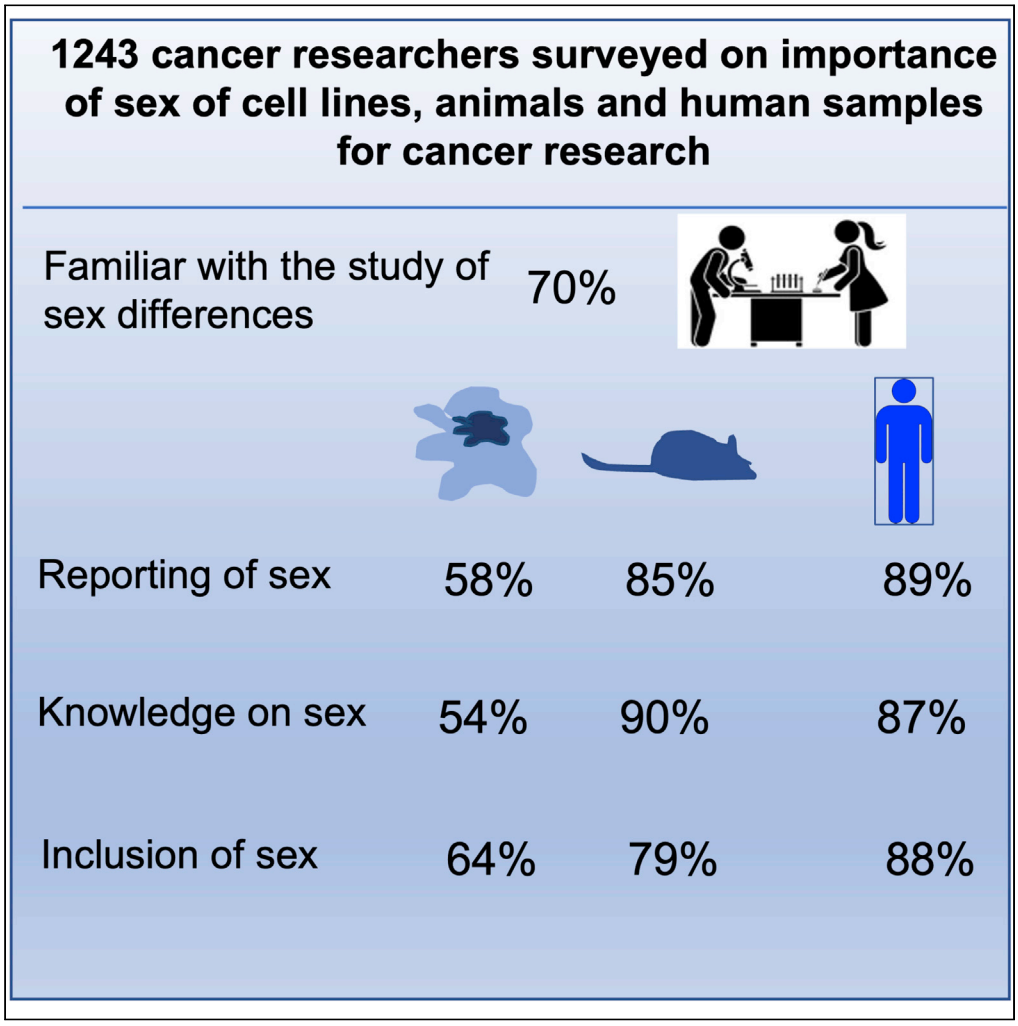
iScience

CellPress
OPEN ACCESS

Article

Cancer researchers' perceptions of the importance of the sex of cell lines, animals, and human samples for cancer biology research



Berna C. Özdemir, Anke Richters, Cristina Espinosa da Silva, Alison May Berner

berna.oezdemir@insel.ch

Highlights

Most cancer researchers agree on importance of including both sexes in experiments

Compared to animals and human samples, sex of cell lines is deemed less important

Nearly half of the researchers do not know the sex of the cell lines they use

Researchers disagree that sex differences should be studied in all tumor types

Özdemir et al., iScience 26, 106212
April 21, 2023
https://doi.org/10.1016/j.isci.2023.106212

iScience

CellPress
OPEN ACCESS

Article

Cancer researchers' perceptions of the importance of the sex of cell lines, animals, and human samples for cancer biology research

Berna C. Özdemir,[1,8,*] Anke Richters,[2,3] Cristina Espinosa da Silva,[4,5] and Alison May Berner[6,7]

SUMMARY

Sex differences in cancer risk and outcome are currently a topic of major interest in clinical oncology. It is however unknown to what extent cancer researchers consider sex as a biological variable for their research. We conducted an international survey among 1243 academic cancer researchers and collected both quantitative and qualitative data. Although most of the participants indicated that they were familiar with the concept of studying sex differences in cancer biology, they did not think it was important to investigate sex differences in every context of cancer research nor in all tumor types. This is in stark contrast to the current recommendations and guidelines and illustrates the need for increased awareness among cancer researchers regarding the potential impact of the sex of cell lines, animals, and human samples in their studies.

INTRODUCTION

Sex disparities in cancer are well documented.[1] While males are more susceptible to cancer and have lower survival rates, females are at higher risk of experiencing toxicities across various anticancer therapies.[2] Both sex and gender affect cancer risk and outcomes. Sex refers to the categorization at birth as male or female based on biological features (e.g., reproductive organs, sex chromosomes, and hormones). Gender, on the other hand, refers to the perception of a person throughout their life (e.g., a woman or man) based on socially constructed roles and behaviors. In scientific literature, "sex" and "gender" are often incorrectly used as synonyms.[3] Sex is typically considered binary, although some of the features used to classify it may exist along a spectrum or be discordant, e.g., high androgen levels in females with congenital adrenal hyperplasia, resulting in masculine external genitalia. Gender is increasingly considered a non-binary, continuous concept, and this may not align with a person's sex. Essentially, while all cells and animal have a sex, each person has both a sex and a gender.

There is growing interest among oncologists to investigate sex and gender differences in order to improve patient outcomes.[4] However, it remains unknown to what extent cancer researchers are familiar with the study of sex differences in cancer biology and whether they include sex as a biological variable in their research. To address this knowledge gap, we conducted an international online survey among cancer researchers and collected both quantitative and qualitative data.

RESULTS

A total of 1243 researchers completed the survey. Half of the participants were identified as women (54%) and 58% were 31–50 years old (Table 1). Nearly two-thirds of the researchers worked in Europe (65%) followed by North America (22%) and Asia (9%). Most participants had a PhD degree (70.7%), 24% had a medical degree (MD/MBBS-Chir), and 39% had worked with patients. Years of research experience was evenly distributed across categories, with 20% of the participants reporting 0–5, 6–10, 11–15, or over 25 years of experience, respectively. With regard to studied cancer type, the majority of the researchers investigated gastrointestinal tumors (upper GI and colorectal, 49%) followed by breast cancer (34%), hematological malignancies (26%), and skin (23%) and lung cancers (21%). Overall, 44% studied sex-specific cancers.

[1]Department of Medical Oncology, Inselspital Bern, Bern University Hospital, University of Bern, Bern, Switzerland

[2]The Netherlands Comprehensive Cancer Organisation, Department of Research and Development, Utrecht, The Netherlands

[3]Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, The Netherlands

[4]Herbert Wertheim School of Public Health and Human Longevity Science, University of California, San Diego, La Jolla, CA, USA

[5]Division of Epidemiology and Biostatistics, School of Public Health, San Diego State University, San Diego, CA, USA

[6]Barts Cancer Institute, Queen Mary University of London, London, UK

[7]Gender Identity Clinic, Tavistock & Portman NHS Foundation Trust, London, UK

[8]Lead contact

*Correspondence: berna.oezdemir@insel.ch

https://doi.org/10.1016/j.isci.2023.106212

Table 1. Characteristics of respondents overall and by gender, n (%)

	All		Women		Men	
Gender						
Women	676	54%	676	100%	–	–
Men	548	44%	–	–	548	100%
Other/prefer not to say	17	1%	–	–	–	–
Unknown	2	0%	–	–	–	–
Age						
≤30	233	19%	161	24%	71	13%
31–40	430	35%	242	36%	180	33%
41–50	297	24%	151	22%	140	26%
51–60	164	13%	77	11%	85	16%
≥61	111	9%	40	6%	70	13%
Unknown	8	1%	5	1%	2	0%
Continent (work)						
Europe	806	65%	462	68%	332	61%
North America	272	22%	130	19%	136	25%
South America	10	1%	5	1%	5	1%
Asia	114	9%	59	9%	54	10%
Pacific	19	2%	10	1%	9	2%
Africa	11	1%	4	1%	7	1%
Unknown	11	1%	6	1%	5	1%
Continent (origin)						
Europe	737	59%	420	62%	308	56%
North America	131	11%	61	9%	66	12%
South America	31	2%	15	2%	16	3%
Asia	236	19%	122	18%	112	20%
Pacific	21	2%	10	1%	11	2%
Africa	23	2%	12	2%	11	2%
Unknown	64	5%	36	5%	24	4%
Worked with patients						
No	754	61%	433	64%	310	57%
Yes	487	39%	242	36%	237	43%
Unknown	2	0%	1	0%	1	0%
Degree						
PhD	880	71%	444	66%	422	77%
MD/MBBS-Chir	298	24%	128	19%	166	30%
Experience as researcher						
0–5 years	261	21%	178	26%	79	14%
6–10 years	248	20%	132	20%	111	20%
11–15 years	232	19%	132	20%	98	18%
16–20 years	159	13%	79	12%	75	14%
21–25 years	112	9%	60	9%	50	9%
25 + years	229	18%	93	14%	135	25%
Unknown	2	0%	2	0%	–	–

(*Continued on next page*)

Table 1. *Continued*

	All		Women		Men	
Works on sex-specific cancer						
No	694	56%	370	55%	313	57%
Yes	550	44%	307	45%	235	43%
Works on cancer type (non-exclusive categories)						
Breast cancer[a]	425	34%	231	34%	187	34%
Gynecologic cancer (ovarian, endometrial)[a]	153	12%	90	13%	62	11%
Male genital cancer (prostate, testicular, penile)[a]	157	13%	81	12%	74	14%
Urinary tract cancer	99	8%	44	7%	53	10%
Upper GI cancer	285	23%	139	21%	137	25%
Colorectal cancer	321	26%	141	21%	171	31%
Lung cancer	255	21%	106	16%	144	26%
Skin cancer	281	23%	152	22%	126	23%
Head and neck cancer	134	11%	61	9%	71	13%
Central nervous system cancer	182	15%	90	13%	91	17%
Haematologic cancer	324	26%	158	23%	159	29%
Other cancers	39	3%	24	4%	15	3%
Field of work (non-exclusive categories)						
Tumor genetics/genomics	446	36%	206	30%	235	43%
Tumor epigenetics	237	19%	121	18%	108	20%
Tumor transcriptomics	323	26%	140	21%	176	32%
Tumor biology	633	51%	332	49%	288	53%
Tumor microenvironment	520	42%	288	43%	223	41%
Tumor angiogenesis	88	7%	46	7%	40	7%
Tumor immunology	476	38%	271	40%	197	36%
Signaling pathways	439	35%	233	34%	205	37%
Drug metabolism	113	9%	65	10%	47	9%
Infectious agents	49	4%	22	3%	27	5%
Other	205	16%	115	17%	88	16%

Percentages may not sum to 100 due to rounding.
[a]sex-specific cancer types.

Most researchers agreed that sex-inclusive research was important, but fewer agreed that including cell lines of both sexes in experiments or reporting the sex of cell lines was as important as in animal or human samples

In terms of our quantitative findings, 70% indicated that they were familiar with the concept of studying sex differences in cancer biology and a majority agreed that experiments should be performed whenever possible with cell lines (62%), animals (81%), and human samples (90%) of both sexes (Figure 1). Most responded that scientific journals should request the reporting of the sex of cell lines (58%), animals (85%), and human samples (89%) (Figures 1A and 1B). Responses did not notably differ by gender. The findings were comparable between researchers who studied sex-specific cancers and those who studied non-sex-specific cancers.

Half of the researchers did not know the sex of the cell lines used in their research

Nearly two-thirds (62%) of the participants indicated that the sex of the cell lines, animals, or human samples was an important consideration in their research. Most participants were aware of the sex of the animals (90%) and human samples (87%) used in their research, but fewer were knowledgeable about the sex of the cell lines (54%) in their experiments. Interestingly, 36% of the cancer researchers in our study felt that

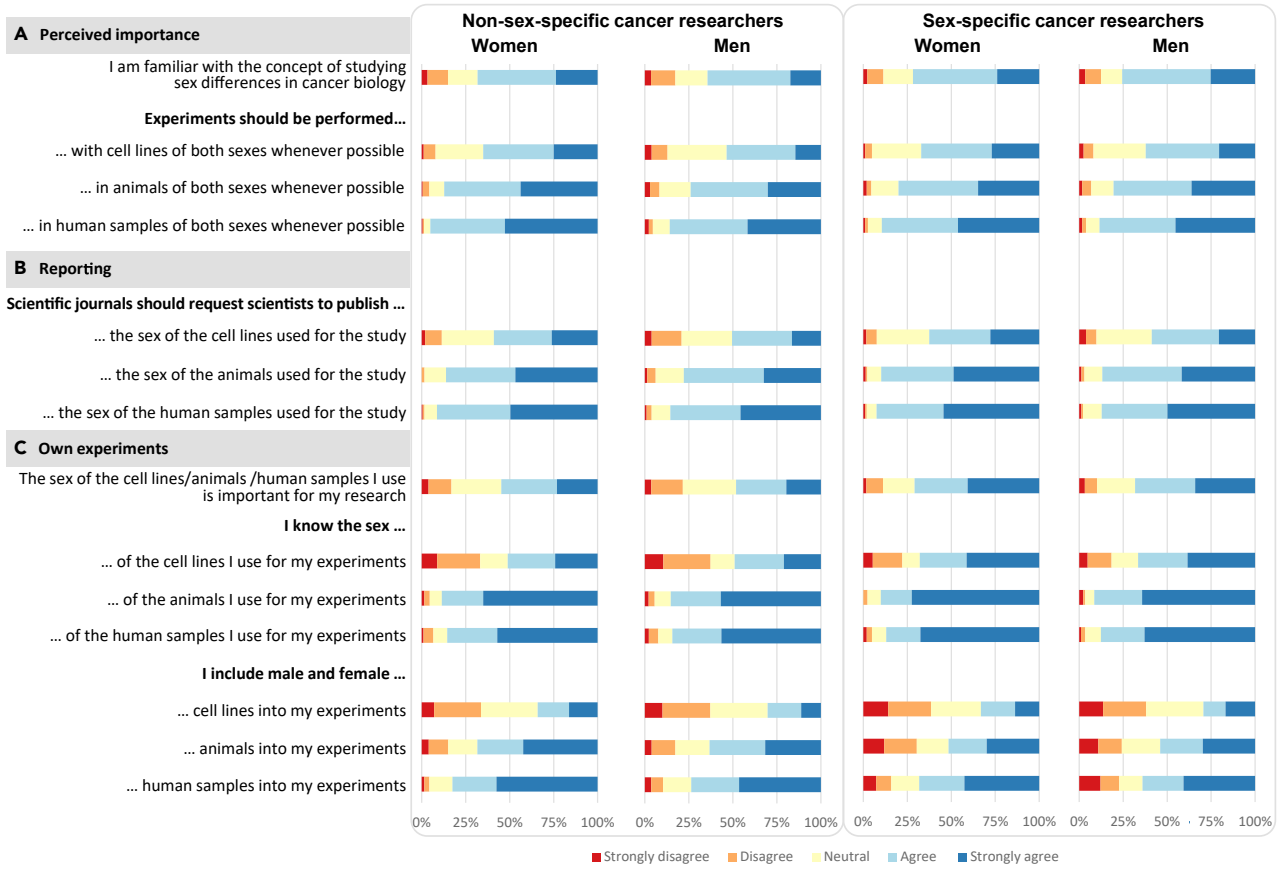


Figure 1. Respondent agreement with statements by gender and subgroups working on sex-specific cancers or non-sex-specific cancers

(A) The perceived importance of sex-inclusive research in general.

(B) The perceived importance of reporting the sex of cell lines, animals, and human samples in cancer research.

(C) The perceived importance of sex-inclusive experiments for the respondent's research overall. The results are stratified by gender and working on sex-specific cancers or focusing on non-sex-specific cancers.

including cell lines of both sexes in their research was unnecessary, as compared to 21% for animals and 12% for human samples of both sexes (Figure 1C).

Researchers disagreed that sex differences should be studied in all tumor types and indicated several barriers to sex-inclusive research

In addition to data from Likert-scaled questions, the survey collected 175 free-text comments with content suitable for thematic analysis. This resulted in 72 codes and 4 themes identified from the data (Figure 2). In our qualitative thematic analysis, researchers felt that it was not important to study sex differences in every context of cancer research and that it did not need to be considered in all tumor types (Figure 2). Some thought that sex differences were only relevant if related to the research question. Some participants indicated that cancers should be studied for effect heterogeneity when published evidence already demonstrated the importance of sex differences while others noted the importance of studying sex in tumors where sex hormones play a role or where there was a clear skew in prevalence by sex. It was frequently commented that sex (or in some cases gender) was not relevant for cancers of reproductive organs or breast. Participants also indicated that the study of sex differences was differentially relevant depending on the field of study (with methylation, immunology, and translational and drug studies highlighted as relevant fields for sex disparity research). Views on whether the study of both sexes was important depended on the mode of study (cell lines, animals, or human samples) and were often contrasting. However, multiple reasons were given as to why sex differences should not be studied in cell lines, including that they are too genetically altered for it to be important but also that they are so well characterized that sex is not an important consideration.

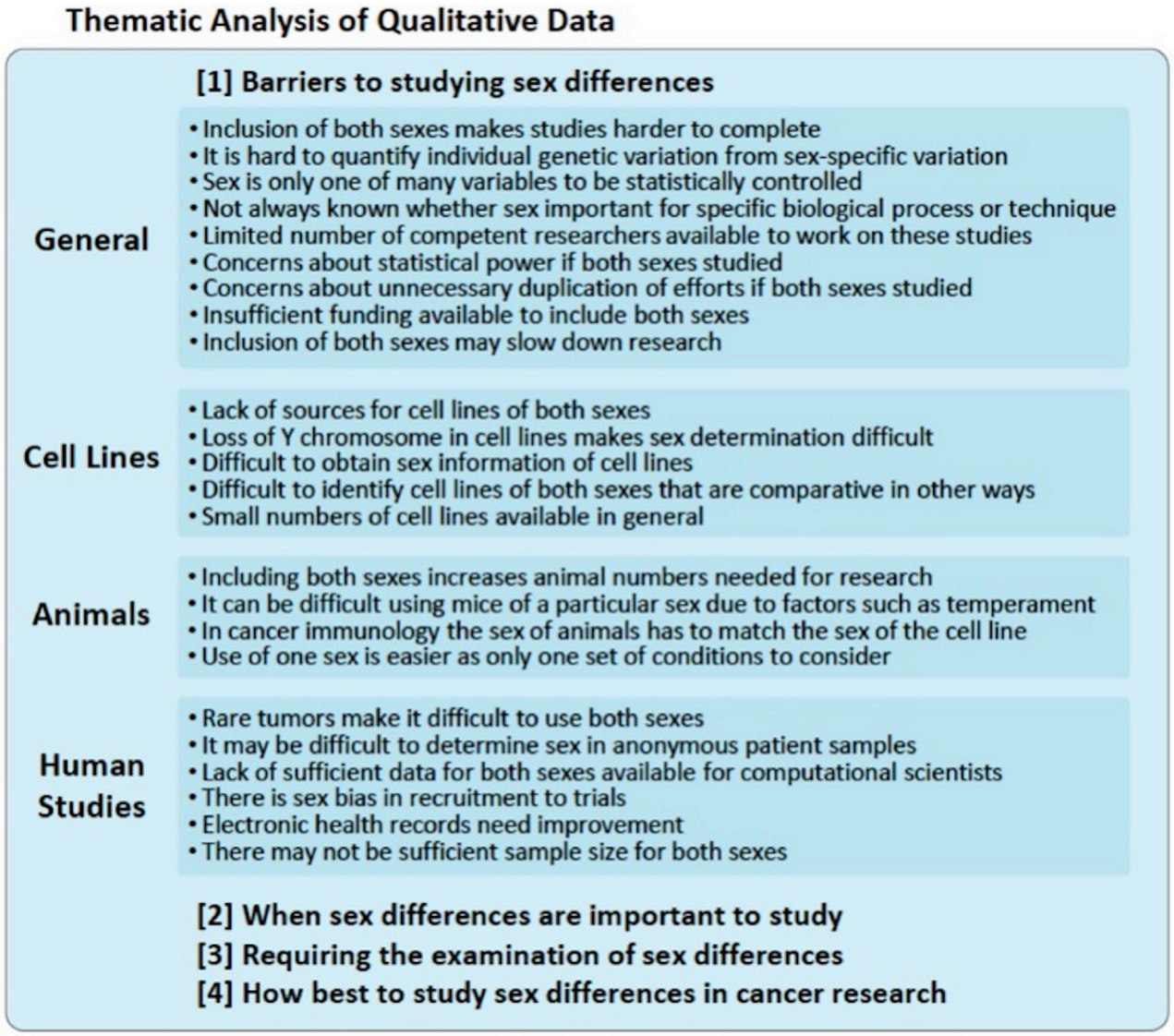


Figure 2. A thematic analysis of 175 free-text comments provided by respondents
Theme 1 had 4 subthemes which are illustrated with examples.

There were four subthemes related to barriers to studying sex differences, general barriers, and barriers to study in cell lines, animals, and human samples/data, respectively (Figure 2).

Researchers had contrasting views about mandating the inclusion of both sexes in cancer biology research studies

Participants had strong and contrasting opinions about mandating the inclusion of both sexes in cancer research studies. Some suggested that it should be, or already was, required by research institutions, funders (for proposals), and journals (via editorial policies). However, other participants felt that such policies were challenging due to the multiple barriers noted previously and that instead, studying sex differences within cancer research should be incentivized or rewarded rather than required.

Researchers proposed strategies to best study sex differences in cancer

Along with multiple comments noting the importance of studying sex differences in cancer, several participants made recommendations around how to best undertake this endeavor. Some participants discussed

the complex nature of biological sex beyond a strict binary and suggested that both hormone levels and chromosomal data should be collected in studies. In addition, the importance of collecting gender as well as biological sex in human studies was noted, along with the relevance of both of these factors in applying findings to the transgender community. While one participant felt that the collection of sex information should be mandatory in public data, another suggested that the unbiased assessment of sex differences might be improved if researchers are blinded to the sex of research subjects.

Several participants also commented that sex is only one variable to be controlled for in cancer research studies and therefore studies needed to be appropriately powered and have appropriate comparison groups.

DISCUSSION

Our quantitative and qualitative results illustrate the need for increased awareness among cancer researchers regarding the potential impact of the sex of cell lines, animals, and human samples in their studies. Our findings provide important insights, including a lack of knowledge of already published data. For instance, some participants indicated that including cell lines of both sexes was unnecessary because cell lines are highly genetically altered. However, evidence exists that the sex of the cancer cell lines[5] and the type of cell culture media (e.g., the concentration of bovine or fetal calf serum, containing growth factors and the concentration of phenol red, which is an estrogenic compound)[6] affect the results of *in vitro* experiments. In a genome-wide CRISPR-based screen on a large number of female and male cancer cell lines, 178 differentially essential genes were found to depend on either the biological sex or the sex chromosomes of the cell line.[7] Given that drug discovery relies on high-throughput screens, potential sex differences in responsiveness of different cells can also become clinically relevant as shown by the higher cytotoxicity detected in male-derived cell lines in a study of 81 antineoplastic compounds.[8] Similarly, important sex differences exist in cancer models, such as the syngeneic B16-F10/BL6 melanoma model which develops significantly slower in female mice possibly due to their stronger immune responses.[9] There is also increasing evidence showing an impact of the sex on gene regulatory networks, resulting in sex differences in activity and connectivity of genes (termed the "sexome").[10] A prominent example of sex-biased gene regulation is TP53, one of the most important tumor suppressor genes. The lower cancer incidence and better outcomes among females could be partially explained by the high number of X-linked genes regulating p53 expression, the silencing of mutated p53-associated X-linked genes in females, and the higher TP53 mutation frequency in males.[11] If most of the transcriptomic research to date in cancer is based on pooled expression data from both male and female samples, this calls into question the validity of many of the results, and applicability to both sexes.

There are also important sex differences in various cellular mechanisms that are fundamental for carcinogenesis which may partly explain the higher cancer risk observed among males. For instance, exposure to ionizing radiation (e.g., atomic bomb survivors)[12] and alkylating agents (e.g., cancer survivors)[13] or germline mutations in some DNA mismatch repair genes (e.g., Lynch syndrome) are associated with higher cancer rates in males compared to females.[14] These sex-related disparities in cancer risk may be linked to the regulation of the p53 axis as well as DNA repair mechanisms by androgen and estrogen receptor signaling.[15] Sex disparities in metabolic activity (e.g., glycolysis rate), expression, and activity of key enzymes are also well described and may contribute to the sex differences in cancer predisposition[16] when considered with the differential activity of signaling pathways (e.g., PI3K/mTOR), mitochondrial activity, and ROS regulation underlying different thresholds for malignant transformation in male and female cells. These data are reported in more detail elsewhere.[16,17]

Most researchers in our survey agreed that the inclusion of animals of both sexes is important in cancer biology research. In a pivotal analysis of articles published in high-impact journals across ten biomedical disciplines, Beery and Zucker illustrated the extent of male bias in research and the lack of sex-based analyses in studies including both sexes.[18] This analysis was repeated and showed an increase from 28% in 2009 to 48% in 2019 in studies including both male and female subjects.[19]

In a national survey of US scientists who use vertebrate animals for their research, Waltz et al. found that women were more likely to report the sex of animals used in their research.[20] Also, an analysis of over 1.5 million medical research publications has shown female gender of the first or last author to be significantly associated with a higher likelihood of a medical study including sex and gender analysis,[21]

suggesting a greater interest and/or awareness of women on sex and gender aspects. In contrast to these results, in our study, we did not observe notable differences in responses by gender (Figure S1).

The Institute of Medicine Committee on Understanding the Biology of Sex and Gender Differences concluded two decades ago that "sex matters" and recommended conducting research on sex at the cellular level to determine the effects of sex chromosome-linked genes on cells, organs, and ultimately on organisms.[22] To address the overreliance on male animals in biomedical research and the underrepresentation of women in clinical trials, the National Institutes of Health (NIH) issued a policy in 2015 requiring that all NIH-funded research consider "sex as a biological variable" for their study design, all clinical trials include women and minorities, and phase III trials be designed and powered to facilitate stratified analyses by sex/gender.[23] Furthermore, the Sex and Gender Equity in Research 2016 Guidelines state that studies should "be designed and conducted in a way that can reveal sex-related differences in the results, even if these were not initially expected."[24] Despite these initiatives and the agreement of the scientists on the importance of this topic, the implementation of sex as a biological variable lags in research practice. An analysis of investigational review board protocols submitted at a major research institution suggests that very few investigators (2%) are in fact considering sex or gender as important variables in their clinical research at the stage of protocol development.[25] Moreover, the lower importance attributed to the reporting of the sex of cell lines compared to that of animals and human samples in our study is comparable to trends in other analyses where the sex of the cell lines was reported in only 50% of the experiments.[26]

The qualitative data from our study revealed that participants felt that the study of sex differences is not important for all aspects of cancer research and in all tumor types and should be studied instead in tumor types with existing evidence for sex differences and in tumors where sex hormones play a role. This view is surprising and contrasts with evidence of the importance of sex in cancer research as well as the current recommendations and guidelines. An ever-increasing number of studies have found significant sex bias in gene expression and the mutation profile of various cancer types[27,28] and that sex hormone signaling (e.g., androgen and/or estrogen receptor) also plays an important role in non-sex-related malignancies such as melanoma, lung cancer, or bladder cancer.[29] Also, in our view, it is not justified to study only cancer types with an already known sex difference, since "absence of evidence is not evidence of absence"[30] and only the unbiased consideration of potential sex differences will move forward the study of sex and gender differences.

Given the clinically relevant differences in treatment toxicity (e.g., 30%–50% higher risk of adverse events among female patients from all types of anticancer treatments[31]), sex should be routinely included in the design of pre-clinical and clinical studies and the results should be analyzed by sex. Early assessment of potential sex differences in exposure, efficacy, and toxicity during drug development is required to improve the outcome of patients with cancer. Indeed, statistically significant differences in pharmacokinetics (e.g., higher plasma levels in females) have been described for 20% of the investigated cytotoxic drugs.[32] In addition, sex differences in body composition and tumor biology are well known and might affect the drug sensitivity of normal and cancer cells as well as dose-response and dose-toxicity relationships.[33] However, currently only half of oncology clinical trials report results by sex. When sex-specific subgroup analyses are reported, they are of insufficient methodological quality which limits drawing any meaningful conclusions for clinical practice.[34] This type of reductionist, biology-centered approach does not allow to identify the dynamic interactions between the individual and their environment and the resulting contextual gendered practices.[35]

Furthermore, some researchers recognized that there may be considerable variation in the attributes typically used to classify sex (e.g., hormones and sex chromosome compliment) and that sex assigned at birth may differ from gender in human subjects.

Considering that all these attributes can have implications for cancer risk factors, disease prognosis, and drug metabolism, appropriate reporting is particularly important for transgender populations and those with variations in sex characteristics. Among transgender people, there is a profound heterogeneity in the degree to which people will adopt gendered behaviors, take exogenous sex hormones, and pursue gender-affirming surgeries that affect their biology. Although there was a previous lack of clinical data, researchers are now beginning to uncover trends in cancer incidence and mortality among the transgender population.[36] Some of these trends (e.g., lower incidence of prostate cancer in transgender women

compared to cisgender men,[37] and the lower incidence of breast cancer in transgender men compared to cisgender women[38]) are likely related to use of gender-affirming hormones, and specifically mastectomies among transgender men. Other trends require greater exploration of both behavior and biology, such as the elevated odds of being diagnosed with advanced stage lung cancer or poorer survival outcomes after being diagnosed with bladder cancer among transgender patients compared to cisgender patients.[36] Interdisciplinary research involving concepts of social sciences will help to improve our understanding of the complex construct of gender and its impact on health and disease.[35]

Interestingly, several researchers seemed to consider sex a variable to be controlled for in studies. However, controlling for sex in an analysis may not be appropriate in all cases and should be justified.[39,40] While we acknowledge that an effect modifier such as sex could also be a confounder, potential effect heterogeneity by sex should be investigated and revealed rather than controlled for in regression models.

In fact, the evidence that we have in part described as well as current guidelines suggest that sex should *always* be a consideration in cancer research where that cancer can affect both sexes. The first question for researchers should be whether there is clear evidence to show a lack of difference between sexes in the aspect of cancer or process studied. If not, then the research should be designed in a way that such a difference can be detected. Depending on the area of study, adjustment for sex alone may be insufficient, as it may mask entirely different underlying biological processes between the sexes. Of course, this represents an ideal scenario, and resources such as funding and sample availability may not make this type of design possible. In this case, researchers need to clearly acknowledge this limitation in their manuscripts in order to justify future analyses and avoid over-generalization to both sexes. SAGER guidelines suggest researchers to discuss the rationale for lack of sex and gender analyses, but we suggest discussion of resource limitations in addition.[24]

In line with this, participants in our survey shared concerns about mandating the inclusion of both sexes and sex-based reporting in their research due to funding and resource concerns. While it is often assumed that the sample size should be doubled to examine findings among both sexes, it is not required for exploratory analyses where researchers can examine the magnitude of effects and corresponding 95% confidence intervals (instead of focusing on the result's statistical significance given a 0.05 significance level).[41]

Given this finding, funding agencies and institutional review boards could have an influential role in promoting sex-inclusive research.

The progress and success of clinical practice in oncology strongly relies on basic research. Sex as a critical modulator of cellular functions deserves more attention. Cancer researchers should be better supported and trained to perform sex-inclusive research and reporting. Increased awareness on the impact of sex-related factors is needed to increase the reporting of patient characteristics, treatment, toxicity, and survival data stratified by sex. This could help to ultimately identify gender-related factors contributing to survival disparities and to improve care for all patients.

Limitations of the study

The convenience sampling used in our study may limit the generalizability of our findings, especially since most of our participants worked in Europe and were of European origin. However, this sampling method allowed us to recruit a large sample of participants from multiple countries, and our findings can provide valuable insights on which future studies can build. Also, most participants researched common cancer types and their views may not reflect the opinions of the researchers working on rare cancer types (e.g., sarcoma or neuroendocrine tumors).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106212.

ACKNOWLEDGMENTS

We thank all the researchers for their participation in the survey. We thank Dr Dennis Hoch for his assistance in collecting the contact information of the researchers.

AUTHOR CONTRIBUTIONS

B.C.Ö. conceived the study, developed the questionnaire, collected, and analyzed data, and wrote the manuscript; A.R. analyzed data; C.E.D.S. wrote the manuscript; A.M.B. developed the questionnaire, analyzed data, and wrote the manuscript. All authors reviewed, edited, and approved the final version of the article.

DECLARATION OF INTERESTS

The authors do not have any competing interests to declare in relation to this work.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

Received: November 9, 2022
Revised: December 13, 2022
Accepted: February 13, 2023
Published: February 16, 2023

REFERENCES

1. Haupt, S., Caramia, F., Klein, S.L., Rubin, J.B., and Haupt, Y. (2021). Sex disparities matter in cancer development and therapy. Nat. Rev. Cancer *21*, 393–407. https://doi.org/10.1038/s41568-021-00348-y.

2. Özdemir, B.C., Csajka, C., Dotto, G.P., and Wagner, A.D. (2018). Sex differences in efficacy and toxicity of systemic treatments: an undervalued issue in the era of precision oncology. J. Clin. Oncol. *36*, 2680–2683. https://doi.org/10.1200/JCO.2018.78.3290.

3. Gogovor, A., Zomahoun, H.T.V., Ekanmian, G., Adisso, É.L., Deom Tardif, A., Khadhraoui, L., Rheault, N., Moher, D., and Légaré, F. (2021). Sex and gender considerations in reporting guidelines for health research: a systematic review. Biol. Sex Differ. *12*, 62. https://doi.org/10.1186/s13293-021-00404-0.

4. Özdemir, B.C., Oertelt-Prigione, S., Adjei, A.A., Borchmann, S., Haanen, J.B., Letsch, A., Mir, O., Quaas, A., Verhoeven, R.H.A., and Wagner, A.D. (2022). Investigation of sex and gender differences in oncology gains momentum: ESMO announces the launch of a Gender Medicine Task Force. Ann. Oncol. *33*, 126–128. https://doi.org/10.1016/j.annonc.2021.11.011.

5. Kim, S.Y., Lee, S., Lee, E., Lim, H., Shin, J.Y., Jung, J., Kim, S.G., and Moon, A. (2019). Sex-biased differences in the correlation between epithelial-to-mesenchymal transition-associated genes in cancer cell lines. Oncol. Lett. *18*, 6852–6868. https://doi.org/10.3892/ol.2019.11016.

6. de Souza Santos, R., Frank, A.P., Palmer, B.F., and Clegg, D.J. (2018). Sex and media: considerations for cell culture studies. ALTEX *35*, 435–440. https://doi.org/10.14573/altex.1806151.

7. Shohat, S., Vol, E., and Shifman, S. (2022). Gene essentiality in cancer cell lines is modified by the sex chromosomes. Genome Res. *32*, 1993–2002. https://doi.org/10.1101/gr.276488.121.

8. Nunes, L.M., Robles-Escajeda, E., Santiago-Vazquez, Y., Ortega, N.M., Lema, C., Muro, A., Almodovar, G., Das, U., Das, S., Dimmock, J.R., et al. (2014). The gender of cell lines matters when screening for novel anti-cancer drugs. AAPS J. *16*, 872–874. https://doi.org/10.1208/s12248-014-9617-4.

9. Dakup, P.P., Porter, K.I., Little, A.A., Zhang, H., and Gaddameedhi, S. (2020). Sex differences in the association between tumor growth and T cell response in a melanoma mouse model. Cancer Immunol. Immunother. *69*, 2157–2162. https://doi.org/10.1007/s00262-020-02643-3.

10. Arnold, A.P., and Lusis, A.J. (2012). Understanding the sexome: measuring and reporting sex differences in gene systems. Endocrinology *153*, 2551–2555. https://doi.org/10.1210/en.2011-2134.

11. Haupt, S., Caramia, F., Herschtal, A., Soussi, T., Lozano, G., Chen, H., Liang, H., Speed, T.P., and Haupt, Y. (2019). Identification of cancer sex-disparity in the functional integrity of p53 and its X chromosome network. Nat. Commun. *10*, 5385. https://doi.org/10.1038/s41467-019-13266-3.

12. Grant, E.J., Brenner, A., Sugiyama, H., Sakata, R., Sadakane, A., Utada, M., Cahoon, E.K., Milder, C.M., Soda, M., Cullings, H.M., et al. (2017). Solid cancer incidence among the life span study of atomic bomb survivors: 1958–2009. Radiat. Res. *187*, 513–537. https://doi.org/10.1667/RR14492.1.

13. R. Curtis, and D.M. Freedman, eds. (2006). New Malignancies Among Cancer Survivors: SEER Cancer Registries (National Cancer Institute). 1973–2000.

14. Dominguez-Valentin, M., Sampson, J.R., Seppälä, T.T., ten Broeke, S.W., Plazzer, J.-P., Nakken, S., Engel, C., Aretz, S., Jenkins, M.A., Sunde, L., et al. (2020). Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the prospective lynch syndrome

database. Genet. Med. *22*, 15–25. https://doi.org/10.1038/s41436-019-0596-9.

15. Schiewer, M.J., and Knudsen, K.E. (2016). Linking DNA damage and hormone signaling pathways in cancer. Trends Endocrinol. Metabol. *27*, 216–225. https://doi.org/10.1016/j.tem.2016.02.004.

16. Rubin, J.B., Lagas, J.S., Broestl, L., Sponagel, J., Rockwell, N., Rhee, G., Rosen, S.F., Chen, S., Klein, R.S., Imoukhuede, P., and Luo, J. (2020). Sex differences in cancer mechanisms. Biol. Sex Differ. *11*, 17. https://doi.org/10.1186/s13293-020-00291-x.

17. Rubin, J.B. (2022). The spectrum of sex differences in cancer. Trends Cancer *8*, 303–315. https://doi.org/10.1016/j.trecan.2022.01.013.

18. Beery, A.K., and Zucker, I. (2011). Sex bias in neuroscience and biomedical research. Neurosci. Biobehav. Rev. *35*, 565–572. https://doi.org/10.1016/j.neubiorev.2010.07.002.

19. Woitowich, N.C., Beery, A., and Woodruff, T. (2020). A 10-year follow-up study of sex inclusion in the biological sciences. Elife *9*, e56344. https://doi.org/10.7554/eLife.56344.

20. Waltz, M., Saylor, K.W., Fisher, J.A., and Walker, R.L. (2021). Biomedical researchers' perceptions of the NIH's sex as a biological variable policy for animal research: results from a U.S. National survey. J. Womens Health *30*, 1395–1405. https://doi.org/10.1089/jwh.2020.8997.

21. Nielsen, M.W., Andersen, J.P., Schiebinger, L., and Schneider, J.W. (2017). One and a half million medical papers reveal a link between author gender and attention to gender and sex analysis. Nat. Human Behav. *1*, 791–796. https://doi.org/10.1038/s41562-017-0235-x.

22. T.M. Wizeman, and M.-L. Pardue, eds. (2001). Exploring the Biological Contributions to Human Health (National Academies Press). https://doi.org/10.17226/10028.

23. NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research (2001.

24. Heidari, S., Babor, T.F., de Castro, P., Tort, S., and Curno, M. (2016). Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use. Res. Integr. Peer Rev. *1*, 2. https://doi.org/10.1186/s41073-016-0007-6.

25. Freeman, A., Stanko, P., Berkowitz, L.N., Parnell, N., Zuppe, A., Bale, T.L., Ziolek, T., and Epperson, C.N. (2017). Inclusion of sex and gender in biomedical research: survey of clinical research proposed at the University of Pennsylvania. Biol. Sex Differ. *8*, 22. https://doi.org/10.1186/s13293-017-0139-5.

26. Kim, J.Y., Min, K., Paik, H.Y., and Lee, S.K. (2021). Sex omission and male bias are still widespread in cell experiments. Am. J. Physiol. Cell Physiol. *320*, C742–C749. https://doi.org/10.1152/ajpcell.00358.2020.

27. Yuan, Y., Liu, L., Chen, H., Wang, Y., Xu, Y., Mao, H., Li, J., Mills, G.B., Shu, Y., Li, L., and Liang, H. (2016). Comprehensive characterization of molecular differences in cancer between male and female patients. Cancer Cell *29*, 711–722. https://doi.org/10.1016/j.ccell.2016.04.001.

28. Lopes-Ramos, C.M., Chen, C.Y., Kuijjer, M.L., Paulson, J.N., Sonawane, A.R., Fagny, M., Platig, J., Glass, K., Quackenbush, J., and DeMeo, D.L. (2020). Sex differences in gene expression and regulatory networks across 29 human tissues. Cell Rep. *31*, 107795. https://doi.org/10.1016/j.celrep.2020.107795.

29. Schweizer, M.T., and Yu, E.Y. (2017). AR-Signaling in human malignancies: prostate cancer and beyond. Cancers *9*, 7. https://doi.org/10.3390/cancers9010007.

30. Altman, D.G., and Bland, J.M. (1995). Statistics notes: absence of evidence is not evidence of absence. BMJ *311*, 485. https://doi.org/10.1136/bmj.311.7003.485.

31. Unger, J.M., Vaidya, R., Albain, K.S., LeBlanc, M., Minasian, L.M., Gotay, C.C., Henry, N.L., Fisch, M.J., Lee, S.M., Blanke, C.D., and Hershman, D.L. (2022). Sex differences in risk of severe adverse events in patients receiving immunotherapy, targeted therapy, or chemotherapy in cancer clinical trials. J. Clin. Oncol. *40*, 1474–1486. https://doi.org/10.1200/JCO.21.02377.

32. Wagner, A.D., Oertelt-Prigione, S., Adjei, A., Buclin, T., Cristina, V., Csajka, C., Coukos, G., Dafni, U., Dotto, G.P., Ducreux, M., et al. (2019). Gender medicine and oncology: report and consensus of an ESMO Workshop. Ann. Oncol. *30*, 1914–1924. https://doi.org/10.1093/annonc/mdz414.

33. Özdemir, B.C., Gerard, C.L., and Espinosa da Silva, C. (2022). Sex and gender differences in anticancer treatment toxicity: a call for revisiting drug dosing in oncology. Endocrinology *163*, bqac058. https://doi.org/10.1210/endocr/bqac058.

34. Jenei, K., Raymakers, A., Meyers, D.E., and Prasad, V. (2021). Challenges with sex-specific subgroup analyses in oncology clinical trials for drug approvals between 2015–2020. J. Cancer Policy *30*, 100311. https://doi.org/10.1016/j.jcpo.2021.100311.

35. Göttgens, I., and Oertelt-Prigione, S. (2022). Moving beyond Gender Identity: The Need for Contextualization in Gender-Sensitive Medical Research (The Lancet Regional Health - Europe), p. 100548. https://doi.org/10.1007/s00415-022.

36. Jackson, S.S., Han, X., Mao, Z., Nogueira, L., Suneja, G., Jemal, A., and Shiels, M.S. (2021). Cancer stage, treatment, and survival among transgender patients in the United States. J. Natl. Cancer Inst. *113*, 1221–1227. https://doi.org/10.1093/jnci/djab028.

37. de Nie, I., de Blok, C.J.M., van der Sluis, T.M., Barbé, E., Pigot, G.L.S., Wiepjes, C.M., Nota, N.M., van Mello, N.M., Valkenburg, N.E., Huirne, J., et al. (2020). Prostate cancer incidence under androgen deprivation: nationwide cohort study in trans women receiving hormone treatment. J. Clin. Endocrinol. Metab. *105*, E3293–E3299. https://doi.org/10.1210/clinem/dgaa412.

38. de Blok, C.J.M., Wiepjes, C.M., Nota, N.M., van Engelen, K., Adank, M.A., Dreijerink, K.M.A., Barbé, E., Konings, I.R.H.M., and den Heijer, M. (2019). Breast cancer risk in transgender people receiving hormone treatment: nationwide cohort study in The Netherlands. BMJ *365*, l1652. https://doi.org/10.1136/bmj.l1652.

39. Bours, M.J.L. (2020). A nontechnical explanation of the counterfactual definition of confounding. J. Clin. Epidemiol. *121*, 91–100. https://doi.org/10.1016/j.jclinepi.2020.01.021.

40. Bours, M.J.L. (2021). Tutorial: a nontechnical explanation of the counterfactual definition of effect modification and interaction. J. Clin. Epidemiol. *134*, 113–124. https://doi.org/10.1016/j.jclinepi.2021.01.022.

41. The Fraught Quest to Account for Sex in Studies Willingham (2022.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
SAS 9	SAS Institute Inc	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and materials may be directed to the lead contact, Dr. Berna C. Özdemir (berna.oezdemir@insel.ch).

Materials availability

This study did not generate new unique reagents or materials.

Data and code availability

- All data produced in this study are included in the published article and its supplemental information or are available from the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODELS AND SUBJECT DETAILS

Figure S2.

METHOD DETAILS

This article used data from a cross-sectional survey that was conducted via email between December 8th 2021 and March 15th 2022 to evaluate the perceptions of researchers regarding the importance of the sex of cell lines, animals, and human samples in cancer research.

Potential participants were recruited online through a variety of methods. A widely circulated newsletter email containing the survey information and link was sent to members of the European Association for Cancer Research in December 2021. Additionally, targeted web searches of universities and public and private academic institutions were used to identify cancer researchers and generate a list of potential participants. These web searches identified 224 academic cancer institutes in 33 countries in Europe (i.e., Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Italy, Lithuania, the Netherlands, Norway, Poland, Portugal, Scotland, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, UK), North America (i.e., Canada, USA), South America (i.e., Argentina, Brazil), Africa (i.e., South Africa), Asia (i.e., China, Israel, India, Japan) and Pacific (i.e., Australia). When available, research profiles and publications were used to determine whether certain individuals met the inclusion criteria. These web searches generated a list of 6925 eligible cancer researchers. Individuals identified from these web searches were sent an email describing the study and including the link to participate in the survey. Two follow-up emails were sent to all individuals, except those who had already participated or those who opted out of the email listing. Information about the study and the link for the survey were also publicly available on certain social media platforms (Linkedin®, Twitter® and Facebook®). Individuals were eligible to participate in the study if they: worked in an academic institution, conducted basic cancer research, and had a publicly available email address.

Survey instrument

The questionnaire collected data on participant demographics, clinical and basic/translational research experience, the cancer type studied and model systems used, the importance of performing experiments including both sexes, participant perception of the general importance of the sex of cell lines, animals, and humans in conducting and reporting experiments, participant perception of the importance of knowing and including the sex of cell lines, animals, and humans in their own experiments, as well as potential comments (see supplemental information for questionnaire).

A five-point Likert scale was used to measure the respondent's level of agreement with various statements. The survey was administered via SurveyMonkey© (an online platform) and was available to respondents during the 13-week study period. No ethics committee approval was required.

QUANTIFICATION AND STATISTICAL ANALYSIS

Descriptive statistics were used to characterize the study population using frequencies. Agreement to the statements was expressed as percentage in each response category, stratified by gender of the respondent and whether the respondent worked on sex-specific cancers. Logistic regression was used to identify associations between respondent characteristics and outcomes of interest. For these analyses, response options corresponding to agreement statements (i.e., agree, strongly agree, neither agree nor disagree, disagree, strongly disagree) were collapsed to binary responses (1=agree or strongly agree, 0=neither agree nor disagree, disagree, strongly disagree).

We performed for all questions logistic regression analysis between gender, age, degree (MD/PhD), experience in research, works in sex-specific cancer, cancer type studied (Figure S1).

Free text comments were filtered to remove 'None', 'Not applicable' and words to this effect, and any comments that only conveyed thanks to the study authors. The remaining comments (n = 175) were analyzed by inductive thematic analysis at the semantic level with an essentialist approach. Following familiarization with the data, author AMB coded the data. AMB reviewed the resulting codes for redundancy then identified common themes within the remaining codes relating to the topic of the survey. Author AMB acknowledges her approach to the data is reflective of her roles as a physician in both cancer and gender identity medicine, as a researcher into the effects of sex on gender on cancer, and as a cisgender woman.