

## Imaging Tumor Phenotype: 1 Plus 1 Is More Than 2

An increasing trend in cancer treatment is personalized therapy, individualized for each patient based on patient characteristics and the biology of the patient's tumor (1). A key component of personalized cancer therapy is the ability to measure tumor phenotypic features to predict clinical behavior, for example, the propensity for progression and metastasis, and to select therapy with a high likelihood of success (2). An early example is seen in the endocrine treatment of breast cancer, in which assay of tumor biopsy material for estrogen receptor (ER) expression predicts both the prognosis—that is, the aggressiveness

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of the cancer—and the likelihood of response to ER-directed therapy such as tamoxifen or letrozole (3). Recent advances in molecular biology have led to more sophisticated methods for measuring tumor phenotype that can simultaneously assay a variety of tumor phenotypic features, up to thousands in the example of gene expression arrays (4). These assays have shown considerable early promise in predicting tumor behavior and response to therapy. For example, a 16-gene quantitative expression panel predicts the likelihood of tumor re-

currence in early-stage breast cancer (5) and is increasingly used to make clinical decisions about whether to use adjuvant chemotherapy. Because tumor behavior is the result of the summed effect of a large number of molecular pathways, and because pathways often interact (6), it is not surprising that assaying multiple tumor phenotypic features at the same time provides a more comprehensive and predictive measure than the measure of any single gene product.

Phenotypic characterization is most important for those tumors that display a range of biologic characteristics and clinical behavior, in which case the need for aggressive therapy and the likelihood of response may vary considerably from patient to patient or even from site to site. This is the case for the group of tumors sometimes referred to as endocrine-related cancer, which includes tumors arising from endocrine organs, for example, thyroid cancer and neuroendocrine tumors, and those tumors whose parent tissue responds to endocrine signaling, including breast, prostate, and uterine cancers. Endocrine-related cancers display a variety of clinical behaviors. They may be well differentiated, in which case they retain much of their endocrine phenotype and are often relatively indolent. They may also be poorly differentiated tumors that lose endocrine function, respond poorly to endocrine treatments, and display aggressive, frequently lethal, clinical behavior. One example is thyroid cancer, which ranges from differentiated and indolent variants that retain their endocrine function and respond to radioiodine (with retention related to endocrine function signaling a cancer of thyroid origin) to dedifferentiated cancers that do not respond to

radioiodine and are among the most aggressive tumors known (7). Another example is breast cancer, of which low-grade, differentiated forms express ER and respond to the interruption of estrogen stimulation, whereas high-grade forms often lose ER expression, respond poorly to endocrine therapy, and carry a poorer prognosis (3). The ability to characterize phenotype, including the retention of endocrine features and the degree of differentiation, can be especially helpful in directing the treatment of endocrine-related cancers.

Thus far, most work in tumor phenotyping has been performed by *in vitro* assay of tumor biopsy material. Molecular imaging, which has largely been used for tumor detection and staging, can also play an important role in measuring the tumor phenotype. Imaging has the advantages of being able to measure *in vivo* tumor behavior, characterize the entire tumor burden, and capture the heterogeneity of tumor phenotype (8). There have been some notable early studies demonstrating the ability of molecular imaging to characterize the *in vivo* phenotype of endocrine-related tumors and to predict clinical behavior. For example, studies of patients with iodine-refractory thyroid cancers have shown that a high tumor glycolytic rate measured by  $^{18}\text{F}$ -FDG PET identifies a subset with a more aggressive and resistant phenotype. The presence or absence of  $^{18}\text{F}$ -FDG uptake is strikingly predictive of survival in this patient population (9), and  $^{18}\text{F}$ -FDG PET has become an important tool for directing the treatment of iodine-refractory thyroid cancer.

As with *in vitro* assays of tumor phenotype, imaging multiple aspects

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of tumor phenotype at the same time has several potential advantages (10). Besides the ability to characterize multiple aspects of tumor biology, there may be important practical advantages in image interpretation and analysis. For example, although the dependency of partial-volume effects on tumor size creates uncertainty in measures of radiopharmaceutical uptake for a single tracer (11), these effects largely cancel when one is considering the ratio of uptake for 2 radiopharmaceuticals measured on the same imaging device. Some examples of the predictive value of imaging more than one aspect of tumor biology in the same patient include the identification of flow or metabolism mismatch as a predictor of poor response to chemotherapy in breast cancer by PET (12), the identification of the signature of iodine-responsive and -resistant cancers by a combination of iodine imaging and  $^{18}\text{F}$ -FDG PET (13), and the ability to measure both ER expression and ER pathway activation to predict response to endocrine therapy in breast cancer (14,15).

In this issue of *The Journal of Nuclear Medicine*, Tsujikawa et al. have used a combination of PET radiopharmaceuticals to image ER expression and glucose metabolism in endometrial lesions (16). Endometrial cancer is the most common gynecologic malignancy in the United States (17). The primary treatment of endometrial cancer is typically surgery (18). Adjuvant therapy is considered for patients at high risk for developing disease recurrence, and the most common forms of adjuvant therapy are chemotherapy, radiotherapy, or a combination of both. The hormonal therapy of metastatic or recurrent endometrial cancer involves mainly the use of progesterone agents, although aromatase inhibitors and tamoxifen are also being used (19). Hormonal therapy with progesterone agents has been shown to be valuable in the treatment of a small subset of patients with asymptomatic or low-grade disseminated metastases with estrogen receptor-positive and pro-

gesterone receptor-positive disease. The main predictors of response in this patient population are well-differentiated tumors, a long disease-free interval, and the location and extent of extrapelvic metastases. Unlike breast cancer, tumor ER and progesterone receptor status are not routinely assessed and are not the basis for selection of hormonal therapy in endometrial cancer.

In addition to  $^{18}\text{F}$ -FDG PET to measure glucose metabolism, Tsujikawa et al. also used  $^{18}\text{F}$ -fluoroestradiol ( $^{18}\text{F}$ -FES) PET to image regional ER expression in endometrial lesions (16).  $^{18}\text{F}$ -FES has properties similar to estradiol (20) and has been validated against in vitro assays as a measure of ER expression in breast cancer (21,22). Studies in breast cancer have demonstrated the ability of  $^{18}\text{F}$ -FES PET to image the heterogeneity of ER expression (14) and to predict the likelihood of response to endocrine therapy (15,23). In their study of endometrial lesions, Tsujikawa et al. tested the ability of  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -FES PET to distinguish between 3 classes of endometrial lesions: benign endometrial hyperplasia, lower-risk endometrial cancers, and high-risk endometrial cancers. Interestingly, whereas neither  $^{18}\text{F}$ -FDG nor  $^{18}\text{F}$ -FES uptake alone was able to classify these lesions, the combination of the 2 imaging studies, reported as the  $^{18}\text{F}$ -FDG-to- $^{18}\text{F}$ -FES SUV ratio, displayed significant differences among all 3 classes of lesions and demonstrated some ability to classify endometrial lesions into correct categories.

Although this is an interesting early result, there are some limitations of the study. The number of patients in this early study was quite small, and there was only a limited comparison between the imaging measures and histopathologic analysis of the endometrial lesions and no comparison between the PET results and in vitro assay of ER expression. It is unlikely that the proposed imaging approach will affect clinical decision making in current clinical practice because endometrial lesions are relatively ame-

nable to biopsy. The imaging studies might help direct the approach to endometrial cancer treatment; however, future studies will need to test whether  $^{18}\text{F}$ -FES or  $^{18}\text{F}$ -FDG PET can play a role in selecting therapy for patients with endometrial cancer. Besides the classification of lesions, the prediction of response to endocrine therapy through the combination of  $^{18}\text{F}$ -FES and  $^{18}\text{F}$ -FDG PET could be tested. Combined imaging may also provide a sensitive tool in monitoring the response to endocrine therapy and thus promote treatment with hormonal agents in this patient population.

Although the immediate clinical impact of the study of Tsujikawa et al. may be limited, the study is notable as an example of how the combination of 2 imaging studies may be particularly helpful in characterizing the phenotype of endocrine-related cancer.  $^{18}\text{F}$ -FES PET provided a measure of the retention of the endocrine phenotype of the parent tissue, which responds to estrogens as part of normal female reproductive physiology. On the other hand, increased  $^{18}\text{F}$ -FDG uptake provided an indication of aberrant glucose metabolism, increasingly recognized as a marker of tumor dedifferentiation from the parent tissue (24) and often associated with loss of endocrine function in endocrine-related tumors (25). The ratio of  $^{18}\text{F}$ -FDG to  $^{18}\text{F}$ -FES uptake may, in essence, provide an index of differentiation. The ratio also provides a practical first-order compensation for partial-volume effects, which are particularly problematic for the relatively flat geometry of many endometrial lesions. The study of Tsujikawa et al. supports the potential for combinations of molecular imaging studies to characterize in vivo tumor phenotype and help move toward the long-term goal of better, more individualized cancer treatment. Future studies will need to be designed to evaluate the individual ability of each imaging study to predict clinically important endpoints and to test, in a statistically rigorous fashion, whether the combination of the 2 approaches yields more

predictive value than either study alone. It may then be possible to show that when it comes to molecular imaging, 1 plus 1 is greater than 2.

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