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Testing ovarian cancer cell lines to train dogs to detect ovarian cancer from blood plasma: A pilot study

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ABSTRACT

Ovarian cancer is known as a "silent killer" because of its nonspecific symptoms and generally late diagnosis due to the lack of reliable early detection tools. Medical detection canines have been shown to recognize the odor profile of malignant tumors of many types, including ovarian cancer, and may be utilized to help produce a much-needed early detection system for ovarian cancer. One significant drawback, however, is that to train detection canines on the odor signature of ovarian cancer, different samples or sets of samples must be used in every training session so that the canines do not begin to recognize an individual patient's odor signature or attune to patient-specific noncancerous olfactory cues. Therefore, numerous patient-derived samples must be obtained and stored for use in the training process. Ovarian cancer cell lines may be able to solve this obstacle, as they have low sample-to-sample variability and do not present patient-specific confounding olfactory cues. In this study, we attempted to train three medical detection dogs to alert to an ovarian cancer cell line (OVKATE) but not the culture media or nonmalignant cultured fallopian tube cells. Only one dog was able to discriminate between OVKATE and culture media and was able to be tested on other ovarian cancer cell lines (OVCAR-4, OVCAR-8, and SK-OV-3). The dog's responses suggest a common or related olfactory signature of malignant ovarian cancer cell lines. We then tested whether an OVKATE-trained dog recognized the blood plasma of patients with confirmed ovarian cancer. We did not find strong evidence that the dog recognized the blood plasma of a patient with confirmed ovarian cancer within three testing trials, with the dog only hesitating at the target odor, suggesting that while training on the cell lines may prepare medical dedication canines to recognize the blood plasma of patient with ovarian cancer, it is not a spontaneous switch to blood plasma. Despite this outcome, the behavior of the dog led us to believe that this method could help further the use of medical detection canines to inform the production of early detection tools for ovarian cancer, which would lead to earlier diagnoses and more favorable outcomes from therapy.

Introduction

Ovarian cancer is the fifth most common cause of death from cancer among women in the United States and the most common cause of death due to gynecological malignancy (US Cancer Statistics Working Group, 2013). Known as a "silent killer," ovarian cancer's lack of symptoms in early stages and nonspecific cues (e.g., bloating, fatigue, abdominal and back pain) make it difficult to identify the disease until it has progressed to an advanced stage (ACOG Committee on Gynecologic Practice, 2006). Late detection limits the therapeutic options available to patients and decreases the efficacy of treatment, which leads to poor prognoses for most patients (Elattar et al., 2009). There are currently no reliable methods for early detection of ovarian cancer; a screening method with high sensitivity and specificity for early

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stages of the disease would improve outcomes for patients, as survival is stage-dependent (NICE, 2011).

This knowledge gap presents an opportunity for innovation in the field of ovarian cancer screening. Previous studies have used dogs to detect multiple forms of cancer (Pirrone and Albertini, 2017). Dogs have more than 220 million olfactory receptors in their nasal cavity (Uemura, 2015); they can detect odors in parts per trillion (Walker et al., 2006). In addition, dogs are extremely trainable, able to work with humans effectively, and can be easily housed and cared for in a domestic context for research. Dogs have been successfully trained to detect a wide array of substances including, but not limited to, drugs, explosives, molds, and contraband (Lesniak et al., 2008). Furthermore, dogs can distinguish single olfactory cues among complex mixtures of odors, as demonstrated by their ability to detect individual scents in exhaled human breath (McCulloch et al., 2006). Previous studies have harnessed this highly developed sense of smell in dogs to detect the odor of samples from lung, breast, bladder, and skin cancers (Moser and McCulloch, 2010).

Dogs must undergo extensive training to successfully recognize biological markers of cancer found in patient-derived plasma samples. However, the use of plasma samples for training presents numerous challenges, including biosecurity, limited availability, high cost, lack of standardization, and the potential presence of confounding olfactory cues unrelated to the underlying cancer (i.e., stress hormones, medications, individual patient variation, and so forth). Cancer cell lines, which are derived from a single cell source and grown *in vitro*, present a potential solution to these challenges. Cell lines are commercially available at relatively low cost, can be cultured to decrease sample-to-sample variability, eliminate the risk of exposure to infectious disease associated with human samples and do not present patient-specific confounding olfactory cues. Cell lines, however, may not have the same metabolism as spontaneous tumors or the ability to induce cancer *in vivo*. Furthermore, there is debate regarding the similarities and relevance of the various different cell lines (Domcke et al., 2013). Despite these potential limitations, by reducing the patient-derived variables and focusing on cell-specific ovarian cancer cell lines, we may find a "gold standard" odor that can target cancer dog training and improve their ovarian cancer detection performance.

A previous study by Yoel et al. (2015) demonstrated the potential of this strategy to be successful by training dogs to detect a breast cancer cell line. The authors found that after being trained on the breast cancer cell line, the dogs were able to detect both skin cancer and lung cancer cell lines, suggesting the possible presence of a general cancer olfactory cue within cancer cell lines. However, this study did not explore whether these dogs could also then detect cancer in patient-derived samples, which is essential for translating these findings to a potential application for patient screening (Lesniak et al., 2008). In a study by Horvath et al. (2010), the authors found evidence that dogs trained on ovarian carcinoma samples were able to recognize the odor in blood samples from patients with ovarian cancer. Although one dog was trained to recognize ovarian carcinoma samples and then tested on their ability to recognize blood samples from patients with ovarian cancer, the other dog had been previously trained to detect ovarian carcinoma samples and trained to recognize blood samples during the course of the study, and then was tested on their ability to recognize both (Horvath et al., 2010). However, this study utilized a forced-choice test design, which has been recognized as a limitation in animal disease detection studies (Edwards et al., 2017).

In this study, we aimed to train three detection dogs using the human ovarian cancer cell line OVKATE (RRID:CVCL_3110) and then test their ability to detect three other ovarian cancer cell lines: OVCAR-4 (RRID:CVCL_1627), OVCAR-8 (RRID:CVCL_1629), and SK-OV-3 (RRID: CVCL_0532). We chose these cell lines because they represent unique cell origins of tissue found in patients with ovarian cancer. If there is a common olfactory signature of malignant ovarian cancer, it should be present in all four cell lines. The final aim was to test plasma samples from patients with ovarian cancer to evaluate how the cancer cell line training translated to a patient-derived sample. If successful, this approach could help further characterize ovarian cancer cell lines and provide a method to facilitate training dogs as part of a larger effort to develop an effective early-detection method for screening patients for ovarian cancer.

Materials and methods

Study subjects

Four dogs in training or trained at the Penn Vet Working Dog Center were used in this study. Before training the three cell culture dogs (two German Shepherds and a Labrador), one German Shepherd, previously trained on plasma samples from patients with ovarian cancer (the fourth dog), was used to test the cell line odors (see Table 1 for more information on the dogs). All dogs were initially trained to use an odor detection wheel for odor detection trials with Universal Detector Calibrant (UDC) through positive reinforcement, with a clicker used as a marker and food or play/toy rewards. UDC is a synthetic odor made in a laboratory setting and not found naturally, which allows the dogs to be trained on how to search for odor without confounding their future searches with a nontarget odor (Furton et al., 2015). As part of their standard training at the Penn Vet Working Dog Center, dogs were trained on UDC from approximately eight weeks to their time of sale (the Labrador) or their placement into the cancer detection program (the two German Shepherds). The German Shepherds were trained for "stand stare" final response/alert, whereas the Labrador was trained for "sit stare" final response/alert (Figure 1A and 1B). These are only trained indications, both of which are valid. The different final responses were a matter of preference for each trainer and the dog's natural response. All dogs used for the study were brought to the Penn Vet Working Dog Center five days a week during the day and spent their evenings and weekends with foster families in a home environment.

Cancer samples

To determine if there was a common or similar odor between the cell lines and the plasma samples, we presented all four cell line odor samples to the dog previously trained to alert on patient-derived ovarian cancer plasma samples. Although the dog did not give a full final response on any sample, it hesitated at one sample: OVKATE. As a result, OVKATE was selected as the training sample for this study. The OVKATE cell line was originally isolated from a high-grade ovarian serous adenocarcinoma from a 40-year-old patient (Bairoch, 2018a). Once trained using the OVKATE cell line, the

Table 1
Individual information for dogs participating in this study

Dog	Breed	Sex	Age (at study start)	Status	Imprinted odor(s)
Osa	German Shepherd	F	33 months	In training	UDC
Bobbie	German Shepherd	F	12 months	In training	UDC
Jake	Labrador Retriever	M	49 months	In training	UDC
Tsunami	German Shepherd	F	50 months	Trained	UDC, ovarian cancer (plasma)

UDC, Universal Detector Calibrant.

Figure 1. (Above) Labrador retriever showing a sit stare final response. (Below) German Shepherd dog showing a stand stare final response.

additional ovarian cancer cell lines were used for testing: OVCAR-4, which was isolated from a high-grade ovarian serous adenocarcinoma from a 42-year-old patient (Bairoch, 2018b), OVCAR-8, which was isolated from a high-grade ovarian serous adenocarcinoma from a 64-year-old patient (Bairoch, 2018c), and SK-OV-3 (Bairoch, 2018d), which was isolated from an ovarian serous cystadenocarcinoma from a 64-year-old patient. All the cancer cell lines were grown in the RPMI 1640 medium supplemented with 10% fetal bovine serum under 5% CO_2 at 37°C, which was used as a control.

Additional controls included fallopian tube (FT) cell lines (FT190 and FT194), associated media without cells, and sterile filter paper. FT190 and FT194 are human immortalized cell lines that were obtained from FT serous epithelial cell lines that express human telomerase reverse transcriptase and SV40 large T and small t antigens. (Karst et al., 2011; Perets et al., 2013). The FT cells were grown in FT medium, which consisted of DMEM/F12 supplemented with Ultroser G serum substitute (Karst and Drapkin, 2012). Cells were cultured under 5% CO_2 at 37°C.

To capture the cell odor, cells were seeded in 100 mm tissue culture dishes, and once the cells reached 80% confluency, the media was replaced with fresh media and a Whatman 90 mm filter paper (Whatman 1004-090 Qualitative Filter Papers—Fisher Scientific) was placed into the lids of the tissue culture dish. The filter paper pieces were aseptically collected 24-hours later in a biological hood and cut into 8 pieces and stored in 28.35 g of clear glass jars (SKS Bottle & Packaging Inc., Stock #40210010.02S) in a −80°C freezer. For each trial, 3 pieces of filter paper were presented to the dog in a single glass jar.

Fifty-microliter patient-derived normal plasma samples and plasma samples from patients with benign ovarian growths were used as controls to ensure that the dogs would not target the smell of plasma odor derived from benign ovarian growths. The same normal plasma samples or plasma samples from patients with benign ovarian growths were never used for more than one session, to ensure that the dogs did associate any control patient-specific odors with no reward. These samples were received from Dr. Janos Tanyi, and the sample collection and use was approved by IRB #818255. All biological samples were stored at −80°C and thawed before use for approximately ten to twenty minutes. We also introduced saline as an additional control at this stage.

The FT odor, FT medium odor, both normal and benign plasma were all added to the wheel simultaneously at the third stage of training (see Table 2). All of these control odors were presented to the dogs at once because we did not expect any of these odors to be similar to the OVKATE cell line odor.

To move from one stage to the next, dogs had to show proficiency at the current level by achieving an 80% success rate across ten trials for three training sessions in a row.

The target odor was in one port, and all remaining ports on the wheel always contained either control samples or distractors; distractors were randomly chosen objects (e.g., paper clip, paper towel, cotton ball, screw, and so forth) and were not expected to resemble any of the target or control samples. Distractors were changed monthly, so the dogs were consistently exposed to new distractor odors.

Experimental setup

The initial wheel used for the study had 12 ports and was rotated between trials to randomize the location of the sample, but the order of samples remained the same. The dogs were then transitioned to using a wheel with 8 ports in which the samples were moved between ports for each trial (Figure 2), and this was the wheel used for testing. We transitioned to this wheel as soon as it became available, as it was an upgrade in the setup and allowed us to change the order of the samples presented to the dogs within a single session. The new wheel only allowed for 8 rather than 12 ports because of the greater space between each port, as well as a stainless-steel barrier between each port, to keep the odor cones more separated. A random number generator was used to determine into which port on the wheel the target sample was placed for each trial, and all ports were equally likely to receive the target sample on any given trial. A video record was taken for all trials along with a written record of the dogs' behavior at each port. Vinyl gloves were used when handling samples and whenever touching the wheel, and gloves were always changed between handling

Table 2
Sequential stages of dog training and testing used in this study

Phase	Target sample	Controls
Stage 1	OVKATE	Filter paper
Stage 2	OVKATE	Filter paper, cancer medium odor
Stage 3	OVKATE	Filter paper, cancer medium odor, FT odor, FTM odor, benign ovarian plasma, normal plasma, saline
Stage 4	OVKATE in normal plasma	Filter paper, cancer medium odor, FT odor, FTM odor, benign ovarian plasma, normal plasma, saline
Test 1	OVCAR-4 OVCAR-8 SK-OV-3	Filter paper, cancer medium odor, FT odor, FTM odor, benign ovarian plasma, normal plasma, saline
Test 2	Malignant ovarian cancer plasma	Filter paper, cancer medium odor, FT odor, FTM odor, benign ovarian plasma, normal plasma, saline

FT, fallopian tube; FTM, fallopian tube medium.
Dogs moved from one stage to the next after achieving an 80% success rate for three sessions in a row.

Figure 2. Picture of the scent wheel used in this project.

samples. The wheel was cleaned between dogs, and the floor beneath the wheel was cleaned daily with 70% isopropyl alcohol. New normal and benign plasma samples were used each day as controls, and each sample presented to the dogs was composed of plasma taken from one, or pooled from two, or three patients.

We defined a pass as each time a dog circled the wheel. We defined a trial as all of the passes a dog did of the wheel during which the order of samples remained the same. We defined a session as all of the consecutive trials completed by the dog. Training sessions contained between five and ten trials and did not occur more than twice per day, with at least two hours between sessions (not more than ten trials per day across both sessions). Dog behavior at each port was scored as either (1) negative, when a dog sniffed a port and moved on or (2) positive, when then the dog sniffed a port and gave their trained final response ("stand stare" or "sit stare"). We considered hesitation as any brief pause in the dog's movement after the presentation of samples that lasted longer than 1 second. Dogs were rewarded when they gave their trained final response at the target odor but were not rewarded for hesitations at the target odor. Hesitations were used to keep track of dogs' interest in target odors even if they did not alert, as well as determine their interest in new controls. We coded the dogs' behavior at each port as shown in Figure 3.

Training

For the initial training stage of the study, the dogs were introduced to the OVKATE cell line odor samples on the wheel along with clean filter paper as a control. Distractors were placed in all the remaining ports. For the subsequent stages, additional controls were added to the wheel as described in Table 2. For the last training stage, the OVKATE sample was put into a jar containing 50 microliters of normal plasma, to confirm the dogs were able to focus on the cancer cell line odor even when the normal plasma odor was in the same port to prepare them for being tested on the patient-derived malignant cancer plasma sample. The dogs participated in 10 trials per day, across one to two sessions, with short breaks between trials and were given free access to water throughout each session.

Training phase 1 also included "blank wheel" training, which we used to train the dogs to give a response if they did not find their target odor in the wheel (stepping on a platform). This allowed the dogs to receive a reward when there was no target odor present, lowering the likelihood of false response. Only in the initial training phase, after sniffing the last port, the dogs were cued to step onto the platform. During blank wheel training, if the dog turned around to indicate on a port in an attempt to receive a reward, or failed to step on the platform, the dog was called out of the room, given a verbal no reward marker (e.g., Good try!), and sent back to the wheel until they performed it correctly. Approximately half of all trials during odor training at all stages were blank wheels, where no target odor was present, but controls were still present.

Testing

Only dogs that passed stage 4 of training proceeded to testing. For testing, the OVKATE cell line was replaced with 3 different cell line (i.e., OVCAR-4, OVCAR-8, or SK-OV-3) odor samples, or plasma samples from patients with ovarian cancer, as the target sample to determine if the dogs were able to identify these samples spontaneously after training only with the OVKATE cell line.

Trials were such that the dog did not know where the target sample was placed on the wheel, but the handler was told the location of the target sample at the beginning of each trial to allow the trainer to mark and reward correct behavior, if the dog did give a final response at the target odor. The wheel was located behind a barrier and monitored via a video feed so that the dogs could not see their handler, the recorder, or any observers during the trials. Dogs performed only three trials on the new cell lines and on the patient-derived ovarian cancer plasma samples before any more information (via a click) was given to them, to capture spontaneous interest or identification by the dogs rather than learning. As the end goal of our dogs is to move on to recognizing ovarian cancer–positive plasma samples, and thus did not want to give the dog many opportunities to pass that target odor, if the dog did not alert on the target sample, the trainer was allowed to give the dog a "click on sniff" by clicking when the dog sniffed the target sample's port.

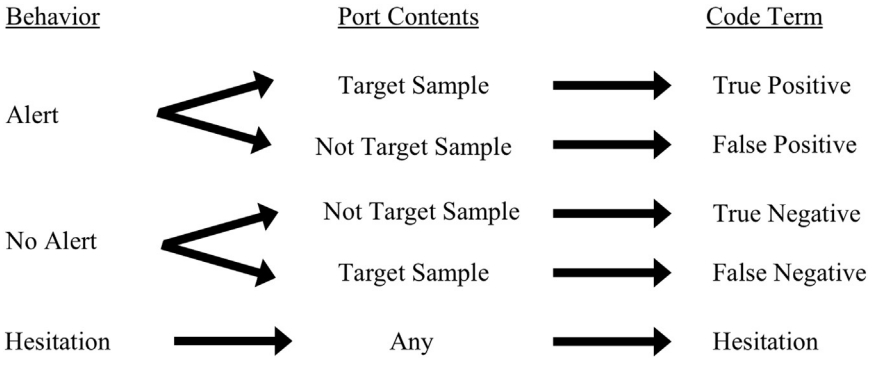

Figure 3. Code term definitions.

We would then test two more times if the dog found the target sample with this further information. The probability of a perfect trial by chance, where the dog correctly exhibited their trained final response on the new target port once, would be 12.5%.

As in training, if the dogs gave a final trained response at the new target odor, the trainer marked the behavior (e.g. clicked), and the dog received a reward. If the dog hesitated at the port, no reward was given. If the dog searched the whole wheel without responding to the test sample and indicated that the wheel was blank by stepping onto the platform, the dog was called off and the trainer asked for other trained behaviors (e.g., sit, paw) which could be rewarded while the wheel was reloaded for the next trial. Thus, the dogs were never rewarded for communicating to us that the wheel with the test samples was blank but were also not told that they were incorrect by indicating that the wheel was blank.

Results

Training

Of the three dogs that began training on the cancer cell lines, only one German Shepherd was able to progress past the second stage of training (see Figure 4); neither of the other dogs were able to reliably distinguish the cancer cell line sample odor from the odor of the cancer medium on which the cancer cell line was grown. The German Shepherd was able to complete all four stages of training; thus, we were able to test its responses to the other cell line and malignant cancer plasma samples.

Cell line recognition

We tested one dog on each of the OVCAR-8, SK-OV-3, and OVCAR-4 cell line odor samples during the same testing session, before we tested the malignant plasma from confirmed patients with ovarian cancer. Each cell line was presented for three trials.

On the first OVCAR-8 trial, the dog gave a false negative on its first pass but turned around a few ports later to return to the target odor and give a full alert before finishing the trial. Within the first pass of its second and third trials, it gave a true-positive alert. Using the SK-OV-3 cell line, it gave a false negative on its first and second trials, but gave a true positive alert on its third trial. On its first OVCAR-4 trial, it hesitated at the port, whereas on its second and third trials, it gave a true-positive alert. See Table 3 for a summary of cell line recognition results. Included is the true-negative rate as a percentage, with true negative being the rate at which the dog correctly passes nontarget odors. In trial 1 of OVCAR-8, the rate is

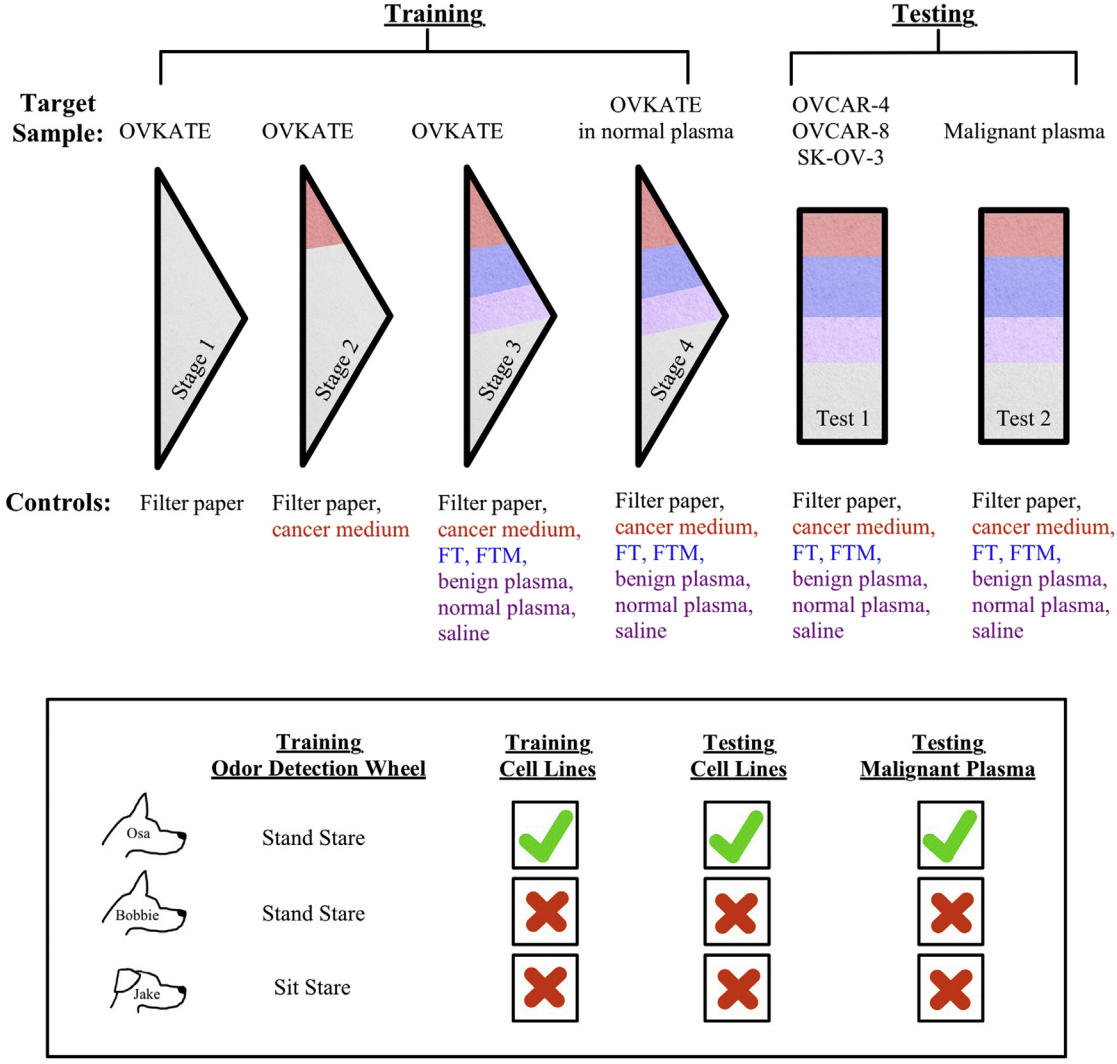

Figure 4. Summary of training and testing procedure and results.

Table 3
Summary of cell line recognition testing results

Target sample	OVCAR-8			SK-OV-3			OVCAR-4		
Trial	1	2	3	1	2	3	1	2	3
Dog's behavior	FN TP	TP	TP	FN	FN	TP	H	TP	TP
True-negative rate (%)	75[a]	100	100	100	100	100	86[b]	100	100

H, hesitation; FN, false negative; TP, true positive.
[a] Dog initially passed target odor then returned to it to alert.
[b] Dog hesitated at a non-cancer odor (cancer media).

not 100% as the dog hesitated at a port before turning to alert on the cell line (that it had initially passed). In trial 1 of OVCAR-4, the dog hesitated once at a noncancer odor (cancer media).

Plasma recognition

When tested on a malignant ovarian cancer plasma sample, the dog passed the target sample without alerting. However, it hesitated at the target sample on its first and third trials, which was identified by an independent observer watching from behind a window. In trial two, the dog passed the target sample with no visible hesitation. After this, we allowed the dog's handler to mark and reward the dog for sniffing the target sample ("click on sniff") once. In two subsequent trials, after having been rewarded for sniffing the target sample once (trial 4), the dog gave a true-positive alert at the target sample. In all trials, the same mix of patient samples from confirmed ovarian cancer cases was used. See Table 4 for a summary of plasma recognition results.

Discussion

One of the three dogs that began this study was able to complete the training protocol and progress to the testing phase. Osa, the dog that successfully completed training, did respond to the odor of the other cell lines and the plasma sample, suggesting that there may be a common ovarian cancer odor that can be identified by dogs. Our results support previous work establishing that dogs can identify cancer cell line odor (Yoel et al., 2015). This provides modest support for the hypothesis that dogs initially trained on cancer cell lines can detect cancer odor in novel patient-derived samples and suggests that it may be a generalized odor that exists across samples and cancer types. This suggests that using cancer cell lines for training cancer detection dogs may be a viable method to accelerate the training process.

Many of the one successful dog's responses were initially hesitations rather than a full-trained response/alert on the new target odors. It is unlikely that these hesitations were merely due to the presence of a new odor in the wheel, as the dogs often received new distractors throughout their training at which they did not hesitate. Moreover, in the first session, when presented with the plasma of patients with benign ovarian growths as well as the plasma of normal controls, it did not hesitate. This suggests that its hesitation on the new cancer odors (cell line and plasma) was more likely due to some similarity to its trained odor, whereas the absence of a full-trained response was due to their dissimilarity. The trained blank wheels further allowed the dogs to be rewarded without placing them into a forced-choice paradigm, reducing the likelihood of them choosing or showing interest in an odor merely to receive a reward. However, we are limited in that we did not test here whether Osa would have spontaneously recognized a second ovarian cancer plasma sample, thus the subsequent trials after the "click on sniff" were likely the dog exhibiting direct memory effects on top of training and did not test whether it would continue to generalize onto other patient samples from confirmed ovarian cancer cases.

Table 4
Summary of plasma recognition testing results

Target sample	Malignant ovarian cancer plasma					
Trial	1	2	3	4	5	6
Dog's behavior	H	FN	H	CoS	TP	TP

CoS, click on sniff; FN, false negative; H, hesitation; TP, true positive.

The dogs that were not able to complete the training protocol were unable to distinguish the cancer growth medium control odor from the cancer cell line odor. The odors of the cancer growth medium and cancer cell line samples may be too similar to be discriminated by these dogs, or the cancer growth medium odor overpowered the cancer cell line odor in these samples to such an extent that only one dog was able to discriminate the cell line odor. This discrimination was the most difficult stage of training. We discontinued the training of the two dogs that were not able to reliably discriminate between cancer cell line odor and cancer growth medium odor after more than six months of training. It is unlikely that these two dogs' inability to pass this stage of training was because of their nose being less sensitive and much more likely that there were behavioral or even personality differences that made them less successful at the task. One of the dogs was dropped from the cancer detection program permanently after his performance on this study. We have no evidence that the initial training on UDC interfered with their ability to learn the odor. The center uses UDC training routinely for all dogs, and all dogs leaving the center for their final job have been rapidly and successfully trained on other odors (e.g., narcotics, bed bugs, accelerant, blood plasma).

As only one dog was able to successfully complete the training phase of this study, we were not able to definitively establish the use of ovarian cancer cell lines for training as a viable method of training dogs to spontaneously detect malignant ovarian cancer in patient-derived samples. Further studies training additional dogs with additional controls are needed to establish whether this method can be successfully implemented for training of dogs to detect malignant cancer in patient-derived samples. Our results suggest that this protocol can be successfully implemented for some dogs because it was successfully used as a precursor for training on malignant ovarian cancer plasma samples. After the completion of this study, Osa was switched from cell line odor to malignant ovarian cancer plasma samples. Osa's future performance on distinguishing ovarian cancer plasma samples was extremely good, suggesting that the training presented in this study may have facilitated the learning process; however, we cannot be sure that this is not also due to Osa having more odor training in general. In addition, our results suggest that there is a common odor for malignant ovarian cancer in cell lines that Osa was able to isolate in all four cell lines presented, which allowed it to recognize the similarity among all the target samples to which it was exposed. It is likely the volatile metabolic waste which is captured by the filter paper, and a pattern in that odor can be detected by canines, may be similar across these ovarian cancer cell lines. This generalizability may be an advantage for those looking to train dogs on cancer cell lines to prepare them for patient-derived samples, as it suggests ovarian cancer cell lines, or at least those tested, may have a common underlying "cancer" odor.

Further studies are needed to confirm that other dogs are able to perform similarly and to establish which variables contribute most significantly to training success. Our protocol used only the OVKATE cell line in the training phase for our dogs. Other ovarian cancer cell lines might have stronger or otherwise easier to detect or distinguish odors. Using alternate cell lines or combinations of cell lines for training could provide support for the feasibility of this training

method and to determine whether different cell lines share an underlying cell type–specific odor or if there is a generalizable cancer odor.

Our study was limited by the number of dogs involved in this study, and the controls we were able to use in the testing phase. We attempted to train three dogs, and only one successfully identified the ovarian cancer cell lines. It is possible that testing the cell lines themselves, instead of the odor that was collected onto the filter paper, may have made training more salient for the dogs and allowed more than one dog to progress through training. A larger sample size of canines and samples would be needed to determine the applicability of this study for general use in training cancer detection canines. In addition, dogs were only trained on ovarian cancer cell lines, so the generalizability to other cancer types is unknown.

Conclusions

Training on the odor of cultured cancer cells has the potential to contribute positively to ovarian cancer odor detection research and may be useful for future projects investigating the odor signature of ovarian cancer. Given the advantages of training using cancer cell lines rather than patient-derived samples (i.e. lower cost, more readily available, less variability, lower risk of infectious disease, and no patient-specific confounding factors), we believe this is a valuable line of investigation for future research into early screening methods for ovarian cancer using detection dogs.

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Authors' contributions: The idea for the paper was conceived by Otto C.M and Drapkin.R. The experiments were designed by Otto.CM and Essler.J.L. The experiments were performed by Essler.J.L., Murarka.M., Smith.P.G., Vesley-Gross.Z.I., and Hooda.J. The data were analyzed by Essler.J.L., Murarka.M., and Vesley-Gross.Z.I. The paper was written by Murarka.M., Vesley-Gross.Z.I., Essler.J.L., Smith.P.G., and Otto C.M.

Ethical statement

This protocol was approved by the Institutional Animal Care and Use Committee (Protocol # 804900).

Conflict of interest

All authors disclose that they have no conflicts of interest regarding this manuscript.

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