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Molecular Imaging of Ovarian Cancer

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ABSTRACT

Ovarian cancer is the most lethal gynecologic malignancy and the fifth leading cause of cancer-related deaths in women. Over the past decade, medical imaging has played an increasingly valuable role in the diagnosis, staging, and treatment planning of the disease. In this *Molecular Imaging Focus* review, we seek to provide a brief yet informative survey of the current state of the molecular imaging of ovarian cancer. The article is divided into sections according to modality, covering recent advances in the magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), ultrasound imaging (US), and optical imaging (OI) of ovarian cancer. While primary emphasis is given to clinical studies, particularly innovative and promising preclinical investigations are discussed as well. Ultimately, we are hopeful that the combination of technological innovations, novel imaging probes, and the further integration of imaging into clinical protocols will lead to significant improvements in the survival rates for ovarian cancer.

INTRODUCTION

Ovarian cancer continues to be the most lethal gynecologic malignancy and the fifth leading cause of cancer-related deaths in women. As of 2015, the 5-year survival rate for patients with advanced stage ovarian cancer is a dismal 27% in United States (*1*). This high mortality rate can be largely attributed to the inability to clinically and radiographically detect ovarian cancer at early stages. The statistics reinforcing the importance of early detection are particularly striking. If detected at an early stage (I), there is a 90% cure rate for ovarian cancers. However, this rate drops precipitously to 20-25% for patients diagnosed at later stages (III-IV)(*2*). The origins of the late detection of ovarian cancer lie at the convergence of a variety of factors, including (a) the ineffective screening tools, tests, and diagnostic methods currently employed in the clinic; (b) the vague presentation of the symptoms of the disease; (c) the long occult period followed by the rapid doubling time of ovarian cancer; and (d) the frequent recurrence of therapy-resistant disease after initial treatment. Clearly, advances across a variety of fields are needed to improve the diagnosis and clinical management of this disease. In the pages that follow, our goal is to focus on a growing part of this puzzle by providing a bird's-eye view of the roles that *molecular imaging* can play in the clinical care of ovarian cancer.

Different imaging modalities provide different types of information. *Anatomical imaging* methods such as ultrasound, magnetic resonance imaging, and computed tomography create exquisitely detailed maps of organ morphology, while *molecular imaging* modalities such as positron emission tomography, single photon emission computer tomography, and optical imaging yield functional information on the biochemistry of tissues. Not surprisingly, both types of imaging play important roles in the clinical management of ovarian cancer. To wit, the use of ultrasound as the first imaging modality in the characterization of adnexal lesions and CT for the evaluation of the extent of disease in patients with suspected ovarian cancer are two excellent examples of the integration of *anatomical* imaging into current clinical protocols. Delving slightly deeper, CT is the imaging modality of choice for the evaluation of ovarian cancer patients prior to the initiation of treatment via surgery or neoadjuvant therapy, and it has found an important place in the staging of the disease as well (*3, 4*). Furthermore, CT has been used following primary cytoreductive surgery for the detection of residual disease, thereby providing a critical prognostic indicator for the post-operative progression-free and overall survival of patients (*5*). CT may be limited in its accuracy for distinguishing recurrent disease from post-operative or post-radiotherapy fibrosis. However, the sensitivity of CT for the detection of recurrent ovarian cancer improves significantly when it is combined with a functional imaging modality such as PET (*6*). Given that CT is a purely *anatomical* imaging technique, further discussion of this modality lies outside of the scope of this review.

Over the past two decades, a variety of *molecular imaging* modalities have emerged to complement traditional anatomical imaging. Indeed, molecular imaging techniques can serve a variety of roles in the clinical care of ovarian cancer patients, including (a) the localization of primary tumors; (b) the determination of the extent of metastatic spread; (c) the stratification of patients for surgery and therapy; (d) the evaluation of the metabolic status of the tumor; (e) the characterization of the expression levels of biomarkers by malignant tissue; and (f) the design, implementation, and monitoring of targeted therapies. In this short review, our goal is to provide a broad overview of the roles that *molecular* imaging plays in the management of ovarian cancer. The review is composed of four sections, each focused on a different imaging modality: magnetic resonance imaging, nuclear imaging, optical imaging, and ultrasound imaging. While primary emphasis is given to clinical studies, particularly innovative and promising preclinical investigations will be discussed as well.

One final clerical note before we begin: recent advances in cancer biology have revealed that the term 'ovarian cancer' is a misnomer, a catch-all that places multiple malignancies (including those that arise from non-ovarian tissues) into a single basket. As a result, it is possible that each histotype of epithelial ovarian cancer — serous, endometrioid, clear cell, and mucinous — may bear unique, signature biomarkers that can be targeted for molecular imaging. It is likewise feasible that molecular imaging targeting surrogate markers of KRAS, BRAF, PI3K mutations, and TP53 mutations could be used to distinguish between indolent ovarian cancer and aggressive disease. Yet as much as we appreciate the molecular and pathophysiological diversity of ovarian cancer, most of this knowledge is relatively new and has yet to be applied to molecular imaging. Consequently, in the pages that fellow, we will yield to simplicity and expedience and use the admittedly anachronistic yet convenient term "ovarian cancer".

MAGNETIC RESONANCE IMAGING

MRI has demonstrated significant utility for ovarian cancer imaging, particularly in distinguishing between benign and malignant ovarian lesions that are indeterminate on ultrasound. For example, a retrospective study performed by Thomassin-Naggara *et al.* including 394 patients with indeterminate adnexal masses showed that pelvic MRI using the ADNEX MR scoring system had a sensitivity of 93.5% and specificity of 96.6% for detecting malignant tissue (*7*). Beyond providing anatomic information, MRI can also be used for functional imaging via diffusion-weighted imaging (DWI), dynamic contrast enhanced (DCE), and magnetic resonance spectroscopy (MRS). DWI measures the Brownian motion of water molecules, which is impacted by the hypercellularity and extracellular architecture of tumor tissue. Apparent diffusion coefficients (ADC) derived from DWI provide a quantitative parameter of the diffusivity of the imaged tissue. In a 32 patient study, Michielsen *et al.* compared DWI and FDG-PET/CT in the context of tumor characterization and staging (*8*). The authors concluded that whole body DWI-

MRI had a 94% accuracy rate for primary tumor characterization and 91% accuracy for peritoneal staging. DCE-MRI, on the other hand, acquires images at different time points following the injection of an MR contrast agent. The transport kinetics of the contrast agent into the tumor are calculated and depend on both the vascularity and the permeability of the malignant tissue. In a clinical study performed by Li *et al.*, DCE-MRI was able to detect a significant difference between the contrast enhancement profiles of benign and malignant ovarian tumors (*9*). In addition, Thomassin-Naggara *et al*. performed a retrospective study concluding that the addition of diffusion-weighted (DWI) and perfusion-weighted (DCE) sequences to conventional MRI can improve the diagnostic accuracy of MRI for complex adnexal masses (*10*).

Magnetic resonance spectroscopy measures the chemical composition of the regions of interest to yield semi-quantitative data on biochemical compounds such as choline, creatine, and lactate. Esseridou *et al.* evaluated MRS in 16 patients with ovarian masses who underwent MRI/MRS followed by histopathological examination of the masses (*11*). Of the 19 malignant tumors identified by histopathology, 17 demonstrated a choline peak on MRS, indicative of metabolic deregulation and malignancy. Multi-parametric MRI can also provide insights into tumor heterogeneity, and quantitative parameters derived from DCE, DWI and MRS may serve as treatment response biomarkers in patients with advanced ovarian cancer. In a prospective study of 21 patients undergoing neo-adjuvant chemotherapy for advanced ovarian cancer, Sala *et al.* found significant differences in baseline ADC values between primary ovarian cancer, omental cake, and peritoneal deposits, indicating that diffusivity profiles may be tumor site-dependent and suggestive of the biological heterogeneity of the disease (Fig. 1) (*12*). In this work, ADC and the fractional volume of the extravascular extracellular space (v_e) correlated with the cytotoxic effects of platinum-based therapy and are thus potentially useful biomarkers of response. In contrast, changes in choline concentration predicted but did not reflect response. Furthermore, ovarian tumors and metastatic peritoneal implants displayed spatial heterogeneity, as perfusion, diffusion, and metabolism can vary markedly between spatially distant tumor sub-regions. This variation likely reflects distinct phenotypic habitats within the tumor, distinct clonality, or a combination thereof.

NUCLEAR IMAGING

Not surprisingly, the most ubiquitous radiotracer in oncologic PET imaging — ^{18}F-FDG — has been used in a variety of ways in the management of ovarian cancer. Several reports point to the superior performance of FDG-PET/CT in the detection of *recurrent* ovarian cancer. FDG-PET/CT is reported to have found positive lesions in many instances in which CT alone was negative (*13*). Furthermore, FDG-PET was able to document the evidence of disease recurrence 6 months prior to findings on CT (*14*). Pre-

operative whole body imaging via FDG-PET/CT has often contributed to the accurate upstaging of ovarian cancer patients as well, especially with regard to lymph node involvement (Fig. 2) (*15, 16*). In addition to the qualitative visualization of lesions on an FDG-PET scan, semi-quantitative parameters such as SUV have been shown to have prognostic and predictive utility during clinical follow-up (*17*). Along these lines, Vallius *et al.* used FDG-PET/CT to predict histopathologic responders from non-responders to neoadjuvant chemotherapy prior to interval debulking surgery (*18*). In another example, a recent clinical study in 12 platinum-resistant patients demonstrated an exposure–response relationship between a pan-AKT inhibitor and the tumoral uptake of FDG (*19*).

Despite its utility in ovarian cancer, FDG-PET has some important limitations, most notably the prevalence of *false negatives* associated with the cystic nature of ovarian cancer and *false positives* stemming from the uptake of FDG in inflammatory cells and benign growths. In response to these limitations, an impressive array of second-generation radiotracers has been developed and translated to the clinic. Generally speaking, these radiotracers target cell surface receptors that are over-expressed in ovarian cancer. The putative clinical advantage of these molecular imaging strategies lies in the ability to provide non-invasive, real-time, and quantitative whole-body information on the *in vivo* receptor status of ovarian tumors. This information can then be leveraged to facilitate the stratification, planning, or monitoring of receptor-targeted therapies. For example, two SPECT radiotracers that target the folate receptor (FR) — ^{111}In-DTPA-folate and ^{99m}Tc-etarfolatide — have shown promise for the delineation of newly-diagnosed ovarian cancer and the stratification of patients based on their levels of FR expression in ovarian tumor lesions, respectively (*20, 21*). Shifting gears to PET, a recent clinical report probing the possibility of imaging estrogen receptor alpha (ERα) levels with ^{18}F-labeled estradiol found that this radiotracer could delineate ERα+ tumors from ERα- tumors with high specificity (*22*). Similarly, mesothelin is over-expressed in ovarian cancer and is known to interact synergistically with MUC16 to promote the peritoneal seeding and spread of ovarian cancer. A recent first-in-human PET imaging study using a ^{89}Zr-labeled anti-mesothelin antibody in 4 ovarian cancer and 7 pancreatic cancer patients found that the mean SUV_{max} was higher in ovarian cancer lesions than in pancreatic cancer lesions (*23*). This approach could help in the identification of metastatic ovarian cancer in patients that may benefit from treatment with anti-mesothelin therapy.

Moving from the clinic to the laboratory, a number of promising imaging agents have been the subjects of preclinical studies in recent years. Sharma *et al.*, for example, have reported the development of a ^{89}Zr-labeled radioimmunoconjugate based on the CA125-targeting murine antibody B43.13 (*24*). Notably, this agent is capable of delineating the metastatic spread of OVCAR3 tumors along the ipsilateral chain of lymph nodes. Ocak *et al.* recently employed a ^{68}Ga-DOTA-albumin-folate conjugate and FR680 for the multimodal PET/FMT (fluorescence molecular tomography) imaging of the

intraperitoneal spread of FR-positive MKP-L cells (*25*). Similarly, Liu and coworkers reported on the multimodal PET/OI of metastatic ovarian tumors within the peritoneum of mice using a ^{64}Cu-labeled pyropheophorbide-folate conjugate (*26*). Such multimodal molecular imaging agents could be clinically useful for both the pre-operative delineation of the extent of disease *and* as intraoperative tools for the efficient resection of residual disease during cytoreductive surgery. On a different note, an increasing understanding of the roles played by components of the immune system — for example, macrophages and cytokines — in the promotion of tumor growth has led to the emergence of yet another class of targets for molecular imaging. Along these lines, Li *et al.* have recently created a SPECT imaging agent to target the interleukin-6 receptor (IL-6R) (*18*). In their work, a radiolabeled IL-6R-targeting peptide — ^{99m}Tc-HYNIC-Aca-LSLITRL — displayed increased uptake in C13K tumors that express high levels of IL-6R and much reduced retention in SKOV3.ip tumors known to express low levels of IL-6R. In theory, molecular imaging of the IL-6R status of tumors could aid in the identification of patients that may benefit from IL-6/IL-6R therapies.

A particularly interesting subset of preclinical investigations has focused on the use of nuclear imaging for the monitoring of therapeutic progress. For example, the HER2-targeted affibody ^{99m}Tc-peptide-$Z_{HER2:342}$ was recently successfully employed for monitoring the efficacy of trastuzumab therapy in a SKOV3 xenograft model via SPECT (*27*). Likewise, Niu *et al.* have shown that the uptake of ^{64}Cu-DOTA-trastuzumab can be used to monitor response to therapy with 17-dimethylaminoethylamino-17-demethoxygeldanamycin (*28*). Finally, Nagengast and coworkers have demonstrated the utility of ^{89}Zr-labeled bevacizumab as an imaging marker for early response to anti-angiogenic therapy with an Hsp90 inhibitor, NVP-AUY922 (*29*).

OPTICAL IMAGING

Optical imaging is emerging as a powerful tool in biomedical research and clinical practice. In oncology, the use of fluorescent probes as tools for image-guided surgery has proven particularly promising. In this setting, the imaging agents assist surgeons in distinguishing malignant from benign tissue with the help of a navigation system that activates the molecular probe and captures and processes the emitted light to generate an image in real time (*30*). The first-in-human studies using this approach were published in 2011 by van Dam et al., who exploited the over-expression of folate receptor alpha (FR-α) in ovarian cancer by developing a fluorescein-isothiocyanate conjugated folate probe (FITC-folate) (Fig. 3) (*31*). Ten patients with suspected ovarian cancer received an injection of the probe and underwent image-guided surgery followed by post-operative histopathological analyses. Critically, all tissue samples that fluoresced *in vivo* and *ex vivo* were confirmed to be malignant, while none of the resected benign tissues exhibited fluorescence. Similarly, a clinical trial is currently underway with data

recently presented by Tummers *et al.* (*32*). Briefly, a FITC-conjugated folate analog EC17 was injected into patients undergoing cytoreductive surgery. Out of the 12 ovarian cancer patients, 44 confirmed malignant lesions were resected, including 6 that were not identified by initial surgical inspection. Recently, the attention of the field has shifted from fluorophores that emit visible light to probes that emit near infrared fluorescence (NIRF). In the clinic, Tummers *et al.* conducted a trial in which 10 patients with suspected ovarian cancer were administered indocyanine green (λ_{em} ~ 830 nm) prior to image-guided cytoreductive surgery. Although all of the metastatic deposits in these patients exhibited NIRF, 13 non-malignant lesions also exhibited fluorescence, leading to a high false-positive rate of 62% (*33*). Preclinical advances in NIRF imaging have been made as well. To wit, Terwisscha van Scheltinga *et al.* targeted epidermal growth factor receptor 2 (HER2) and vascular endothelial growth factor using IRDye 800CW-labeled monoclonal antibodies (*34*), while Lee and coworkers targeted FR-α with an activatable NIRF probe that only emits fluorescence following cleavage of a linker by the lysosomal enzyme cathepsin B (*35*).

ULTRASOUND

Both photoacoustic imaging and traditional ultrasound have also shown promise for ovarian cancer imaging. As its name suggests, photoacoustic imaging is predicated on the photoacoustic effect: the generation of ultrasonic waves following the absorption of photons by biomolecules such as hemoglobin or melanin. Because ultrasonic waves exhibit less scattering in biological tissue compared to photons, photoacoustic imaging has the potential to visualize tumors deep within the body (*36*). Aguirre *et al.* have developed a co-registered ultrasound/photoacoustic imaging system and used it to image 33 ovary samples collected from patients undergoing oophorectomy, ultimately determining a sensitivity and specificity of 83% for diagnosing ovarian cancer in postmenopausal ovaries (*37*). More recently, several preclinical papers have been published improving upon the hybrid technology, including the creation of a system for the real-time co-registration of images (*38*) and a miniaturized illumination probe for transvaginal photoacoustic imaging (*39*). Exogenous contrast agents can also be used for photoacoustic imaging, as Jokerst *et al.* have illustrated in their work using non-targeted gold nanorods as contrast agents for photoacoustic *and* Raman imaging (*40*).

Traditional ultrasound continues to be further improved upon as well. Specifically, a number of promising preclinical investigations have shed light on the potential of microbubble-based contrast agents for the imaging of tumor angiogenesis. For example, Willmann *et al.* observed increased ultrasound signal in ovarian tumor tissue after the administration of perfluorocarbon-filled microbubbles conjugated to knottin peptides targeting integrin $\alpha_v\beta_3$ (*41*). More recently, Lutz *et al.* observed a significantly higher ultrasound signal using microbubbles engineered to target the vascular marker CD276 (Fig. 4) (*42*).

Ultimately, the real-time acquisition and processing of both traditional ultrasound and photoacoustic imaging — combined with the fact that ultrasound systems are inexpensive and widespread in the clinic — make these modalities primed for increased use as diagnostic tools for ovarian cancer.

CONCLUSION

With the advent of personalized medicine, the field of ovarian cancer research has reached a fascinating crossroads. Much of the silence of the '*silent killer'* has been dispelled, and recent years have witnessed a surge in the understanding of the etiology and molecular characteristics of the disease. In light of these advances, we believe that imaging will play a central role in both the preclinical study and clinical management of ovarian cancer in the years to come. As we have discussed in these pages, an array of extremely promising imaging strategies have *already* impacted clinical care. The future is even brighter. Indeed, there are a variety of different avenues that are primed for innovation. In our opinion, two stand out. First, the use of hybrid imaging systems — such as PET/MR — that provide simultaneous anatomical and functional data have shown potential in the ovarian cancer setting and certainly merit further investigation (Fig. 5). Second, the current push to unravel the molecular fingerprints of the sub-types of ovarian cancer has yielded a list of new biomarkers that is growing by the day. Molecular imaging agents targeting these biomarkers have the potential to serve two exciting roles: as *diagnostic tools* for the identification of a patient's tumor type and as *theranostic tools* for the selection of personalized targeted therapies. In the end, we are optimistic and hopeful that the combination of technological innovations, novel imaging probes, and the further integration of imaging into clinical protocols will lead to significant and lasting improvements in the prognosis and care of ovarian cancer patients.

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FIGURES AND FIGURE CAPTIONS

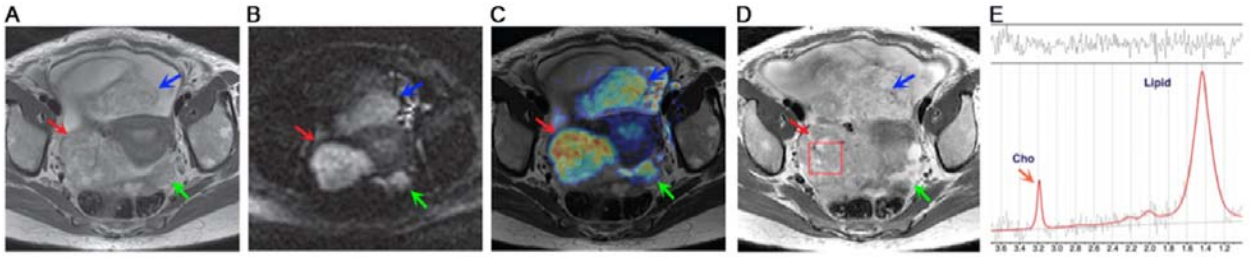

Figure 1. Multiparametric pre-treatment MR images (3.0 T) of the primary ovarian tumor (red arrow), the omental cake (blue arrow) and the peritoneal implant (green arrow) in a 58-year-old woman with advanced ovarian cancer. (A) T2-weighted image; (B) DW image (b = 500 sec/mm^2); (C) fused T2 and DW image; (D) T2-weighted image showing the ROI for MR spectroscopy (red) from the primary tumor; (E) spectral fit (red) overlaid on the raw MRS data (grey), illustrating a strong choline (Cho) signal (orange arrow). Adapted and reprinted with permission from Sala *et al.* (*12*).

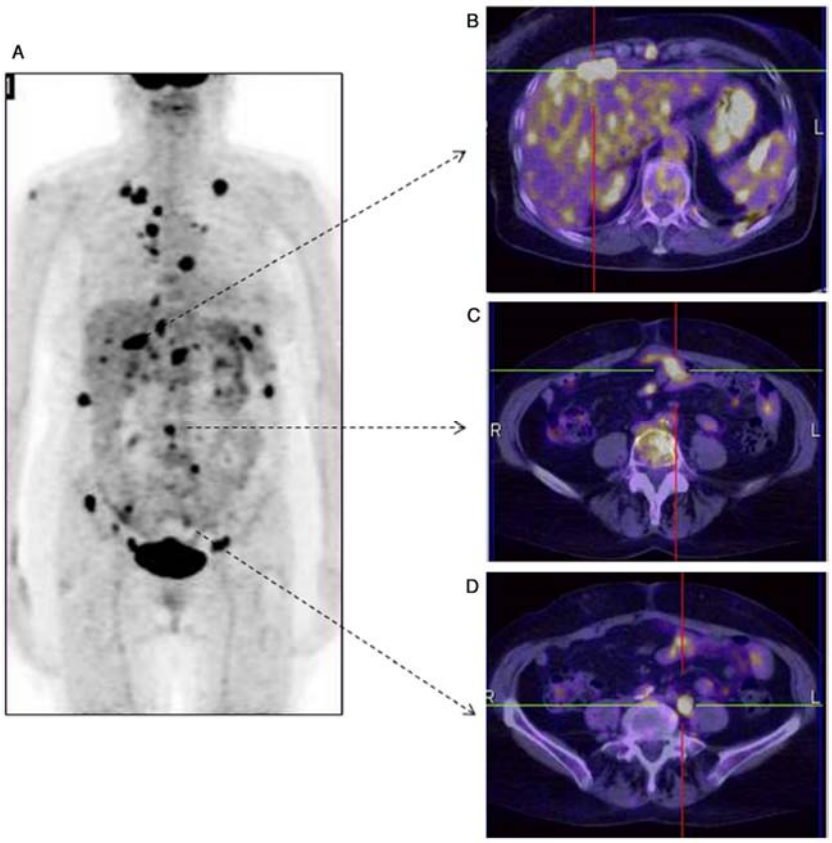

Figure 2. FDG-PET/CT of recurrent ovarian cancer in a patient 10 months after treatment with radical surgery and adjuvant chemotherapy. (A) Maximum intensity projection FDG-PET; (B-D) transaxial fused PET/CT images delineating uptake of the radiotracer in the liver (B), the peritoneum (C), and the locoregional lymph nodes (D). Adapted and reprinted with permission from Caobelli *et al.* (*16*).

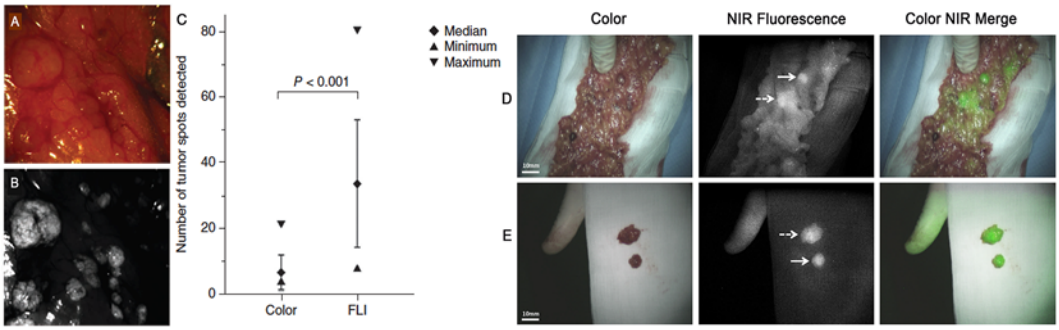

Figure 3. Identification of metastatic ovarian cancer deposits via intraoperative and *ex vivo* fluorescence imaging. Comparative images of tumors within the abdominal cavity visualized under white light (A) versus fluorescence emissions from a folate receptor-targeted probe (B); (C) statistical analysis revealing the benefit of intraoperative fluorescence imaging; (D) identification of two metastatic lesions in the greater omentum using the indocyanine green dye for intraoperative near infrared imaging; (E) *ex vivo* visualization of the resected metastatic lesions. Adapted and reprinted with permission from van Dam *et al.* (*31*) and Tummers *et al.* (*33*).

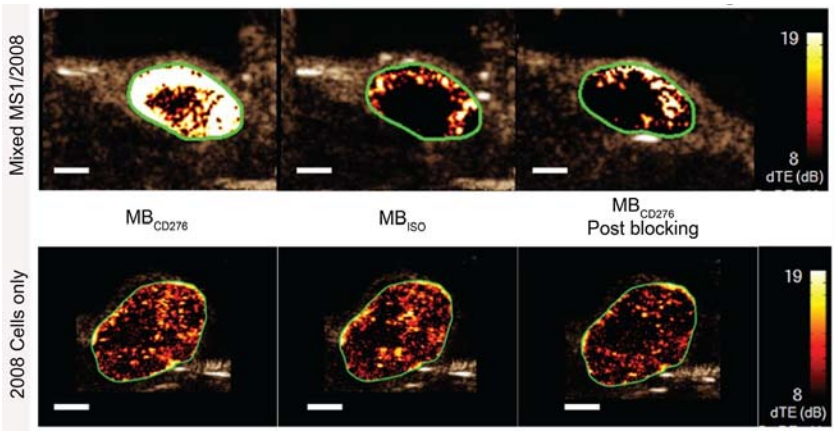

Figure 4. Preclinical contrast-enhanced ultrasound imaging of angiogenesis. Microbubbles functionalized with a CD276-targeting antibody (MB_{CD276}) effectively targeted subcutaneous xenografts composed of 2008 human endometrioid ovarian cancer cells mixed with the CD276-expressing MS1 mouse endothelial cells. The specificity of targeting was demonstrated via comparison with an isotype antibody-functionalized microbubbles (MB_{ISO}) as well as a blocking experiment in which an excess of CD276 antibody was administered. Adapted and reprinted with permission from Lutz *et al.* (*42*).

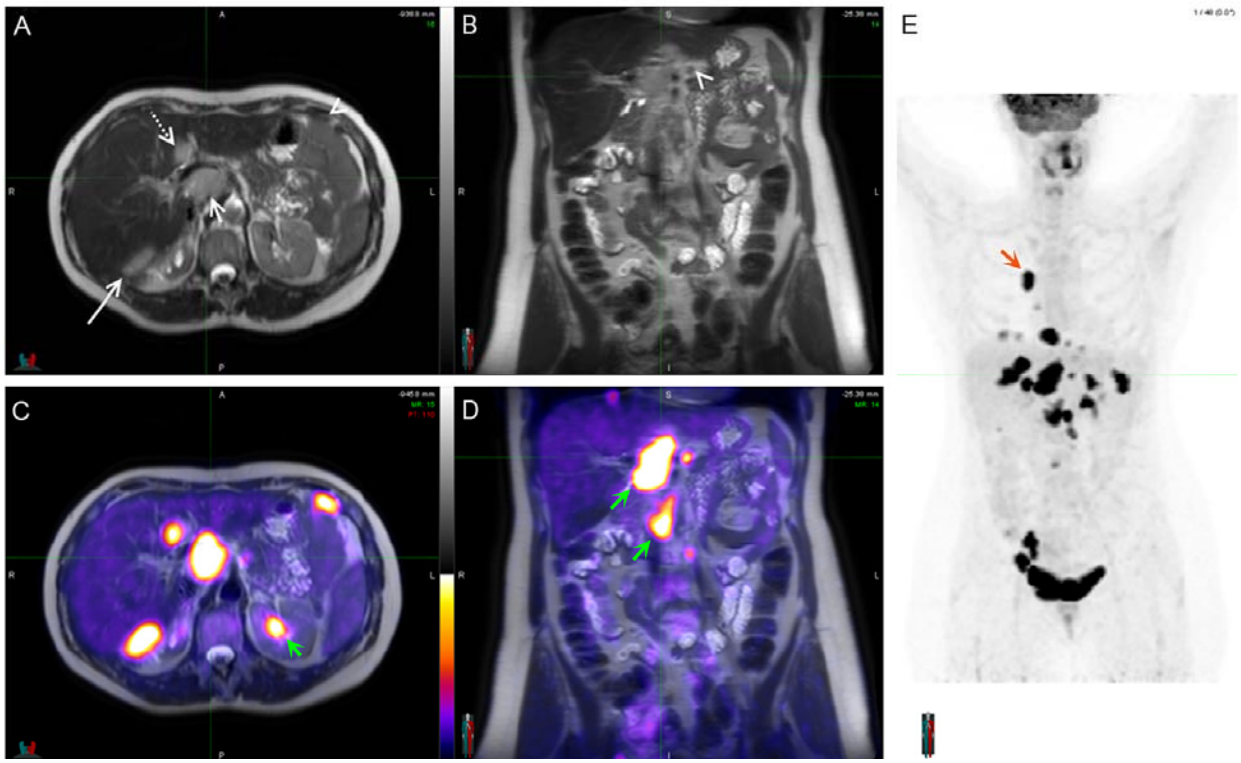

Figure 5. PET/MRI: An emerging hybrid imaging technique. Axial (A) and coronal (B) T2-weighted MRI scans of an ovarian cancer patient, in which tumor lesions were seen adjacent to the liver (long arrow), segment IV of the liver (dotted arrow), *porta hepatis* (short arrow), and peritoneum (arrowheads); fused axial (C) and coronal (D) PET/MRI scans wherein FDG-PET not only demonstrated excellent correlation with lesions identified previously by MRI alone but also highlighted new lesions (green arrows); (E) whole body MIP from FDG-PET showing multiple lesions in the chest (orange arrow) and abdomen. Adapted and reprinted with permission from Partovi *et al.* (*43*).