – THE NEXT BIG LEAP?

One key area of interest for DC vaccines is the development of neoantigen-targeted vaccines. Neoantigens are proteins expressed by tumors that differ from other tumor-associated antigens in several keys. First, neoantigens arise from DNA mutations within the tumor and thus produce peptides that are specific to tumor cells. Because these antigens are not expressed on other cell types in the body, they are often highly immunogenic in comparison to other tumor-associated antigens, which are typically non-mutated self-antigens that are simply overexpressed on cancer cells. This increased immunogenicity is in part driven by increased affinity for major histocompatibility complexes (MHCs) (31). Further, because neoantigens are only expressed by tumor cells, this limits potential off-target effects. Thus, neoantigens are ideal targets for an anti-tumor T cell response (31). While ovarian cancers have lower mutational burdens than most other cancer types, recent analyses have shown that some patients can express moderate to high levels of neoantigens (32). In other cancers, neoantigen-loaded DC vaccines have shown promising results in small phase I trials in melanoma patients and non-small cell lung cancers (33, 34).

Neoantigens can be classified as being shared or personalized. Shared neoantigens are common in some tumor types and can be used to broadly treat patients who have the same tumor type. However, not all patients express these shared neoantigens, and even if they are expressed, different patients may mount different immune responses to them (35). Thus, personalized neoantigens, which are specific to individual patients and tumors, have become a point of interest for DC vaccine design across all cancer types (22). However, identifying a patient's neoantigen repertoire has only recently become possible with the development of next-generation sequencing (NGS) such as whole exome sequencing, mass spectrometry analysis of the immunopeptidome (i.e., peptides associated with HLAs), as well as highly predictive bioinformatics tools (36-38).

Initial evidence suggests that recognition and targeting of tumor-specific neoantigens improves the effectiveness of dendritic cell vaccines in ovarian cancers. For example, patients with tumor lysate-pulsed DCs were found to have activated high-avidity CD8+ T cell clonal expansion specific for de novo neoantigens, which improved progression-free survival compared to patients without neoantigen-specific T cell responses (29).

However, multiple research groups are still investigating the true benefit of this approach compared to using overexpressed self-antigens or whole tumor lysates, particularly because the process of identifying a patient's neoantigens remains costly and resource-intensive (22). Currently, one trial is underway to assess a personalized neoantigen-pulsed DC vaccine in ovarian cancer patients. This trial will investigate the feasibility and safety of a personalized neoantigen-loaded DC vaccine in patients with ovarian cancer (ClinicalTrials.gov NCT04024878).

CONCLUSIONS

Dendritic cell vaccines have been shown to be an effective immunotherapy for ovarian cancer, however it remains an active area of innovation in the wake of new technological

advances in the realms of NGS and bioinformatics. Further, combinations of various immunomodulatory treatments with DC vaccines will likely be required to capitalize on the immunogenicity of ovarian malignancies and will be a crucial area of clinical investigation going forward.

DISCLOSURES

Funding: Not applicable.
Conflicts of interest: None.
Availability of data and material: Not applicable.
Code availability: Not applicable.
Authors' contributions: Authors listed in the manuscript have contributed per submission guidelines and standards for authorship.
Ethics approval: Not applicable.
Consent to participate: Not applicable.

REFERENCES

1. Siegel, R.L., Miller, K.D., Jemal, A. (2020). Cancer statistics. CA: A Cancer Journal for Clinicians, 70(1), 7-30. doi:10.3322/CAAC.21590
2. Gadducci, A., Cosio, S., Conte, P.F., Genazzani, A.R. (2005). Consolidation and maintenance treatments for patients with advanced epithelial ovarian cancer in complete response after first-line chemotherapy: a review of the literature. Critical reviews in oncology/hematology, 55(2), 153-166. doi:10.1016/J.CRITREVONC.2005.03.003
3. Stuart, G.C.E. (2003). First-line treatment regimens and the role of consolidation therapy in advanced ovarian cancer. Gynecologic oncology, 90(3 Pt 2), S8. doi:10.1016/S0090-8258(03)00472-4
4. Vonderheide, R.H., Hahn, W.C., Schultze, J.L., Nadler, L.M. (1999). The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. Immunity, 10(6), 673-679. doi: 10.1016/s1074-7613(00)80066-7
5. Sato, E., Olson, S.H., Ahn, J., et al. (2005). Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proceedings of the National Academy of Sciences of the United States of America, 102(51), 18538-43. doi: 10.1073/pnas.0509182102
6. Kandalaft, L.E., Odunsi, K., Coukos, G. (2019). Immunotherapy in ovarian cancer: Are we there yet? Journal of Clinical Oncology, 37(27), 2460-2471. doi:10.1200/JCO.19.00508
7. Varga, A., Piha-Paul, S., Ott, P.A., et al. (2019). Pembrolizumab in patients with programmed death ligand 1–positive advanced ovarian cancer: Analysis of KEYNOTE-028. Gynecologic Oncology, 152(2), 243-250. doi:10.1016/J.YGYNO.2018.11.017
8. Wculek, S.K., Cueto, F.J., Mujal, A.M., Melero, I., Krummel, M.F., Sancho, D. (2020). Dendritic cells in cancer immunology and immunotherapy. Nature Reviews Immunology, 20(1), 7-24. doi:10.1038/S41577-019-0210-Z
9. Labidi-Galy, S.I., Sisirak, V., Meeus, P., et al. (2011). Quantitative and functional alterations of plasmacytoid dendritic cells contribute to immune tolerance in ovarian cancer. Cancer Research, 71(16), 5423-5434. doi:10.1158/0008-5472.CAN-11-0367
10. Labidi-Galy, S.I., Treilleux, I., Goddard-Leon, S., et al. (2012). Plasmacytoid dendritic cells infiltrating ovarian cancer are associated with poor prognosis. OncoImmunology, 1(3), 380-382. doi:10.4161/ONCI.18801
11. Harimoto, H., Shimizu, M., Nakagawa, Y., et al. (2013). Inactivation of tumor-specific CD8+CTLs by tumor-infiltrating tolerogenic dendritic cells. Immunology and Cell Biology, 91(9), 545-555. doi:10.1038/ICB.2013.38
12. Munn, D.H. and Mellor, A.L. (2016). IDO in the Tumor Microenvironment: Inflammation, Counter-Regulation, and Tolerance. Trends in Immunology, 37(3), 193-207. doi:10.1016/J.IT.2016.01.002
13. Conrad, C., Gregorio, J., Wang, Y.H., et al. (2012). Plasmacytoid dendritic cells promote immunosuppression in ovarian cancer via ICOS costimulation of Foxp3+ T-regulatory cells. Cancer Research, 72(20), 5240-5249. doi:10.1158/0008-5472.CAN-12-2271
14. Lluesma, S.M., Wolfer, A., Harari, A., Kandalaft, L.E. (2016). Cancer Vaccines in Ovarian Cancer: How Can We Improve? Biomedicines, 4(2). doi:10.3390/BIOMEDICINES4020010
15. Bol, K.F., Schreibelt, G., Gerritsen, W.R., de Vries, I.J.M., Figdor, C.G. (2016). Dendritic cell-based immunotherapy: state of the art and beyond. Clin Cancer Res, 22(8), 1897-1906. doi:10.1158/1078-0432.ccr-15-1399
16. Zhang, X., He, T., Li, Y., et al. (2021). Dendritic Cell Vaccines in Ovarian Cancer. Frontiers in Immunology, 11, 613773. doi:10.3389/FIMMU.2020.613773/BIBTEX

17. Zhou, Y., Slone, N., Chrisikos, T.T., et al. (2020). Vaccine efficacy against primary and metastatic cancer with in vitro-generated CD103 + conventional dendritic cells. Journal for ImmunoTherapy of Cancer, 8(1). doi:10.1136/JITC-2019-000474
18. Zhang, W., Lu, X., Cui, P., et al. (2019). Phase I/II clinical trial of a Wilms' tumor 1-targeted dendritic cell vaccination-based immunotherapy in patients with advanced cancer. Cancer Immunology, Immunotherapy, 68(1), 121-130. doi:10.1007/S00262-018-2257-2
19. Chu, C.S., Boyer, J., Schullery, D.S., et al. (2012). Phase I/II randomized trial of dendritic cell vaccination with or without cyclophosphamide for consolidation therapy of advanced ovarian cancer in first or second remission. Cancer Immunology, Immunotherapy, 61(5), 629-641. doi:10.1007/S00262-011-1081-8
20. Rahma, O.E., Ashtar, E., Czystowska, M., et al. (2012). A gynecologic oncology group phase II trial of two p53 peptide vaccine approaches: Subcutaneous injection and intravenous pulsed dendritic cells in high recurrence risk ovarian cancer patients. Cancer Immunology, Immunotherapy, 61(3), 373-384. doi:10.1007/S00262-011-1100-9
21. Schietinger, A., Philip, M., Schreiber, H. (2008). Specificity in cancer immunotherapy. Seminars in Immunology, 20(5), 276-285. doi:10.1016/j.smim.2008.07.001
22. Tang, L., Zhang, R., Zhang, X., Yang, L. (2021). Personalized Neoantigen-Pulsed DC Vaccines: Advances in Clinical Applications. Frontiers in Oncology, 11, 701777. doi:10.3389/FONC.2021.701777/BIBTEX
23. Hatfield, P., Merrick, A.E., West, E., et al. (2008). Optimization of dendritic cell loading with tumor cell lysates for cancer immunotherapy. Journal of Immunotherapy, 31(7), 620-32. doi: 10.1097/CJI.0b013e31818213df
24. Cho, D., Yang, W., Lee, H., et al. (2012). Adjuvant immunotherapy with whole-cell lysate dendritic cells vaccine for glioblastoma multiforme: a phase II clinical trial. World Neurosurgery, 77(5-6), 736-44. doi: 10.1016/j.wneu.2011.08.020
25. Di Nicola, M., Zappasodi, R., Carmelo, C.S., et al. (2009). Vaccination with autologous tumor-loaded dendritic cells induces clinical and immunologic responses in indolent B-cell lymphoma patients with relapsed and measurable disease: a pilot study. Blood, 113(1), 18-27. doi:10.1182/blood-2008-06-165654
26. Palucka, A.K., Ueno, H., Connolly, J., et al. (2006). Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8 + T-cell immunity. J Immunother, 29(5), 545-557. doi:10.1097/01.cji.0000211309.90621.8b
27. Chiang, C.L.L., Kandalaft, L.E., Tanyi, J., et al. (2013). A dendritic cell vaccine pulsed with autologous hypochlorous acid-oxidized ovarian cancer lysate primes effective broad antitumor immunity: from bench to bedside. Clinical cancer research : an official journal of the American Association for Cancer Research, 19(17), 4801-4815. doi:10.1158/1078-0432.CCR-13-1185
28. Kobayashi, M., Chiba, A., Izawa, H., et al. (2014). The feasibility and clinical effects of dendritic cell-based immunotherapy targeting synthesized peptides for recurrent ovarian cancer. Journal of ovarian research, 7(1). doi:10.1186/1757-2215-7-48
29. Tanyi, J.L., Bobisse, S., Ophir, E., et al. (2018). Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. Science translational medicine, 10(436). doi:10.1126/SCITRANSLMED.AAO5931
30. Kandalaft, L.E., Powell, D.J., Chiang, C.L., et al. (2013). Autologous lysate-pulsed dendritic cell vaccination followed by adoptive transfer of vaccine-primed ex vivo co-stimulated t cells in recurrent ovarian cancer. OncoImmunology, 2(1). doi:10.4161/ONCI.22664
31. Lawrence, M.S., Stojanov, P., Polak, P., et al. (2013). Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature, 499(7457), 214-218. doi:10.1038/nature12213
32. O'Donnell, T., Christie, E.L., Ahuja, A., et al. (2018). Chemotherapy weakly contributes to predicted neoantigen expression in ovarian cancer. BMC Cancer, 18(1), 87. doi:10.1186/s12885-017-3825-0
33. Ding, Z., Li, Q., Zhang, R., et al. (2021). Personalized neoantigen pulsed dendritic cell vaccine for advanced lung cancer. Signal Transduction and Targeted Therapy, 6(1). doi:10.1038/S41392-020-00448-5
34. Carreno, B.M., Magrini, V., Becker-Hapak, M., et al. (2015). A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science, 348(6236), 803-808. doi:10.1126/SCIENCE.AAA3828
35. Mullard, A. (2016). The cancer vaccine resurgence. Nature Reviews Drug Discovery, 15(10), 663-665. doi:10.1038/NRD.2016.201
36. Boisguérin, V., Castle, J.C., Loewer, M., et al. (2014). Translation of genomics-guided RNA-based personalised cancer vaccines: towards the

bedside. British Journal of Cancer, 111(8), 1469-1475. doi:10.1038/bjc.2013.820

37. Bassani-Sternberg, M., Gfeller, D. (2016). Unsupervised HLA peptidome deconvolution improves ligand prediction accuracy and predicts cooperative effects in peptide-HLA interactions. Journal of Immunology, 197(6), 2492-2499. doi:10.4049/jimmunol.1600808
38. Gfeller, D., Guillaume, P., Michaux, J., et al. (2018). The length distribution and multiple specificity of naturally presented HLA-I ligands. Journal of Immunology, 201(12), 3705-3716. doi:10.4049/jimmunol.1800914

"The School of Rome" by Felice Giani. Courtesy National Gallery of Art, Washington.

Suggestions Implemented During the COVID-19 Pandemic to Improve Medical Student Dermatology Exposure and Education

Scout M. Treadwell[1], Maxwell R. Green[1], Sowmya Ravi[1]

[1]Tulane University School of Medicine, New Orleans, Louisiana, 70112
Correspondence: STreadwell@tulane.edu

The COVID-19 pandemic has drastically changed medical education for both preclinical and clinical students. Virtual learning, shortened rotation schedules, cancelled away rotations, decreased interactions with faculty and mentors, and other curriculum adaptions have had a profound effect on the learning opportunities students receive during their medical training. Research studies surveying US medical schools show students interested in dermatology have limited exposure to this specialty in the medical school curriculum, and the COVID pandemic has exacerbated this lack of clinical experience. This article serves as a review of proposed improvements and current adjustments that have been implemented amid the COVID-19 pandemic in medical schools across the country to address the unforeseen changes in dermatology medical education. General changes have included virtual dermatology electives, student involvement in teledermatology, and online mentorship programs.

INTRODUCTION

Beginning in 2020, the COVID-19 pandemic led to extraordinary disruptions in medical education across the United States. To maintain CDC guidelines and prevent the

spread of infection and decrease mortality rates, classes were moved online, instructors were delivering lectures virtually to maintain the integrity of medical student education, and students were deemed nonessential in the hospital on clinical rotations. Shadowing opportunities and clinical rotations for students were limited as minimum room occupancy guidelines were implemented and students were isolated from their peers, faculty, and advisors. In response to this shift of learning, innovative methods to continue to provide patient care and train medical students have been established.

Dermatological assessment is a vital diagnostic skill in clinical practice. Understanding skin pathologies is significant for all physicians; however, many medical graduates feel they were not adequately exposed to dermatology. Additionally, the COVID-19 pandemic has exacerbated this lack of experience. Patients often present to non-dermatologists who may not know how to diagnose and treat dermatologic conditions. Research has shown that dermatologic diagnoses made by the primary care physician were concordant with that made by the dermatologists only 57% of the time (1). Thus, incorporating strategies to improve clinical education for all future physicians in the diagnosis and management of dermatologic conditions is imperative. Pre-pandemic, dermatology education was limited in the medical school curriculum (2). Research has shown that although dermatologic conditions have a high disease of burden, dermatology is not widely included in medical school curricula (3). Specifically, a study surveying 137 Allopathic US medical schools showed that only sixteen of the 137 schools had a course dedicated to dermatology in the first two preclinical learning years (3). Furthermore, only two of the surveyed schools required a third-year dermatology clinical rotation (3). While most medical schools incorporate dermatology lectures throughout broader educational systemic blocks, students must take the initiative to reach out to mentors in the field, set up shadowing opportunities, and plan for away rotations to establish a relationship with residency programs. During the COVID-19 pandemic, these opportunities have been increasingly limited, and restrictions have made it difficult to learn from experts in this field (4). Without exposure to the field of dermatology throughout the entirety of the one's medical school education, it may present as challenge for students matching into this field. This review serves a summary of the literature on how the COVID-19 pandemic has led to new innovative teaching methods in a virtual learning environment as well as suggestions on ways to improve student exposure to the field of dermatology.

METHODS

Two of us (S.M.T. and M.R.G.) independently identified studies published in February of 2020 to current date to account for the current pandemic that reported impact of COVID-19 on curriculum and learning changes in United States medical students' preclinical and clinical dermatology education. We systematically searched PubMed, Embase, and Web of Science were searched in December of 2021 using the terms ("Dermatology education" or "dermatology curriculum" or "dermatology knowledge") and ("medical school" or "medical student"). A total of 892 results were returned from this search. Following Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA), we exported our search results into Covidence for systematic review management (5). After removal of duplicates, full-text articles were obtained if their abstracts were considered eligible by at least 1 of us. Each full-text article was assessed independently for final inclusion in this systematic review and meta-

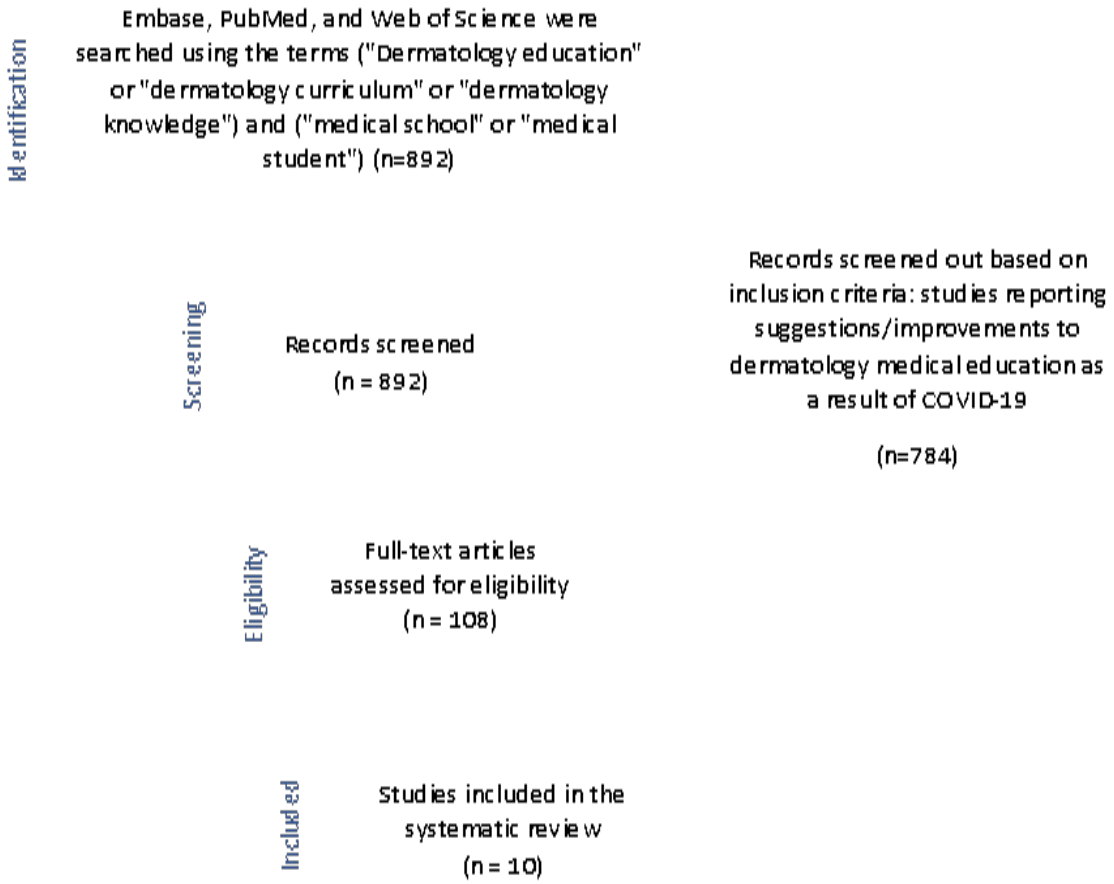


Figure 1: *Flow diagram of the literature search using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Adapted from* http://prisma-statement.org.

analysis, and disagreements were resolved by consensus. Studies were included if they met all criteria below.

Inclusion Criteria

1. COVID-19 on curriculum and learning changes in United States medical students' preclinical and clinical dermatology education.
2. Teledermatology in medical education.
3. Virtual mentoring opportunities for students interested in dermatology.

Results were limited to those published in 2020 to account for published articles on the current pandemic.

Exclusion Criteria

1. Dermatology residency training during the COVID-19 pandemic.
2. Dermatological manifestations of COVID-19 infection.

RESULTS

A total of 10 articles were included in this review, see **Figure 1**. A discussion of the role of the COVID-19 pandemic or its resulting effects on medical student dermatology curriculum and learning is summarized. The characteristics of the included studies are summarized in **Table 1**.

Virtual/Remote Learning

The traditional hands-on clerkship learning environment has been deconstructed due to the virtual changes the COVID-19 pandemic has imposed. To combat these alterations in

Table 1. Characteristics of the 10 Selected Studies

Source	Type of Article	Region	Focus of Article	Principle Findings
Stewart *et al*. 2020	Letter to the Editor	United States	Recommendations for medical student preparedness during COVID-19 pandemic	Creative new solutions are necessary in response to the changes by the COVID-19 pandemic. Students must be adequately prepared for applications in the process.
Patel *et al*. 2020	Letter to the Editor	United States	Recommendations to improve virtual learning	Implementation of teledermatological and integration of supplemental online resources into the medical student curriculum.
Lipner *et al*. 2021	Research Article	United States	Improving virtual learning	Integrating virtual didactics, teledermatology, and self-directed learning into the dermatology curriculum using online platforms.
Ashrafzadeh *et al*. 2021	Letter to the Editor	United States	Strategies to improve virtual/remote learning	4-week elective virtual rotation: positives and negatives were observed in a fully remote rotation.
Su *et al*. 2020	Letter to the Editor	United States	Teledermatology in medical student education	Participation in asynchronous teledermatology as a paired educational experience for medical students will expand access to dermatology care.
Belzer *et al*. 2021	Letter to the Editor	United States (Yale School of Medicine)	Teledermatology in improving efficiency of care	5-week pilot study to enhance efficiency of care provision during the pandemic in a dermatology continuity clinic.
Linggonegoro *et al*. 2021	Research Article	United States (Massachusetts)	Teledermatology student run clinic	Study followed a clinic that aims to provide high quality dermatologic care to a diverse, underserved pediatric patient population while teaching trainees how to diagnose and manage common skin conditions.
Muzumdar *et al*. 2020	Letter to the Editor	United States	Dermatology away rotations	Many students will not have the opportunity to rotate or obtain letters of recommendation. Evaluate students upon intrinsic values.
Alikhan *et al*. 2009	Research Article	United States	Mentorship advice from 4th year students post-residency match	Opinions and advice from four 4th year students who matched regarding what they believe were the most important factors in their success.
Fernandez *et al*. 2021	Letter to the Editor	United States	Finding virtual mentorship	Survey of 4th-year students to assess the level of mentorship they received in dermatology.

learning, Patel et al. suggest the addition of a dermatology elective in the medical student curriculum to ensure students are comfortable and competent in the art of history taking, physical examination, documenting, and therapeutic management of common dermatological conditions (4). This proposed elective includes lessons on describing dermatology morphology and understanding the underlying pathophysiology causing the most common dermatology diagnoses. Virtual and online lectures can achieve this mission. Patel et al. also propose a flipped classroom approach that includes video learning so students can learn dermatologic procedures (4). Virtual platforms have been essential in continuing medical student dermatology education during the pandemic. The implementation of prerecorded dermatology lectures into medical student curriculum, proposed by Lipner et al., allows for students to learn at their own pace without the constraints of a socially distanced and limited occupancy classroom (6). Increasing access to virtual dermatology learning models for medical students may improve dermatology education and exposure.

A piloted virtual 4-week dermatology elective provided students the unique opportunity to participate in virtual patient care was well as learn from faculty. Students were able to collect a history via phone call conversations with patients. Students were also able to describe dermatology morphologies based on the photographs patients submitted. This allowed students to formulate a differential diagnosis and recommend further diagnostic screening tests to their assigned faculty mentor. Students wrote clinical notes on the patients they evaluated as well as gave oral pretentions of the patients they spoke with. Students consulted approximately 100 patients and were exposed to a variety of dermatology conditions including rashes, sarcoidosis or immunotherapy induce skin toxicities (7). Individuals involved in this pilot program received valuable feedback from their faculty mentor on their oral presentation and clinical consulting skills. All students reported a substantial increase in their prior dermatology knowledge and confidence (7). Because COVID-19 limits student presence in dermatology clinics, teledermatology serves a critical role in medical student education. This pilot virtual dermatology elective created by Brigham and Women's Hospital Department of Dermatology serves as a positive model for other medical schools interesting in increasing student exposure to the field of dermatology amid the international pandemic (7).

Teledermatology

The role of telehealth visits has become increasingly important amid the COVID-19 pandemic. Specifically, many dermatology practices have relied on telemedicine to virtually assess and treat patients. Su et al. states that this shift in care provides a unique opportunity to educate budding dermatologists. They encourage student involvement in teledermatology appointments as this aspect of dermatology will remain an important component of future patient care (8). A teledermatology rotation can provide participating medical students the opportunity to eConsult cases in a self-directed manner which allows for an individual formulation of a differential diagnoses. Medical students are also expected to learn the importance of longitudinally monitoring patients with chronic dermatologic conditions during a global pandemic. The implementation of teledermatology visits expands access to dermatologic care and is complementary to traditional trainee education (8).

The burden of managing both virtual and in-person patients presents a challenge to dermatology practices. Nine Yale School of

Medicine students created the Teledermatology Student Task Force aiming to enhance the efficacy of care provided during the pandemic in a dermatology continuity clinic (9). Volunteers of this task force were able to help schedule patient video appointments and upload any clinically important images to patient charts. Students were able to assist in patient education ensuring patients were familiar with operating a smartphone or tablet for the telemedicine visit as well as how to access and open their own patient chart during the appointment. This pilot program was able to contact 104 patients (9). Of the 104, 87 patients were successfully reached by phone by the student task force volunteers and 93% reported that the outreach was helpful (9). 78% of these patients were able to successfully complete a video visit with their dermatologist (9). This study shows the potential for medical students to learn from telemedicine visits as well as the student opportunity to support both physicians and patients leading to optimal utilization of teledermatology.

In 2020, a pediatric dermatology student-run clinic was established to provide dermatologic care to an underserved population whose health care disparities and inequities were widened by the COVID-19 pandemic. Due to pandemic restrictions, care was delivered virtually. Patients were able to submit photographs of their dermatology complaints and three pre-clinical students were able to learn from dermatology resident's teaching sessions on the specific presentations (10). Students were also able to virtually take patient history under the supervision of the dermatology resident or attending. These interactions increased students' skills in history taking and improved their basic dermatology knowledge (10). Creating more teledermatology student run clinics can provide students interested in dermatology pre-clinical mentors as well as increasing exposure to the field of dermatology while still delivering care to underserved patients amid a global pandemic.

Away Rotations

Due to program restrictions, away rotations have been limited for visiting medical students. Traditionally, away rotations were critical for developing connections with faculty and obtaining letters of recommendation (2). With these clinical experiences delayed or cancelled, many students are concerned that they will be at a disadvantage for matching into a residency program. Stewart et. al proposes recommendations ensuring the application process is equitable and fair for medical students applying into dermatology. Measures such as virtual didactics and grand rounds may allow student to interact with dermatology faculty (2). Muzumdar et al. also outlines the challenge away rotations in midst of a pandemic presents. They too note that limiting student rotations negatively affects students interested in applying to the highly competitive field of dermatology, specifically those with no home dermatology departments or limited experience in the field. Creative options for students limited to virtual away rotations include virtual lectures and grand rounds sessions, student participation in teledermatology care, and virtually engaging in case-based learnings sessions (11). This provides an opportunity for students to ask questions to current residents and faculty and have experience with a program they may have not had otherwise due to COVID restrictions.

Mentorship

Mentorship is a critical aspect of a successful medical career. Advice from a supportive mentor can change the trajectory for medical students. According to a study completed by Alikhan et al., 4 students who matched in

Dermatology noted that having an encouraging mentor that provided invaluable advice was crucial in a successful match (12). Amidst the COVID-19 pandemic, students are in an unprecedented position in which their access to mentorship is limited. Therefore, it is imperative to improve methods promoting more access to mentorship in dermatology. Minority students have reported that lack of a supportive mentor is an obstacle in applying to a dermatology residency program. In 2018, only 6% of dermatology faculty at medical schools across the United States identified as Black or Latino (13). Creating virtual dermatology mentorship programs can diversify student's access to mentors. These programs can include alumni directories of clinicians in specific specialties as a resource for students. Web-based open houses hosted by program directors may also provide opportunities for students to establish connections with mentors, especially for students with no home institution (13). While forming a relationship with a mentor in a virtual setting may not develop as organically as an in-person interaction, the goal of a virtual mentoring program can increase access for students to find support within the field of dermatology.

DISCUSSION

In this systematic review of alterations to dermatology teachings in medical education in response to the COVID-19 pandemic, we found suggestions that have been implemented to improve curriculum in areas of virtual and remote learning, teledermatology, away rotations, and mentorship. These proposals can continue to be implemented to further improve medical school dermatology curriculum.

As with many other medical specialties, the field of dermatology has had to adjust to COVID-19 by improving virtual teaching options for students and expanding teledermatology care and education. In addition, the pandemic has limited student access to both away-rotations and mentorship opportunities, both vital components to the traditional undergraduate dermatology education for those interested in the field.

Although many adjustments were made to create virtual medical school curricula, students overall see a benefit in virtual learning for its increased flexibility with their study schedule (14). Additionally, no differences in the effectiveness of online versus offline medical education have been shown (15). These results argue in favor for the benefits of widespread online medical education that took place during the COVID-19 pandemic; however, increased stress and burnout have also been associated with online learning platforms, suggesting that hybrid curricula with both on and offline components may be the most effective for medical students (16). A hybrid plan could include virtual lectures with in-person case-based team learning sessions.

The reduced access to both mentorship and away rotations is more challenging to tackle, but the creation of virtual away rotations may be an effective solution to both these issues. Virtual opportunities in the field of radiology increased student access to both rotation experience and mentorship while lowering the costs associated with traditional away experiences; the largest reported drawbacks were delays for students and technical difficulties (17). Increased access to rotations was also observed with virtual plastic surgery rotations, but students felt that it was difficult to connect and stand out through a virtual platform (18). Although these same challenges faced by other medical specialties with virtual rotations would likely be experienced in dermatology, the benefits of creating more virtual programs to improve access may be worthwhile. Traditional away rotations add an extra cost for medical

students and the cost associated may disadvantage students from completing rotations and establishing relationships with non-home programs. Establishing more virtual away rotations will decrease financial burden and may increase opportunities and equity for all students interested in going into dermatology.

CONCLUSIONS

The effects of COVID-19 on undergraduate dermatology education will likely outlast the pandemic itself, so using the lessons learned over the past two years is essential for continuing to strengthen the field of dermatology. The current literature demonstrates improvements and suggestions that have been implemented to account for the rapid classroom changes imposed by the ongoing pandemic. These recommendations have taken into account the importance of continuity in medical student education and have increased exposure for students virtually in a time defined by isolation. Furthermore, as the pandemic continues to evolve, it is crucial to continue to adapt current models to ensure students interested in dermatology have access to knowledge, mentorship, and feel confident applying for a residency position in this field.

DISCLOSURES

Conflicts of interest: None.
Availability of data and material: Not applicable.
Code availability: Not applicable.
Authors' contributions: Authors listed in the manuscript have contributed per submission guidelines and standards for authorship.
Ethics approval: Not applicable.
Consent to participate: Not applicable.

REFERENCES

1. Lowell, B. A., Froelich, C. W., Federman, D. G., & Kirsner, R. S. (2001). Dermatology in primary care: Prevalence and patient disposition. Journal of the American Academy of Dermatology, 45(2), 250–255.
2. Stewart, C. R., Chernoff, K. A., Wildman, H. F., & Lipner, S. R. (2020). Recommendations for medical student preparedness and equity for dermatology residency applications during the COVID-19 pandemic. Journal of the American Academy of Dermatology, 83(3), e225–e226.
3. Cahn, B. A., Harper, H. E., Halverstam, C. P., & Lipoff, J. B. (2020). Current Status of Dermatologic Education in US Medical Schools. JAMA dermatology, 156(4), 468–470.
4. Patel, P. M., Tsui, C. L., Varma, A., & Levitt, J. (2020). Remote learning for medical student-level dermatology during the COVID-19 pandemic. Journal of the American Academy of Dermatology, 83(6), e469–e470.
5. Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., McGuinness, L. A., … Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ (Clinical research ed.), 372, n71.
6. Lipner, S. R., Shukla, S., Stewart, C. R., & Behbahani, S. (2021). Reconceptualizing dermatology patient care and education during the COVID-19 pandemic and beyond. International journal of women's dermatology, 7(5), 856–857.
7. Ashrafzadeh, S., Imadojemu, S. E., Vleugels, R. A., & Buzney, E. A. (2021). Strategies for effective medical student education in dermatology during the COVID-19 pandemic. Journal of the American Academy of Dermatology, 84(1), e33–e34.
8. Su, M. Y., Lilly, E., Yu, J., & Das, S. (2020). Asynchronous teledermatology in medical education: Lessons from the COVID-19 pandemic. Journal of the American Academy of Dermatology, 83(3), e267–e268.
9. Belzer, A., Olamiju, B., Antaya, R. J., Odell, I. D., Bia, M., Perkins, S. H., & Cohen, J. M. (2021). A novel medical student initiative to enhance provision of teledermatology in a resident continuity clinic during the COVID-19 pandemic: a pilot study. International journal of dermatology, 60(1), 128–129.
10. Linggonegoro, D., Rrapi, R., Ashrafzadeh, S., McCormack, L., Bartenstein, D., Hazen, T. J., Kempf, A., Kim, E. J., Moore, K., Sanchez-Flores, X., Song, H., Huang, J. T., & Hussain, S. (2021). Continuing patient care to underserved communities and medical education during the COVID-19 pandemic through a teledermatology

student-run clinic. Pediatric dermatology, 38(4), 977–979.
11. Muzumdar, S., Grant-Kels, J. M., & Feng, H. (2020). Medical student dermatology rotations in the context of COVID-19. Journal of the American Academy of Dermatology, 83(5), 1557–1558.
12. Alikhan, A., Sivamani, R. K., Mutizwa, M. M., & Aldabagh, B. (2009). Advice for medical students interested in dermatology: perspectives from fourth year students who matched. Dermatology online journal, 15(7), 4.
13. Fernandez, J. M., Behbahani, S., & Marsch, A. F. (2021). A guide for medical students and trainees to find virtual mentorship in the COVID era and beyond. Journal of the American Academy of Dermatology, 84(5), e245–e248.
14. Creagh, S., Pigg, N., Gordillo, C., & Banks, J. (2021). Virtual medical student radiology clerkships during the COVID-19 pandemic: Distancing is not a barrier. Clinical imaging, 80, 420–423.
15. Pei, L., & Wu, H. (2019). Does online learning work better than offline learning in undergraduate medical education? A systematic review and meta-analysis. Medical education online, 24(1), 1666538.
16. Mheidly, N., Fares, M. Y., & Fares, J. (2020). Coping With Stress and Burnout Associated With Telecommunication and Online Learning. Frontiers in public health, 8, 574969.
17. Janopaul-Naylor, J., Qian, D., Khan, M., Brown, S., Lin, J., Syed, Y., Schlafstein, A., Ali, N., Shelton, J., Bradley, J., & Patel, P. (2021). Virtual Away Rotations Increase Access to Radiation Oncology. Practical radiation oncology, 11(5), 325–327.
18. Tucker, A. B., Pakvasa, M., Shakir, A., Chang, D. W., Reid, R. R., & Silva, A. K. (2022). Plastic Surgery Away Rotations During the Coronavirus Disease Pandemic: A Virtual Experience. Annals of plastic surgery, 88(6), 594–598.

"Two Women" by Edgar Degas. Courtesy National Gallery of Art, Washington.

The Role of Estrogen in Ovarian Cancer and the Pathways by Which Estrogen Acts

Timothy Ehmann[1], Stephanie Barel[1], Amitabha Ray[2], Daniel Borsch[1]

[1]Lake Erie College of Osteopathic Medicine, Seton Hill University, Greensburg, Pennsylvania, 15601
[2]Alderson Broaddus University, Philippi, West Virginia, 26416, United States
Correspondence: TEhmann08651@med.lecom.edu

Ovarian cancer remains a prevalent and deadly cancer in females. While anti-estrogen therapies have been useful in the treatment of other cancer types, their effectiveness in the treatment of ovarian cancers remains limited due to the limited understanding of estrogen's effects on carcinogenesis and growth promotion in these tissues. This paper aims to summarize the role estrogen plays in ovarian cancer tumorigenesis and its potential value in targeted therapeutics. Estrogen's effects are secondary to interactions with estrogen receptor (ER) α, ER β, and the G protein coupled receptor, GPR30. Genomic signaling of ER-αhas been shown to be primarily carcinogenic, while that of ER-βhas been shown to be a negative regulator of carcinogenesis. Additionally, ER-αhas been found to have carcinogenic effects through non-genomic signaling of the *p53*, MAPK, EGFR/Her2, PI3K, and IGF/IGFR pathways. GPR30's effects have been found to be more variable and specific to tumor classification. For example, GPR30 is primarily carcinogenic in ovarian epithelial tumor types but appears to have protective effects when highly expressed in granulosa cell tumors. While the above generalizations can be made, a better understanding of estrogen's effects on molecular signaling pathways will potentially allow for development of more effective targeted therapies against ovarian cancer.

INTRODUCTION

In the United States, ovarian cancer remains the 5th most common cancer among females today and the second most common gynecological/urinary (GU) cancer among females. Ovarian cancer had an estimated 21750 new cases in 2020 it ranks 4th in mortality among all cancers in females (1,2). Stage I, low grade tumors may be managed with surgery and observation while stage II-IV lesions are typically managed with surgical debulking as well as multiple cycles of chemotherapy; 60% of ovarian cancer cases are stage III on detection (3,4). Chemotherapy, which is typically in the form of platinum-based agents and taxane agents, and surgery still produce a 5 year survival rate of 85-90% for stage I, 57-70% for stage II, 39-59% for stage III, and 17% for stage IV (4). Unfortunately, anti-estrogen treatments are still in their early years with regards to the treatment of ovarian cancer and have shown limited efficacy (5), necessitating a better understanding of this pathway in cancer.

Estrogen interacts with three primary receptors: α, β, and a G protein coupled receptor, GPR30 (17, 26) (**Table 1** defines the abbreviations/terms used throughout the paper). These receptors interact with downstream signaling pathways regulating, cell cycle progression, cell division, and migration (10, 37). These pathways may represent a novel target for ovarian cancer treatment, pending a better understanding of their role in carcinogenesis. The treatment of ovarian cancer requires further inroads into the understanding of the pathogenesis of these tumors and the effects of estrogen as it pertains to carcinogenesis and growth promotion. In theory, this will allow for the development of more effective targeted therapies.

Histology of ovarian carcinoma

There are numerous subtypes of ovarian carcinoma, each defined by the cells of origin. **Table 2** summarizes some of the major characteristics/statistics regarding each subtype.

ESTROGEN RECEPTORS AND CARCINOGENIC EFFECTS IN OVARIAN CANCER

Estrogen α receptor (ER-α)

The α receptor exists as one of three variants, based on variable splicing of mRNA: ER-α66, ER-α46, and ER-α36 (6, 7). ER-α66 is the primary receptor referenced in the literature. ER-α46 lacks the activating function domain, AF-1,while ER-α36 lacks both AF-1 and AF-2 domains; ER-α46 has been shown to be inhibitory towards ER-α66 in MCF-7 breast cancer cells (6,7). The α receptor (ER-α66) is commonly found in ovarian cancer, but shows a particular predominance in serous carcinomas. In a study performed by Sieh et.al (2013) (8), samples from 2933 females diagnosed with ovarian cancer were examined showing: 87.5% of low-grade serous carcinomas, 80.7% of high grade serous carcinomas, 76.6% of endometrioid carcinomas, 20.8% of mucinous carcinomas, and 19.4% of clear cell carcinomas stained positive for ER (1). In a smaller study of serous and mucinous carcinomas, 60% of these cancers had a ratio of estrogen receptor α:β greater than 1, meaning that cancer cells expressed a significantly greater number of estrogen receptor α molecules when compared to benign ovarian tissues (9). Furthermore, when exposed to estradiol, 5/16 ovarian cancer cell lines all PEO1 and PEO4 cells, staining strongly positive for ER α, showed increased growth compared to ER negative cell lines e.g., PEO14, PEO16, 41M, 59M, OVCAR-3, OVCAR-4, OVCAR-5, A2780, CAOV3 and OAW42 (10). ER positive staining ovarian serous carcinoma PEO4 cells have been shown to proliferate significantly more when exposed to 17-B estradiol; ER negative PEO14 cells displayed

Table 1. Definition of terms and abbreviations used throughout the text	
Akt	Aka Protein kinase B (PKB); A serine/threonine protein kinase which functions in the control of the cell cycle/proliferation
Aloesin	An active component of the aloe vera plant with antiproliferative effects
ATF3	(Activating transcription factor 3); Member of the ATF/CREB family
Bcl-2	Anti-apoptotic protein that inhibits release of caspases from within mitochondria
β-catenin	Signaling protein important to the WNT signaling pathway; Involved in cell adhesion as well as transcription/cell proliferation
BRAF	Proto-oncogene; A serine/threonine kinase of the RAF family
BTG2	(B-Cell Translocation Gene 2); A tumor suppressor of the BTG/TOG gene family
Cadherin 6	Transmembrane glycoprotein. Aids formation of desmosomes and cell-cell adhesion.
CaMKIV	Calmodulin kinase IV: A calcium-dependent protein kinase
Caspase	Pro-apoptotic enzymes activated when leaked into cytosol from within mitochondria
Cathepsin	A gene promoter activated by ERα and Sp1
c-fos	Proto-oncogene that produces pro-growth transcription factors
c-myc	Proto-oncogene found on Chromosome 8
CTF-1	(CCAAT-binding transcription factor 1); Acts as a DNA-bound cofactor for NF-KB via activation by CaMKIV increasing transcription of p53
CTNNB1	Gene that encodes β-catenin
Cyclin A	Protein driving cell cycle progression during the S phase
Cyclin B1	Protein involved in driving cell cycle progression from G2 to M phase
Cyclin D1	Protein involved in driving cell cycle progression from G1 to S phase
Cyclin E	Protein involved in driving cell cycle progression from G1 to S phase
Delphinidin	An anthocyanidin; Natural pigment found in berries, red cabbage, grapes, and sweet potatoes with anticarcinogenic effects
E-cadherin	Transmembrane adhesion molecules within belt desmosomes that interacts with actin thereby controlling cell motility, adhesion, and shape
EGFR	(Epidermal growth factor receptor); Induces growth of keratinocytes, fibroblasts, and granulation tissue
Transactivation	Increased expression of EGFR mediated by GPR30 expression
G-1	(GPER-1-specific compound 1); agonist of GPER1 that inhibits proliferation of cancer cells
GRIP1	(Glucocorticoid receptor-interacting protein 1); Transcriptional coactivator, SRC family
IL-6	(Interleukin-6); Cytokine involved in acute systemic inflammation
Jun	Proto-oncogene that produces pro-growth transcription factors
MDR-1	(Multidrug resistance gene 1); promoter targeted by p53 to repress transcription
MEK1/2	Protein kinases; Involved in the Ras-Raf-Mek-Erk cascade
MMP 11	(Matrix metalloproteinase 11); Protein that helps break down the extracellular matrix
MMP 17	(Matrix metalloproteinase 17); Protein that helps break down the extracellular matrix
mTOR	(Mammalian target of rapamycin); A substrate of Akt that regulates cell metabolism, stimulating protein and lipid synthesis
NF-KB	(Nuclear factor kappa B); Transcription factor involved in secretion of cytokines, DNA transcription, and cell adhesion.
p21	Cyclin-dependent kinase inhibitor 1 (CDKN1A). Target of p53 leading to cell cycle arrest.
p73	A tumor suppressor of the p53 family, with strong pro-apoptotic activity to tissues with DNA damage secondary to chemotherapy
PTEN	(Phosphate and tensin homologue) Tumor suppressor gene that inhibits PI3K/Akt signaling
RAS	Proto-oncogene; Family of G proteins involved in signal transduction
SDF-1	(Stromal cell-derived factor 1); Chemokine promoting autocrine and paracrine cell migration
SP-1	(Specificity protein 1); Transcription factor that regulates fibulin-1
Src (SRC-3)	(Steroid hormone receptor coactivator-3) Has the ability to target nuclear hormone receptors as well as act as a transcription factor in other pathways
Survivin	Promoter targeted by p53 to repress transcription; Part of IAP (Inhibitor of apoptosis) family
TNFα	(Tumor necrosis factor α); Inflammatory cytokine
TRAF4	(Tumor necrosis factor [TNF] receptor-associated factor 4); Target of p53 leading to apoptosis

Table 2. ***In vitro*** **and** ***in vivo*** **cell models used for testing of estrogen responsivity**

	ER α +	ER α —	ER β +	ER β —	GPR30 +	Unknown —
Epithelial Cell Tumors						
Serous	PEO1	PEO14	SKOV-3	SKOV-3	SKOV-3	HEYA8
Low grade: 87.5% ER α positive[1]		PEO4	PEO16			OVCAR-3
High grade: 80.7% ER α positive[1]	PEOR1	41M				
49.6% ER β positive[82]		59M				
GPR30 expression high[21]		OVCAR-3				
	OVCAR-4	OVCAR-5				
		CAOV3				
		OAW42				
Endometrioid 76.6% ER α positive[1] ER β status unknown GPR30 expression high[21]		A2780				
Mucinous 20.8% ER α positive[1] ER β status unknown GPR30 expression high[21]						
Clear Cell 19.4% ER α positive[1] ER β status unknown GPR30 status unknown						ES-2
Sex Cord Stromal Tumors						
Granulosa Cell 66% ER α positive 53% GPR30 positive[20] ER β status unknown						
Sertoli/Leydig Cell 79% ER α positive ER β status unknown GPR30 status unknown						
Sertoli/Leydig Cell 79% ER α positive ER β status unknown GPR30 status unknown						
Unknown origin	BG-1		BG-1		BG-1	

no significant response to estradiol (11).

Estrogen receptor β (ER β)

The β receptor appears more frequently in normal ovarian tissue and benign tumors as opposed to malignant tissue/tumor (9, 12). The β receptor has multiple splicing variants: ERB1, ERB2, and ERB5. ERB1 is the primary isoform while ERB2 lacks ligand/DNA binding abilities and serves as a negative regulator of ER α; ERB5 serves a similar inhibitory function towards ER αand ERB1 (12, 13). ERB1 is thought to interfere with ER α activities, inhibit production of ER α, and inhibit cellular proliferation. These effects were exhibited in ER α positive, ER β negative BG-1 cells subsequently infected with adenovirus carrying the ER β gene (14).

Furthermore, ovarian cancer SKOV-3 cells expressing ERB1 exhibited decreased proliferation while caspase activity was found to be increased in ERB1 positive SKOV-3 cell lines compared to ERB1 negative SKOV-3 lines, increasing apoptosis (15). ER β receptor particularly the ERB1 isoform, seems to be protective against malignant evolution/progression. This is further elucidated by He et al, who showed that ER β agonist LY500307 decreased cell viability, promoted tumor suppressor gene expression, and increased apoptotic gene expression (89). Similarly, OSU-ERb-12, a ER β agonist, demonstrated both in vitro and in vivo inhibition of ovarian cell proliferation by inhibiting epithelial to mesenchymal transition, a key transition for malignancies (90). As such, the loss or suppression of ER β appears to be a key to malignant transformation.

GPR30

GPR30 is a membrane bound, G protein coupled receptor, sensitive to estradiol. The GPR30 receptor mediates signaling through multiple mechanisms (16, 17). The primary role of GPR30 in ovarian carcinogenesis is disputed currently. In BG-1 ovarian cancer cells, GPR30 was shown to activate the c-fos gene independently of ER α activity, suggesting that GPR30 may have effects on carcinogenesis independent of the estrogen receptor (17).

In ovarian cysts, higher levels of GPR30 mRNA were found in malignant lesions compared to benign, correlating with increased tumor size, advanced stage, and increased metastatic ability (18). Smith et. al (2009) showed that GPR30 was present more frequently in ovarian carcinoma than in lower risk ovarian tumors and correlated with a lower survival rate (19).

In granulosa cell tumors, GPR30 was found to be present in 53% of samples and correlated with poor survival in newly diagnosed cases (20). Comparison of 42 samples of various benign, borderline, and malignant epithelial ovarian carcinoma samples revealed that malignant samples were more likely to have increased amounts of GPR30 (21). However, GPR30 was shown to exhibit an anti-proliferative role in SKOV-3 and OVCAR-3 ovarian cancer cell lines. In these cells, GPR30 was found to mediate cell cycle arrest and inhibit cell proliferation (22).

MOLECULAR SIGNALING PATHWAYS

Estrogen receptors contain a ligand domain, a DNA binding domain, as well as domains for the binding and interaction with coactivators and corepressors (23). When estrogen enters the cell, it binds these receptors, inducing a conformational change and allowing for binding to estrogen response elements (ERE) within DNA as well as transcriptions factors (24, 25). Estrogen receptors can localize to the cytosol and nucleus, both initiated via a common pathway (26, 27). Cytosolic receptors, after binding estrogen, do not necessarily localize to the nucleus but rather associate with various other cytosolic proteins, affecting intracellular signaling cascades. ER activity can be divided into genomic and non-genomic, with interplay between the two groups (28).

Genomic activities of ER

Genomic signaling includes the activation of transcription factors and coactivators or corepressors via estrogen receptors α and β, as well as the direct binding of the ER to ERE regions within DNA. In ovarian cancer cells, genes directly regulated by estradiol through ERE regions include the genes for: AP4 DNA binding protein, cathepsin, cyclin B1, caspase 4, IGFBP3, cadherin 6, matrix metalloproteinases 11 and 17, in PEO1 cells, (10) and stromal cell-derived factor 1 (SDF-1) in BG-1 cells (29), as shown in **Figure 1.**

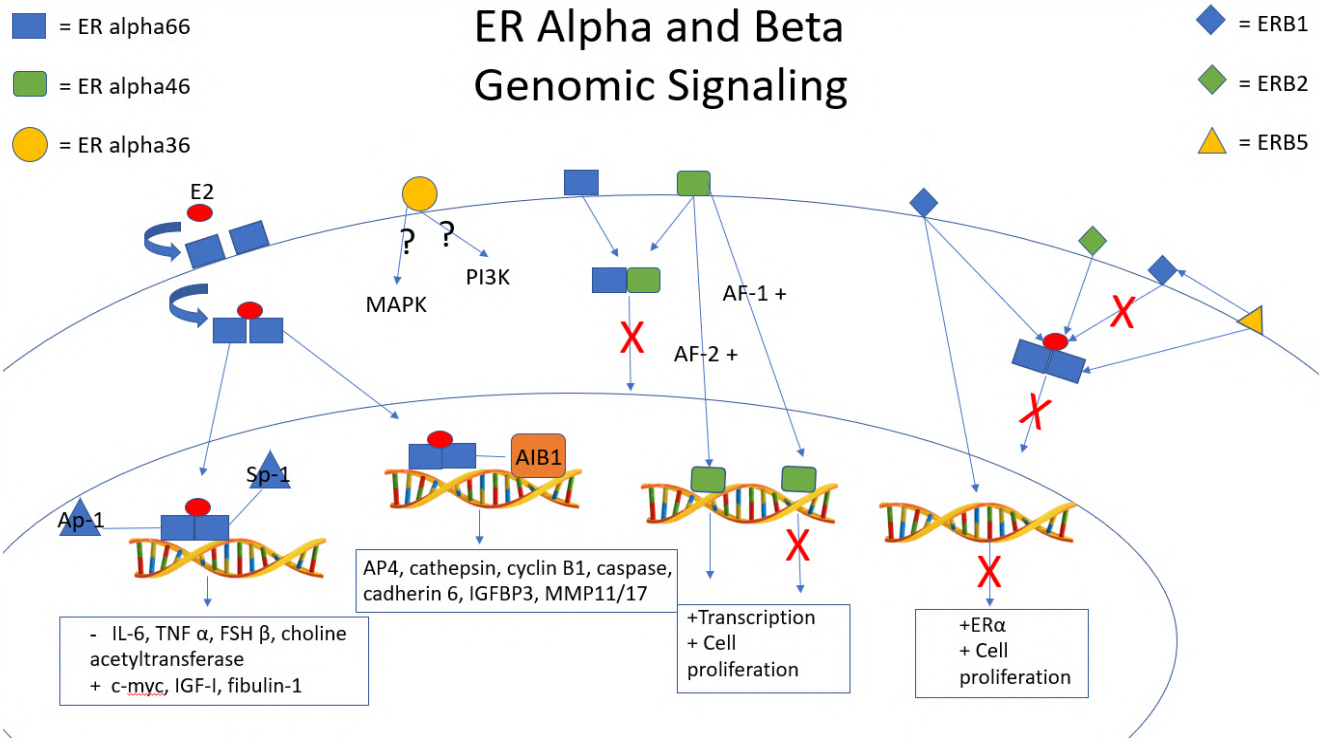


Figure 1: ***Through mRNA splicing, three variants of estrogen receptor α (ERα) exist: ERα66, ERα46, and ERα36.*** *ERα66 interacts intranuclearly with DNA bound transcription factors AP-1 and SP-1, leading to both the upregulation of c-myc, IGF-1, and fibulin-1 as well as the downregulation of IL-6, tumor necrosis factor α (TNFα), follicle stimulating hormone β (FSHβ), and choline acetyltransferase. Through a separate pathway, amplified in breast cancer-1 (AIB1) acts as a coactivator to ERα66, leading to the production of AP-4 (transcription factor), cathepsin, cyclin B1, caspase, cadherin 6, insulin-like growth factor binding protein 3 (IGFBP3), and matrix metalloproteinases 11 and 17 (MMP11/17). Via the activating factor 2 (AF2) binding site, ERα46 acts intranuclearly to increase transcription and cell proliferation. Binding at activating factor 1 (AF1) inhibits ERα46 from this process. Interactions with ERα66 inhibit ERα46 from entering the nucleus entirely. ERα36 lacks the AF1 and AF2 domains, and therefore is not involved in ovarian tumorigenesis. Three forms of estrogen receptor β (ERβ) are involved in ovarian tumorigenesis: ERβ1, ERβ2, and ERβ5. ERβ1 inhibits ERα activity, leading to a decrease in ERα production as well as inhibition of cell proliferation. ERβ2 negatively regulates ERα within the cytoplasm, and as it has no ligand/DNA binding abilities, it does not directly act intranuclearly. Similarly, ERβ5 acts as an ERα inhibitor, and has additional ERβ1 inhibitory effects.*

Furthermore, a large family of p160 coactivators have been found to mediate ER/estrogen mediated genomic actions. This includes AIB1/SRC-3 and GRIP1 (24, 30). AIB1 (SRC-3) has been shown to be overexpressed in BG-1 ovarian cancer cells. These same AIB1 overexpressing cells have shown increased transcription activity when treated with estradiol, eluding to AIB1's role as a coactivator for ER (31). AIB1 is present in 68.7% of epithelial ovarian cancer samples with a significantly higher expression in cancerous tissue (32) and correlates with worse overall survival in epithelial cancer patients as well as higher grade tumors (33).

AP1 and Sp1 are two highly important transcription factors regulated by the estrogen receptors. Estrogen receptor α has been shown to bind and activate the AP1 complex, which requires intact activating function domains, AF-1 and AF-2. Interestingly, the estrogen β receptor lacks an AF-1 domain and as such only interacts with AP1 when treated with anti-estrogens, which normally block estrogen-α receptor mediated effects (30). Estradiol has been shown to have an inhibitory effect on the AP1 transcriptional pathway, suppressing

expression of IL-6, TNF α, FSH β, and choline acetyltransferase (34) as shown in **Figure 1**. However, in CAOV-3, OVCAR-3, and A2780-ER ovarian cancer cells expressing ER α, estradiol was found to induce the expression of c-myc and IGF-I through an ER/estradiol/AP1 binding complex, suggesting a gene dependent ER mechanism (35), shown in **Figure 1**. Furthermore, components of AP-1, fos and jun proteins, have been found in elevated levels within ovarian tumors (36). Fibulin-1, a protein that plays a role in migration and motility of cells, has been shown to be overexpressed in BG-1, PEO4, and OVCAR-3 ovarian cancer cells and is responsive to estradiol treatment through ER α mediation (37). The fibulin-1 promoter was subsequently found to have 2 Sp1 binding sites, both required for estradiol/ER mediated fibulin transcription (37).

P53: Cross-talk between genomic and non-genomic

P53 serves as a "guardian" for the cellular genome, arresting the cell cycle, promoting DNA repair, and when necessary, promoting apoptosis. A large percentage of high grade serous and endometrioid carcinomas have shown loss of function mutations in p53, a gateway to carcinogenesis (38, 39). P53 has been shown to crosstalk with ER, forming a complex interaction between the two. ER α has been found to increase the transcription of the p53 gene within MCF7 breast cancer cells lines, both through a ligand dependent binding to an ERE in the p53 promoter (40) and through a calmodulin kinase IV mediated activation of NF-kB/CTF-1 transcription complex (41). P53 and its downstream mediator p21 have both been found to be upregulated with estradiol treatment within RhOSE ovarian cells as well (42). Furthermore, a polymorphism of p73, itself a homolog of p53 involved in the apoptosis of germ cells, has been found to be positively associated with increased ER positive human ovarian cancer development; this study surveyed epithelial, germ cell, and sex cord stromal cell tumors from human patients (43). However, ER α also plays an inhibitory role in regard to p53 regulated repression of survivin and MDR-1 (44) as well as p53 activation of ATF3, BTG2, and TRAF4 (45) as seen in **Figure 2**.

Wild type, non-mutated, p53 has been shown to increase the transcription of ER (46). In epithelial ovarian cancer, a mutated p53 protein that stabilizes wild type p53 was shown to increase levels of the ESR1 gene (47). It is hypothesized that the increased ESR1 expression is due to increased p53 mediated transcription of the ESR1 gene itself (47). However, owing to its anti-proliferative mechanism, a lack of p53 in mouse ovarian surface epithelial tumor cells correlated with estradiol mediated upregulation of growth and tumor invasion through upregulation of the estrogen receptor ESR1 (48). P53 and the estrogen receptor have a complex relationship, each increasing the transcription of the other while simultaneously inhibiting the transcriptional activity of that same molecule, preventing its downstream effects. A similar paradox can be seen in type 2 diabetes mellitus induced insulin resistance, in which high glucose levels drives increased insulin secretion but this subsequently results in resistance to insulin's effects.

Non-genomic activities of ER

Non genomic activities include those in which the estrogen receptor interacts with proteins other than transcription factors and do not immediately affect the genome. Ultimately many of these pathways lead to genomic alterations in the form of upregulation/downregulation of various genes, but begin independently of the genome. **Table 3** details some of the therapies currently being studied, targeting

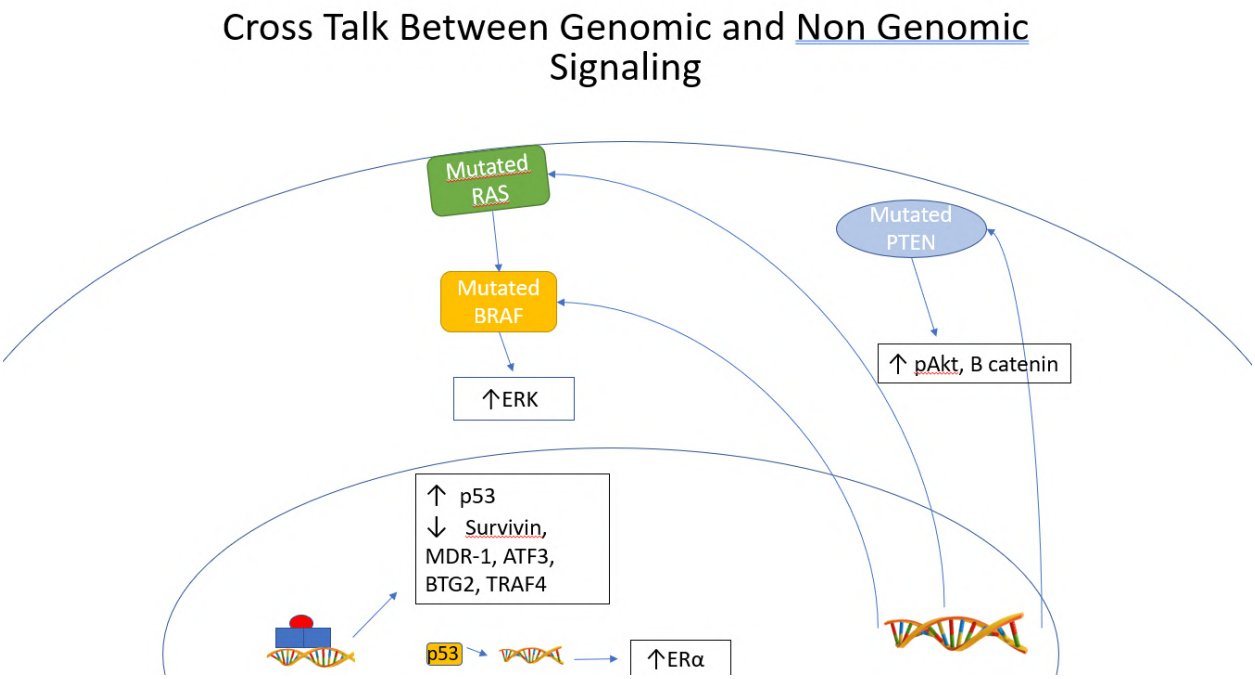


Figure 2: ***Mutations of the PTEN, RAS, and BRAF proteins can be found in multiple subtypes of ovarian carcinoma.*** *PTEN down regulation/mutation leads to increased signaling in the PI3K pathway while mutations in RAS/BRAF lead to upregulation of the MAPK pathway. P53 exhibits a complex relationship with the estrogen receptor, increasing transcription of ERα, while ERα increases transcription of p53 with altered transcription of many p53 related genes.*

these pathways.

MAPK pathway and ER

Mutations in both RAS and BRAF are present in higher frequency in low grade serous and mucinous ovarian carcinomas, upregulating the MAPK pathway, illustrated in **Figure 2** (49, 50). Rap1A, a RAS associated protein, has been found to increase cell proliferation, migration, and invasion in human HEYA8 ovarian cancer cells. This carcinogenic activity appeared to be mediated by induced expression of MAPK pathways constituents, MEK1 and 2, as well as ERK 1 and 2, illustrated in **Figure 3** (51). Furthermore, high amounts of phosphorylated/active MAPK have been observed in high grade serous carcinomas, corresponding to a worse survival rate (52). This increased level of MAPK correlated with a higher level of EGFR as well (52). Inhibition of the MAPK pathway, through the use of Aloesin (53) and delphinidin (54), has actually been shown to decrease the growth of SKOV3 ovarian cancer cells. The inhibition of Src kinase, which acts as a key regulator of both the MAPK and EGFR mediated pathways, has been investigated as well. Src was found to be highly expressed in ovarian cancer cell lines activated by the estrogen/ER complex (55). Subsequently, inhibition of Src inhibited cancer growth and reduced levels of c-myc expression within PEO1R and BG-1 ovarian cancer cells (55) seen in **Figure 3**.

EGFR/Her2 and ER

Her2/neu is a growth factor receptor commonly associated with carcinogenesis and has been shown to bind membrane associated ER, modulating its ligand responsive properties (56, 57). Her2 interacts with the MAPK pathway and has been shown to activate both nuclear ER and its coactivator AIB1 in order to increase ER mediated transcription (58). Therefore, the

Table 3. Summary of Treatments Targeting Non-Genomic Pathways

Agent	Mechanism of action	Studies Performed	References
Trastuzumab	Her2 receptor targeting antibody	Phase II study - 41 patients with primary peritoneal/recurrent or refractory ovarian carcinoma; 11.4% overexpression of Her2; response rate low at 7%	Bookman, 83
Trastuzumab/Pertuzumab	Both Her2 receptor targeting antibodies	Case report - high grade serous epithelial carcinoma stage IV; focal amplification of Her2 gene found; treated with combination trastuzumab/pertuzumab with persistent partial response of 37 months	Thouvenin, 84
Pertuzumab	Her2 receptor targeting antibody	Phase III study - 156 patients with platinum resistant ovarian carcinoma randomized to topotecan, gemcitabine, or paclitaxel monotherapy followed by adjuvant pertuzumab; no significant improvement in PFS/overall survival	Lorusso, 85
Gefitinib	EGFR targeting antibody	Phase Ib/II study - 19 patients with platinum resistant ovarian carcinoma, expressing EGFR, treated with topotecan and gefitinib; 16% had stable disease, 11% had partial response	Chelariu-Raicu, 86
Cetuximab	EGFR targeting antibody	Phase II study - 25 patients selected for single agent treatment with cetuximab; 1 patient achieved partial response, 9 achieved stable disease	Schilder, 87
Trametinib	MEK inhibitor, targeting the MAPK pathway	Phase II/III study - 260 patients with low grade serous carcinoma selected; 101 progression free survival events in treatment group vs 116 in control group; median progression free survival 13 months in treatment group vs 7.2 months in control	Gershenson, 88

presence of Her2/neu in various carcinomas may enhance estrogen's role in carcinogenesis and tumor progression. High levels of Her2 have been reported in ovarian cancer, which reportedly caused increased stimulation of both MAPK and PI3K pathways. However, when inhibited by pertuzumab (a monoclonal anti-Her2 dimerization antibody), Her2 and ER α mediated activity is inhibited (59). Furthermore, EGFR has been found to be more highly expressed in ovarian cancer tissue when compared to normal ovarian tissue, with a 56.8% difference between the two groups (60). Higher EGFR expression in malignant ovarian tissue corresponds to a worse survival rate, owing to the anti-apoptotic effects of EGFR (61). As shown in **Figure 3**, EGFR gain of function mutations were found to promote increased phosphorylation of both Akt and ERK in ovarian cancer (62), ERK playing a role in the estrogen responsive MAPK pathway already mentioned above.

PI3K and ER

Lower grade endometrioid as well as clear cell carcinomas of the ovary showed higher rates of PTEN and PI3K pathway mutations, leading to an upregulation of this pathway and carcinogenesis (49, 39). In cases of low grade carcinoma, CTNNB1 (16-38% of cases) and PTEN (14-21% of cases) mutations result in increased activity of the

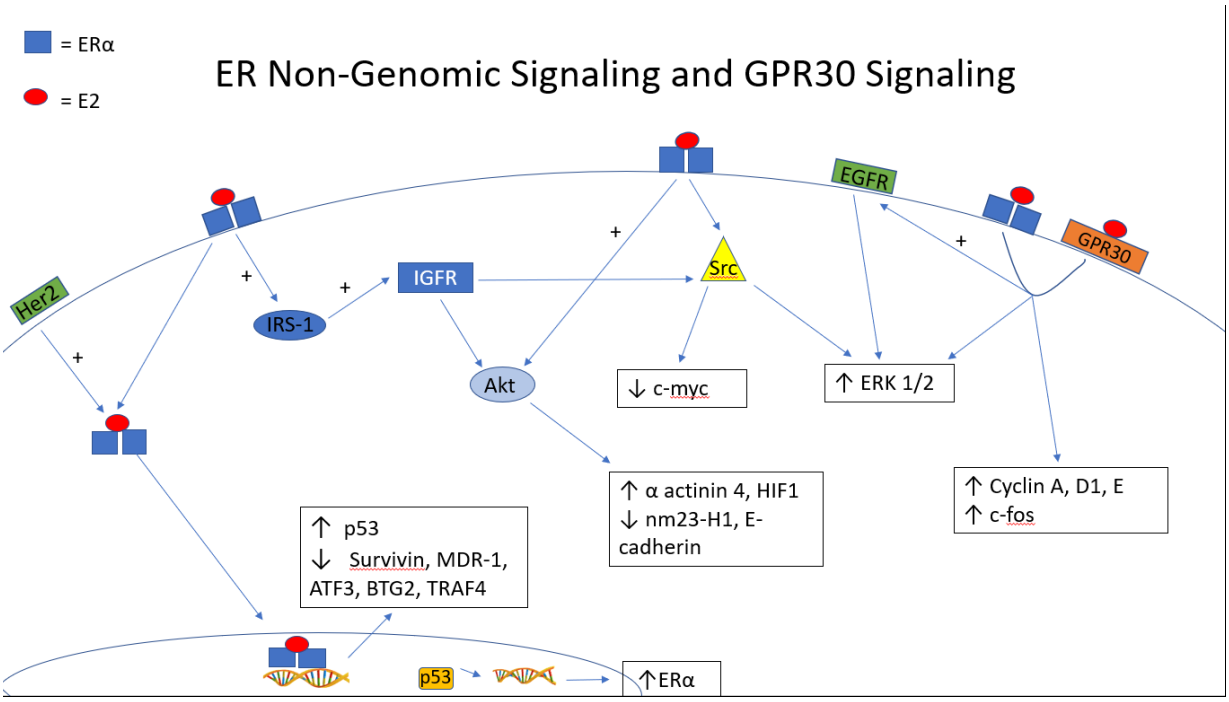


Figure 3: ***Estrogen receptor α (ERα) is involved in ovarian tumorigenesis and cellular proliferation through multiple non-genomic signaling pathways.*** *Intranuclear effects of ERα lead to the upregulation of p53 as well as downregulation of survivin, multi-drug resistance gene 1 (MDR-1), activating transcription factor 3 (ATF3), B-cell translocation gene 2 (BTG2), and tumor necrosis factor [TNF] receptor-associated factor 4 (TRAF4). This process is enhanced via human epidermal growth factor receptor 2 (Her2) activity at the cell membrane. ERα directly increases activity of Akt (protein kinase B) and steroid hormone co-receptor (Src), and exerts similar action indirectly through the insulin receptor substrate/insulin-like growth factor receptor (IRS-1/IGFR) pathway. Downstream, Akt upregulates α-actinin 4 and hypoxia inducible factor 1 (HIF1), and downregulates the tumor suppressor gene, nm23-H1, and E-cadherin. Src upregulates both c-myc and extracellular signal related kinases 1 and 2 (ERK1/2). Furthermore, the G protein coupled receptor 30 (GPR30)/ERα co-dependent mechanism leads to cell proliferation via upregulation of c-fos and cyclins (A, D1, and E) as well as the epidermal growth factor receptor/extracellular related kinase (EGFR/ERK) pathway. Lastly, intranuclear p53 acts on DNA upregulating the production of ERα.*

B-catenin protein, causing unregulated cell proliferation through the PI3K cascade illustrated in **Figure 2**. However, in cases of high grade endometrioid carcinoma, mutations in P53 were found to be more prevalent (60% of cases), ultimately leading to unregulated DNA replication and cell proliferation (39). Shown in **Figure 3**, downstream of PI3K, Akt/pAkt and mTOR/pmTOR were shown to be phosphorylated in 55% of ovarian cancer tissue regulating transcription of Bcl-2 and survivin (63, 64). In ES-2 and SKOV-3 ovarian cancer cells, estradiol was found to reduce the expression of the nm23-H1 tumor suppressor gene (65). This inhibition of nm23-H1 expression was found to be mediated by pAkt, pointing to a PI3K/Akt mediated pathway in ovarian carcinogenesis (65). Furthermore, α-actinin 4, a metastatic promoter, and E-cadherin, a tumor suppressor gene, are both regulated by the PI3K pathway (fig 3). In SKOV3 cells, positive for both the α and β receptor, treatment with estradiol caused an increase in pAkt and increased α-actinin 4 expression, with concurrent decreased E-cadherin expression and increase in growth and migration (66). Another tumorigenic promoter, HIF-1α, expressed under low oxygen conditions, was found to be elevated post-estradiol treatment in ES-2 and SKOV3 cells, promoting cell proliferation. This elevation was found to be dependent on

estradiol's activation of Akt (67).

IGF/IGFR and ER

IGF is involved in normal function and development of many tissues including the ovary. Given its role in steroidogenesis and overall cell growth, it has been thought that IGF-I and IGF-II, as well as their receptors and the related 6 IGF binding proteins (IGFBP), might play a role in carcinogenesis. IGF-I has been found to facilitate proliferation, invasion and angiogenesis in tumor cells and IGF-II has been shown to mediate cell adhesion and invasion in ovarian cancer (68). IGFBPs play an inhibitory role in normal physiologic development, acting as a trap for IGF-I and IGF-II (69), but in ovarian cancer, a downregulation of IGFBP3 and 5 with a concurrent increase in IGFBP4 (70) and IGFBP2 (71) levels has been observed. The mechanism behind this selective regulation of IGF binding proteins is unknown but has been suggested to be a result of ER α influence (72). Furthermore, as shown in **Figure 3**, the IGF-I receptor has been found to upregulate both the MAPK and PI3K pathways mentioned above (73-76), as well as downstream constituents Akt and ERK1 and ERK2 (68). This signaling generally occurs through the phosphorylation of the insulin receptor substrate-1 (IRS-1), which has been shown to be activated by the estradiol/ER α complex (73).

GPR30 Receptor

The G protein coupled receptor is referred to as either GPR30 or GPER1 and both labels will be used interchangeably in the subsequent section. The ligands to which GPR30 responds include estradiol, G-1 (G protein coupled receptor agonist), as well as tamoxifen and other estrogen agonists (77). GPR30 has been found to localize primarily in 2 locations: the plasma membrane and the endoplasmic reticulum (78, 77), with estrogen responsivity at both locations. Ultimately, GPR30 exerts its effects in ovarian cancer through calcium mobilization (77) and EGFR transactivation with subsequent MAPK activation (79,80). GPR30 expression is higher in ovarian carcinomas, as opposed to benign and borderline malignancies, with its highest expression in serous, endometrioid and mucinous tumors (80). In BG-1 ovarian cancer cells, EGFR/ERK activation was found to be dependent on both ER α and GPR30, effectively promoting cell proliferation (79). Furthermore, estradiol was found to upregulate cyclins A, E, and D1 as well as the c-fos gene, through this same ER α/GPR30 mechanism, further promoting proliferation and cell cycle progression (79), shown in **Figure 3**. GPR30/EGFR co-expression in ovarian carcinomas has been shown to correlate with worse survival rates in these tumors (80). Paradoxically, GPER1 has been found to be highly expressed in granulosa cell tumors and was found to be associated with reduced migration and invasion in these tumors specifically. This reduced migratory/invasive property was shown to be mediated by GPER1 and repression of ERK1 and ERK2 (81).

CONCLUSION

Estrogen has been linked to breast, endometrial, and ovarian cancer development. Its involvement in the pathogenesis of ovarian cancer is complicated, given the presence of ER α and β, as well as GPR30 and the individual mechanisms by which each acts. ER-αhas been shown to be primarily carcinogenic while ER-β is a known negative regulator of carcinogenesis; GPR30 is primarily carcinogenic but there is evidence to suggest it could be a negative regulator of the cell cycle as well in ovarian tissue. Furthermore, these receptors interact with the p53, MAPK, EGFR/Her2, PI3K, and IGF/IGFR pathways, with unique interactions with each pathway.

Estrogen and its effects in ovarian cancer are still poorly characterized, particularly regarding these molecular signaling pathways, an area of research that may yield unique insights into treatment of this disease.

DISCLOSURES

Conflicts of interest: None.
Availability of data and material: Not applicable.
Code availability: Not applicable.
Authors' contributions: Authors listed in the manuscript have contributed per submission guidelines and standards for authorship.
Ethics approval: Not applicable.
Consent to participate: Not applicable.

REFERENCES

1. Siegel, R.L., Miller, K.D. and Jemal, A. (2020). Cancer statistics, 2020. CA: a cancer journal for clinicians, 70(1), 7–30.
2. Ehmann T, Barel S, Xu G, et al. (2019). A comparative analysis of estrogenic pathways between ovarian and cervical cancers. Proceedings of the LECOM Inter-Professional Research Day (Erie, PA), A7.
3. Orr, B. and Edwards, R.P. (2018). Diagnosis and treatment of ovarian cancer. Hematology/Oncology Clinics, 32(6), 943-964.
4. Howlader N, Noone AM, Krapcho M, et al. (2020). SEER Cancer Statistics Review, 1975-2017 [Online]. Available from: https://seer.cancer.gov/archive/csr/1975_2017/. [Accessed Aug 1 2021]
5. Simpkins, F., Garcia-Soto, A. and Slingerland, J. (2013). New insights on the role of hormonal therapy in ovarian cancer. Steroids, 78(6), 530-537.
6. Flouriot, G., Brand, H., Denger, S., et al. (2000). Identification of a new isoform of the human estrogen receptor-alpha (hER-α) that is encoded by distinct transcripts and that is able to repress hER-α activation function 1. The EMBO journal, 19(17), 4688-4700.
7. Shi, L., Dong, B., Li, Z., et al. (2009). Expression of ER-α36, a novel variant of estrogen receptor α, and resistance to tamoxifen treatment in breast cancer. Journal of clinical Oncology, 27(21), 3423.
8. Sieh, W., Köbel, M., Longacre, T.A., et al. (2013). Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. The lancet oncology, 14(9), 853-862.
9. Pujol, P., Rey, J.M., Nirde, P., et al. (1998). Differential expression of estrogen receptor-α and-β messenger RNAs as a potential marker of ovarian carcinogenesis. Cancer research, 58(23), 5367-5373.
10. O'Donnell, A.J., Macleod, K.G., Burns, D.J., et al. (2005). Estrogen receptor-α mediates gene expression changes and growth response in ovarian cancer cells exposed to estrogen. Endocrine-related cancer, 12(4), 851-866.
11. Langdon, S.P., Hawkes, M.M., Lawrie, S.S., et al. (1990). Oestrogen receptor expression and the effects of oestrogen and tamoxifen on the growth of human ovarian carcinoma cell lines. British Journal of Cancer, 62(2), 213-216.
12. Voutsadakis, I.A. (2016). Hormone receptors in serous ovarian carcinoma: prognosis, pathogenesis, and treatment considerations. Clinical Medicine Insights: Oncology, 10, CMO-S32813.
13. Herynk, M.H. and Fuqua, S.A. (2004). Estrogen receptor mutations in human disease. Endocrine reviews, 25(6), 869-898.
14. Bossard, C., Busson, M., Vindrieux, D., et al. (2012). Potential Role of Estrogen Receptor Beta as a Tumor Suppressor of Epithelial Ovarian Cancer. PLoS ONE, 7(9), e44787.
15. Treeck, O., Pfeiler, G., Mitter, D., et al. (2007). Estrogen receptor β1 exerts antitumoral effects on SK-OV-3 ovarian cancer cells. Journal of Endocrinology, 193(3), 421-433.
16. Prossnitz, E.R. and Barton, M. (2014). Estrogen biology: new insights into GPER function and clinical opportunities. Molecular and cellular endocrinology, 389(1-2), 71-83.
17. Albanito, L., Madeo, A., Lappano, R., et al. (2007). G protein–coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17β-estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. Cancer research, 67(4), 1859-1866.
18. Long, L., Cao, Y. and Tang, L.D. (2012). Transmembrane estrogen receptor GPR30 is more frequently expressed in malignant than benign ovarian endometriotic cysts and correlates with MMP-9 expression. International Journal of Gynecologic Cancer, 22(4).
19. Smith, H.O., Arias-Pulido, H., Kuo, D.Y., et al. (2009). GPR30 predicts poor survival for ovarian cancer. Gynecologic oncology, 114(3), 465-471.
20. Heublein, S., Mayr, D., Friese, K., et al. (2014). The G-protein-coupled estrogen receptor (GPER/GPR30) in ovarian granulosa cell tumors. International journal of molecular sciences, 15(9),

15161-15172.
21. Kolkova, Z., Casslén, V., Henic, E., et al. (2012). The G protein-coupled estrogen receptor 1 (GPER/GPR30) does not predict survival in patients with ovarian cancer. Journal of ovarian research, 5(1), 1-11.
22. Ignatov, T., Modl, S., Thulig, M., et al. (2013). GPER-1 acts as a tumor suppressor in ovarian cancer. Journal of ovarian research, 6(1), 1-10.
23. Katzenellenbogen, B.S., (1996). Estrogen receptors: bioactivities and interactions with cell signaling pathways. Biology of reproduction, 54(2), 287-293.
24. O'Malley, B.W. (2005). A life-long search for the molecular pathways of steroid hormone action. Molecular Endocrinology, 19(6), 1402-1411.
25. Weigel, N.L. (1996). Steroid hormone receptors and their regulation by phosphorylation. Biochemical Journal, 319(3), 657-667.
26. Pietras, R.J. and Márquez-Garbán, D.C. (2007). Membrane-associated estrogen receptor signaling pathways in human cancers. Clinical Cancer Research, 13(16), 4672-4676.
27. Razandi, M., Pedram, A., Greene, G.L. et al. (1999). Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERα and ERβ expressed in Chinese hamster ovary cells. Molecular endocrinology, 13(2), 307-319.
28. Bjornstrom, L. and Sjoberg, M. (2005). Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Molecular endocrinology, 19(4), 833-842.
29. Hall, J.M. and Korach, K.S. (2003). Stromal cell-derived factor 1, a novel target of estrogen receptor action, mediates the mitogenic effects of estradiol in ovarian and breast cancer cells. Molecular Endocrinology, 17(5), 792-803.
30. Webb, P., Nguyen, P., Valentine, C., et al. (1999). The estrogen receptor enhances AP-1 activity by two distinct mechanisms with different requirements for receptor transactivation functions. Molecular Endocrinology, 13(10), 1672-1685.
31. Anzick, S.L., Kononen, J., Walker, R.L., et al. (1997). AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science, 277(5328), 965-968.
32. Liang, M. and Zhao, J. (2018). Protein expressions of AIB1, p53 and Bcl-2 in epithelial ovarian cancer and their correlations with the clinical pathological features and prognosis. Eur Rev Med Pharmacol Sci, 22(16), 5134-5139.
33. Palmieri, C., Gojis, O., Rudraraju, B., et al. (2013). Expression of steroid receptor coactivator 3 in ovarian epithelial cancer is a poor prognostic factor and a marker for platinum resistance. British journal of cancer, 108(10), 2039-2044.
34. Jakacka, M., Ito, M., Weiss, J, et al. (2001). Estrogen receptor binding to DNA is not required for its activity through the nonclassical AP1 pathway. Journal of Biological Chemistry, 276(17), 13615-13621.
35. Sasaki, H., Hayakawa, J., Terai, Y., et al. (2008). Difference between genomic actions of estrogen versus raloxifene in human ovarian cancer cell lines. Oncogene, 27(19), 2737-2745.
36. Hein, S., Mahner, S., Kanowski, C., et al. (2009). Expression of Jun and Fos proteins in ovarian tumors of different malignant potential and in ovarian cancer cell lines. Oncology reports, 22(1), 177-183.
37. Bardin, A., Moll, F., Margueron, R., et al. (2005). Transcriptional and posttranscriptional regulation of fibulin-1 by estrogens leads to differential induction of messenger ribonucleic acid variants in ovarian and breast cancer cells. Endocrinology, 146(2), 760-768.
38. Cotran, R., Kumar, V. and Robbins, S. (2015). Pathologic basis of disease. Philadelphia, PA: Saunders Elsevier.
39. Cho, K.R. and Shih, I.M. (2009). Ovarian cancer. Annual review of pathology: mechanisms of disease, 4, 287-313.
40. Berger, C.E., Qian, Y., Liu, G., et al. (2012). p53, a target of estrogen receptor (ER) α, modulates DNA damage-induced growth suppression in ER-positive breast cancer cells. Journal of Biological Chemistry, 287(36), 30117-30127.
41. Qin, C., Nguyen, T., Stewart, J., et al. (2002). Estrogen up-regulation of p53 gene expression in MCF-7 breast cancer cells is mediated by calmodulin kinase IV-dependent activation of a nuclear factor κB/CCAAT-binding transcription factor-1 complex. Molecular Endocrinology, 16(8), 1793-1809.
42. Wright, J.W., Stouffer, R.L. and Rodland, K.D. (2005). High-dose estrogen and clinical selective estrogen receptor modulators induce growth arrest, p21, and p53 in primate ovarian surface epithelial cells. The Journal of Clinical Endocrinology & Metabolism, 90(6), 3688-3695.
43. Guan, X., Zhang, N., Yin, Y., et al. (2012). Polymorphisms in the p63 and p73 genes are associated with ovarian cancer risk and clinicopathological variables. Journal of Experimental & Clinical Cancer Research, 31(1), 1-8.
44. Sayeed, A., Konduri, S.D., Liu, W., et al. (2007). Estrogen receptor α inhibits p53-mediated transcriptional repression: implications for the

regulation of apoptosis. Cancer research, 67(16), 7746-7755.
45. Bailey, S.T., Shin, H., Westerling, T., et al. (2012). Estrogen receptor prevents p53-dependent apoptosis in breast cancer. Proceedings of the National Academy of Sciences, 109(44), 18060-18065.
46. Berger, C., Qian, Y. and Chen, X. (2013). The p53-estrogen receptor loop in cancer. Current molecular medicine, 13(8), 1229-1240.
47. Ren, Y.A., Mullany, L.K., Liu, Z., et al. (2016). Mutant p53 promotes epithelial ovarian cancer by regulating tumor differentiation, metastasis, and responsiveness to steroid hormones. Cancer research, 76(8), 2206-2218.
48. Mullany, L.K., Liu, Z., Wong, K.K., et al. (2014). Tumor repressor protein 53 and steroid hormones provide a new paradigm for ovarian cancer metastases. Molecular Endocrinology, 28(1), 127-137.
49. Rosen, D.G., Yang, G., Liu, G., et al. (2009). Ovarian cancer: pathology, biology, and disease models. Frontiers in bioscience: a journal and virtual library, 14, 2089.
50. Ho, C.L., Kurman, R.J., Dehari, R., et al. (2004). Mutations of BRAF and KRAS precede the development of ovarian serous borderline tumors. Cancer research, 64(19), 6915-6918.
51. Lu, L., Wang, J., Wu, Y., et al. (2016). Rap1A promotes ovarian cancer metastasis via activation of ERK/p38 and notch signaling. Cancer medicine, 5(12), 3544-3554.
52. Hew, K.E., Miller, P.C., El-Ashry, D., et al. (2016). MAPK activation predicts poor outcome and the MEK inhibitor, selumetinib, reverses antiestrogen resistance in ER-positive high-grade serous ovarian cancer. Clinical Cancer Research, 22(4), 935-947.
53. Zhang, L.Q., Lv, R.W., Qu, X.D., et al. (2017). Aloesin suppresses cell growth and metastasis in ovarian cancer SKOV3 cells through the inhibition of the MAPK signaling pathway. Analytical Cellular Pathology, 2017, 8158254.
54. Lim, W. and Song, G. (2017). Inhibitory effects of delphinidin on the proliferation of ovarian cancer cells via PI3K/AKT and ERK 1/2 MAPK signal transduction. Oncology Letters, 14(1), 810-818.
55. Simpkins, F., Hevia-Paez, P., Sun, J., et al. (2012). Src Inhibition with saracatinib reverses fulvestrant resistance in ER-positive ovarian cancer models in vitro and in vivo. Clinical cancer research, 18(21), 5911-5923.
56. Chung, Y.L., Sheu, M.L., Yang, S.C., et al. (2002). Resistance to tamoxifen-induced apoptosis is associated with direct interaction between Her2/neu and cell membrane estrogen receptor in breast cancer. International journal of cancer, 97(3), 306-312.
57. Arpino, G., Wiechmann, L., Osborne, C.K. et al. (2008). Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance. Endocrine reviews, 29(2), 217-233.
58. Osborne, C.K., Bardou, V., Hopp, T.A., et al. (2003). Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. Journal of the National Cancer Institute, 95(5), 353-361.
59. Mullen, P., Cameron, D.A., Hasmann, M., et al. (2007). Sensitivity to pertuzumab (2C4) in ovarian cancer models: cross-talk with estrogen receptor signaling. Molecular cancer therapeutics, 6(1), 93-100.
60. Ranjbar, R., Nejatollahi, F., Ahmadi, A.S.N., et al. (2015). Expression of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) in patients with serous ovarian carcinoma and their clinical significance. Iranian journal of cancer prevention, 8(4).
61. Psyrri, A., Kassar, M., Yu, Z., et al. (2005). Effect of epidermal growth factor receptor expression level on survival in patients with epithelial ovarian cancer. Clinical Cancer Research, 11(24), 8637-8643.
62. Tanaka, Y., Terai, Y., Tanabe, A., et al. (2011). Prognostic effect of epidermal growth factor receptor gene mutations and the aberrant phosphorylation of Akt and ERK in ovarian cancer. Cancer biology & therapy, 11(1), 50-57.
63. Altomare, D.A., Wang, H.Q., Skele, K.L., et al. (2004). AKT and mTOR phosphorylation is frequently detected in ovarian cancer and can be targeted to disrupt ovarian tumor cell growth. Oncogene, 23(34), 5853-5857.
64. Cheaib, B., Auguste, A. and Leary, A. (2015). The PI3K/Akt/mTOR pathway in ovarian cancer: therapeutic opportunities and challenges. Chinese journal of cancer, 34(1), 4-16.
65. Hua, K., Feng, W., Cao, Q., et al. (2008). Estrogen and progestin regulate metastasis through the PI3K/AKT pathway in human ovarian cancer. International journal of oncology, 33(5), 959-967.
66. Lu, Z., Zhang, Y., Yan, X., et al. (2014). Estrogen stimulates the invasion of ovarian cancer cells via activation of the PI3K/AKT pathway and regulation of its downstream targets E-cadherin and α-actinin-4. Molecular Medicine Reports, 10(5), 2433-2440.
67. Hua, K., Din, J., Cao, Q., et al. (2009). Estrogen and progestin regulate HIF-1α expression in

ovarian cancer cell lines via the activation of Akt signaling transduction pathway. Oncology reports, 21(4), 893-898.
68. Beauchamp, M.C., Yasmeen, A., Knafo, A. et al. (2010). Targeting insulin and insulin-like growth factor pathways in epithelial ovarian cancer. Journal of oncology, 2010, 257058.
69. Foulstone, E., Prince, S., Zaccheo, O., et al. (2005). Insulin-like growth factor ligands, receptors, and binding proteins in cancer. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 205(2), 145-153.
70. Walker, G., MacLeod, K., Williams, A.R., et al. (2007). Insulin-like growth factor binding proteins IGFBP3, IGFBP4, and IGFBP5 predict endocrine responsiveness in patients with ovarian cancer. Clinical Cancer Research, 13(5), 1438-1444.
71. Flyvbjerg, A., Mogensen, O., Mogensen, B., et al. (1997). Elevated serum insulin-like growth factor-binding protein 2 (IGFBP-2) and decreased IGFBP-3 in epithelial ovarian cancer: correlation with cancer antigen 125 and tumor-associated trypsin inhibitor. The Journal of Clinical Endocrinology & Metabolism, 82(7), 2308-2313.
72. Lu, L., Katsaros, D., Wiley, A., et al. (2006). The relationship of insulin-like growth factor-II, insulin-like growth factor binding protein-3, and estrogen receptor-alpha expression to disease progression in epithelial ovarian cancer. Clinical Cancer Research, 12(4), 1208-1214.
73. Kahlert, S., Nuedling, S., Van Eickels, M., et al. (2000). Estrogen receptor α rapidly activates the IGF-1 receptor pathway. Journal of Biological Chemistry, 275(24), 18447-18453.
74. Chen, J., Zhao, K.N., Li, R., et al. (2014). Activation of PI3K/Akt/mTOR pathway and dual inhibitors of PI3K and mTOR in endometrial cancer. Current medicinal chemistry, 21(26), 3070-3080.
75. Fukushima, T., Nakamura, Y., Yamanaka, D., et al. (2012). Phosphatidylinositol 3-kinase (PI3K) activity bound to insulin-like growth factor-I (IGF-I) receptor, which is continuously sustained by IGF-I stimulation, is required for IGF-I-induced cell proliferation. Journal of Biological Chemistry, 287(35), 29713-29721.
76. Cao, Z., Liu, L.Z., Dixon, D.A., et al. (2007). Insulin-like growth factor-I induces cyclooxygenase-2 expression via PI3K, MAPK and PKC signaling pathways in human ovarian cancer cells. Cellular signalling, 19(7), 1542-1553.
77. Qian, H., Xuan, J., Liu, Y. et al. (2016). Function of G-protein-coupled estrogen receptor-1 in reproductive system tumors. Journal of immunology research, 2016, 7128702.
78. Revankar, C.M., Cimino, D.F., Sklar, L.A., et al. (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science, 307(5715), 1625-1630.
79. Albanito, L., Madeo, A., Lappano, R., et al. (2007). G protein–coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17β-estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. Cancer research, 67(4), 1859-1866.
80. Fujiwara, S., Terai, Y., Kawaguchi, H., et al. (2012). GPR30 regulates the EGFR-Akt cascade and predicts lower survival in patients with ovarian cancer. Journal of ovarian research, 5(1), 1-10.
81. François, C.M., Wargnier, R., Petit, F., et al. (2015). 17β-estradiol inhibits spreading of metastatic cells from granulosa cell tumors through a non-genomic mechanism involving GPER1. Carcinogenesis, 36(5), 564-573.
82. Schüler-Toprak, S., Weber, F., Skrzypczak, M., et al. (2018). Estrogen receptor β is associated with expression of cancer associated genes and survival in ovarian cancer. BMc cancer, 18(1), 1-9.
83. Bookman, M.A., Darcy, K.M., Clarke-Pearson, D., et al. (2003). Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the Gynecologic Oncology Group. Journal of clinical oncology, 21(2), 283-290.
84. Thouvenin, L., Charrier, M., Clement, S., et al. (2021). Ovarian cancer with high-level focal ERBB2 amplification responds to trastuzumab and pertuzumab. Gynecologic Oncology Reports, 37, 100787.
85. Lorusso, D., Hilpert, F., Martin, A.G., et al. (2019). Patient-reported outcomes and final overall survival results from the randomized phase 3 PENELOPE trial evaluating pertuzumab in low tumor human epidermal growth factor receptor 3 (HER3) mRNA-expressing platinum-resistant ovarian cancer. International Journal of Gynecologic Cancer, 29(7).
86. Chelariu-Raicu, A., Levenback, C.F., Slomovitz, B.M., et al. (2020). Phase Ib/II study of weekly topotecan and daily gefitinib in patients with platinum resistant ovarian, peritoneal, or fallopian tube cancer. International Journal of Gynecologic Cancer, 30(11).
87. Schilder, R.J., Pathak, H.B., Lokshin, A.E., et al. (2009). Phase II trial of single agent cetuximab in

patients with persistent or recurrent epithelial ovarian or primary peritoneal carcinoma with the potential for dose escalation to rash. Gynecologic oncology, 113(1), 21-27.

88. Gershenson, D.M., Miller, A., Brady, W.E., et al. (2022). Trametinib versus standard of care in patients with recurrent low-grade serous ovarian cancer (GOG 281/LOGS): an international, randomised, open-label, multicentre, phase 2/3 trial. The Lancet, 399(10324), 541-553.
89. He, Y., Alejo, S., Venkata, P.P., Johnson, J.D., Loeffel, I., Pratap, U.P., Zou, Y., Lai, Z., Tekmal, R.R., Kost, E.R. and Sareddy, G.R. (2022). Therapeutic targeting of ovarian Cancer stem cells using estrogen receptor Beta agonist. International journal of molecular sciences, 23(13), 7159.
90. Banerjee, A., Cai, S., Xie, G., Li, N., Bai, X., Lavudi, K., Wang, K., Zhang, X., Zhang, J., Patnaik, S. and Backes, F.J. (2022). A Novel Estrogen Receptor β Agonist Diminishes Ovarian Cancer Stem Cells via Suppressing the Epithelial-to-Mesenchymal Transition. Cancers, 14(9), 2311.

HMSR

Connect with HMSR!

@HMSReview

Executive Board

Editor-in-Chief: @AryaRao_
Executive Director: @KaushikVenkates
Managing Editors: Nico Poux and @MercyMazurek
Production Directors: Daniel Kim and @Annadromeda_

Read more at hmsreview.org