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Building the Bridge: Molecular Imaging Biomarkers for 21st Century Cancer Therapies

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ABSTRACT (146 words)
Precision medicine, where the molecular underpinnings of the disease are assessed for tailored therapies, has greatly impacted cancer care. In parallel, a new pillar of therapeutics has emerged with profound success, including immunotherapies such as checkpoint inhibitors and cell-based therapies. Nonetheless, it remains essential to develop paradigms to predict and monitor for therapeutic response. Molecular imaging has the potential to add substantially to all phases of cancer patient care: predicative, companion diagnostics can illuminate therapeutic target density within a tumor, and pharmacodynamic imaging biomarkers can complement traditional modalities to judge a favorable treatment response. This “Focus on Molecular Imaging” article discusses the current role of molecular imaging in oncology and highlights an additional step in clinical paradigm termed a “therapeutic biomarker,” which serves to assess whether next generation drugs reach their target to elicit a favorable clinical response.

INTRODUCTION

There has been rapid progress in the development of targeted cancer therapies over the past 20 years. Cytotoxic chemotherapeutic regimens are still effective and often used, but lack specificity and frequently result in significant side-effects. One of the goals of the precision medicine era is to better tailor treatments to the individual's particular cancer. This has evolved to targeted treatments that are based on biomarkers present in the tumor (1). The therapeutic strategies have diversified in the post-genomic era, taking advantage of biologic agents, recombinant proteins, chimeric approaches, radiotherapies, and even so-called "living drugs" based on engineered cells or viruses that can sense and respond to a particular pathology. As the diversity of approaches has advanced, molecular imaging is uniquely positioned to broaden its critical role in modern therapy development, therapeutic monitoring, and response assessment (2, 3).

Molecular diagnostics such as tissue or blood-based biomarkers continue to play an important role and are often a gold-standard in terms of being both predictive and prognostic biomarkers. To ensure that the therapeutic potential of targeted drugs is realized, there has been a push for a drug-diagnostic co-development model where diagnostic tests and drugs are developed in parallel (4). For example, immunohistochemistry for estrogen receptor expression plays an important role as a biomarker forecasting tumor aggressivity and response to ER targeted therapies in breast cancer (5), and molecular characterization of hematologic malignancies such as diffuse large B cell lymphoma guides the use of modern therapies such as CAR T cells and bi-specific antibodies targeting cell surface markers like CD19 and CD20 (6). Other recent examples include peripheral blood sampling for cytokines associated with T cell activation (e.g. IL-12), and circulating tumor DNA (ctDNA) (7, 8). These examples highlight how direct tissue sampling approaches can complement circulating biomarkers that capture the state of the pathology and/or therapy in action. However, despite their benefits, all of these biomarkers are currently being assessed using *in vitro* assays with biopsied tissues and blood samples. Direct sampling approaches are limited by their invasive nature which makes repeated sampling impractical. Furthermore, direct sampling comes with the potential trade-off of failing to capture tumor heterogeneity, or assess multifocal disease, and can be prone to sampling errors and artifacts (9). Indeed, even robust gold standard techniques with biologically relevant results can be misleading as to whether a therapy may be successful. For example, PD-L1 expression was not predictive of overall response to immune checkpoint therapy with nivolumab in patients with recurrent metastatic urothelial carcinoma (10). This highlights that not only are current methods of assessing biomarkers invasive and impractical, but also might not yet be powerful enough to predict accurately whether the patient will respond to certain treatments. Thus, key developments in molecular imaging are needed to address the current limitations and to provide the information to clinicians to best tailor cancer therapies.

Here we briefly review the molecular imaging paradigm that has evolved in recent years and consider new ways of applying molecular imaging to predict and assess response to 21st century cancer therapeutics, including the unique ability of molecular imaging to capture targeted therapy delivery to tumor sites. Concepts such as integrated and integral biomarkers and the evolving use of biomarkers in oncology clinical trials – early response indicators and surrogate end-points – were discussed in more depth previously (3).

Section 1: Introduction of Types of Biomarkers

We consider a clinical imaging paradigm of informed decision-making using several branch points that includes predictive markers, "therapeutic markers", and pharmacodynamic (PD) markers (2), guided by definitions used in oncology (11). Predictive markers measure the

therapeutic target and ideally give quantitative measures of target expression at the disease site. We define the term “therapeutic biomarkers” as markers that can measure target engagement and/or occupancy to guide drug dosing, for example. PD biomarkers measure biochemical processes or phenotypic outcomes that are downstream of the target to assess whether the drug has had its intended action after treatment. This approach, illustrated in (Fig. 1), highlights serial branch points in treatment decision making. The schema emphasizes the important strength of molecular imaging markers to assess target engagement, described later in the text. These biomarkers leverage the capability of molecular imaging to assess drug-targeted engagement at each cancer site and can guide therapeutic dosing to determine whether the drug reaches its target at a sufficient dose.

Before discussing each type of biomarker in more depth with relevant examples, it should be noted that there are numerous pathologic biomarkers and their partner radiotracers which can be used in different capacities at multiple points in the paradigm. For example, the PET estrogen receptor imaging described below can be used as both a predictive and therapeutic biomarker. A key concept in our framework (Fig. 1) is that the timing of imaging dictates the primary capacity in which the biomarker is functioning and providing useful information. In general, a predictive biomarker describes an assessment before treatment. Therapeutic and PD biomarkers are assessed during treatment, typically early after treatment has been applied. While all markers are in some sense predictive of later response, a distinction is made between marker that predict response in advance of treatment (predictive) versus those that require a short exposure to treatment (therapeutic, PD).

Section 2: Predictive Markers

A well-known example of a predictive biomarker is PET imaging of ER expression using [^{18}F]-fluoroestradiol (FES). [^{18}F]-FES uptake has been shown to strongly correlate with ER expression measured by conventional tissue-based assays (12). Clinical studies have demonstrated a robust correlation between baseline [^{18}F]-FES uptake levels and therapeutic response, with [^{18}F]-FES uptake being highly predictive of breast cancer responsiveness to ER-targeted endocrine therapies and aromatase inhibitors. More importantly, [^{18}F]-FES PET has a high negative predictive value with the lack of uptake strongly suggesting a lack of response, demonstrating how the assessment of ER status with [^{18}F]-FES can thus be used to select patients whose tumor expresses the therapeutic target and guide therapy.

Predictive marker imaging can also be used to guide radionuclide therapy. An example includes the theranostic pairing of [^{68}Ga]-DOTATATE (NETSPOT) with [^{177}Lu]-DOTATATE (LUTATHERA), a somatostatin-targeted peptide receptor radionuclide therapy (PRRT) for the treatment of neuroendocrine tumors (NETs) (13). For radionuclide therapy, one can also consider radiopharmaceutical dosimetry, specifically the estimation of radiation dose to normal organs from the radiopharmaceutical, as an important predictive marker for guiding therapy (14). An important area of ongoing dosimetry research is the ability of imaging to assess tumor dose in addition to normal organ exposure, as a key predictive measure for tumor response. For this task, closely paired diagnostic agents and quantitative tomographic imaging can provide a good estimate of disease targeting to optimize treatments choices and radiopharmaceutical dose selection (14, 15), in an elegant use of paired diagnostic/therapeutic radiopharmaceutical - i.e., a “theranostic” approach.

While the above examples have been in development for over a decade, many promising predictive markers are in the pre-clinical stage for emerging therapies. Poly[adenosine diphosphate (ADP) ribose] polymerase-1 (PARP-1) has emerged as an attractive anti-cancer

target given its role in DNA damage repair and the development of PARP inhibitors (PARPis) are on the rise for the treatment of various types of cancers (16). A radiotracer developed based on the PARPi AG14699, [^{18}F]-FluorThanatrace (FTT), is currently at the stage of validation against tissue-based studies for breast and ovarian cancer to assess its predictive values, and has the potential to be a clinical predictor of response to PARPi therapies (17-19).

The use of predictive markers has extended into new classes of therapies to support patient selection and response prediction. The emergence of immunotherapies has motivated antibody-based, immuno-PET probes to be developed for imaging therapeutic targets. For example, [^{89}Zr]-atezolizumab, an anti-PD-L1 antibody, has been developed to assess PD-L1 expression on cancer cells to predict benefit from PD-1/PD-L1 checkpoint blockade therapy (20) (Fig. 2A). Initial results from clinical studies have demonstrated that [^{89}Zr]-atezolizumab tumor uptake positively correlates to the responsiveness of tumor to PD-L1 blockade therapy with atezolizumab and to both progression-free survival (PFS) and overall survival (OS). Furthermore, PD-L1 status evaluated by PET imaging has been shown to better predict the clinical response compared to immunohistochemistry or RNA-sequencing-based biomarkers (20). Imaging of checkpoint protein receptor CTLA-4 with [^{89}Zr]-ipilimumab is currently being studied in a clinical trial setting (NCT03313323) to determine the correlation between tumor uptake of radiolabeled ipilimumab and response to ipilimumab therapy.

To date, tumor-specific and therapy-specific markers have been the dominant classes of predictive PET imaging biomarkers. A deviation in this trend is noted with the emergence of PET tracers developed to image pan-tumor markers, such as fibroblast activation protein (FAP) in the tumor microenvironment (TME) (21). FAP-specific enzyme inhibitor (FAPI) has been radiolabeled with ^{68}Ga to image FAP+ reactive stromal content in various solid tumors with high contrast ([^{68}Ga]-FAPI) (22). The DOTA-coupled, chelated nature of the radiotracer highlights that the radioisotope can easily be switched with a therapeutic isotope such as ^{177}Lu, enabling the potential theranostic pairing of [^{68}Ga]-FAPI imaging with [^{177}Lu]-FAPI therapy. Given its selective expression in tumor stromal cells, FAP has also emerged as a promising stromal cell target for solid cancer immunotherapy, including CAR T cell therapy, portending the use of FAP PET imaging as companion diagnostics for FAP CAR T cell therapy to assess the biodistribution of the target (23).

Section 3: Therapeutic Biomarkers

Therapeutic biomarkers, which are focused on assessing whether the therapy has reached the target, is a rapidly evolving area in molecular imaging. Traditional examples include diagnostic and therapeutic radiopharmaceuticals (e.g. previously mentioned neuroendocrine tumor agents), including therapeutic agents that intrinsically emit a measurable signal, such as ^{131}I therapy (24). Dosimetry with ^{131}I allows optimal dosing in refractory thyroid cancers yielding measurements that optimize the radiotherapy dose to the target without surpassing toxicity limits to key organs such as the lungs and the bone marrow. With advent of quantitative molecular imaging biomarkers, imaging can also assess drug dosing for standard therapeutics. An initial study performed to measure estrogen receptor (ER) availability with [^{18}F]-FES before and during fulvestrant therapy demonstrated that residual [^{18}F]-FES uptake following treatment is associated with early clinical disease progression (discussed in (12)). This observation motivated the use of [^{18}F]-FES to measure target engagement of selective estrogen receptor degrader (SERD) drugs for determining optimal dose to achieve clinically significant ER inhibition (Fig. 2B), and is now increasingly applied in the development of new SERD agents (25).

The expansion and translation of imaging-based therapeutic monitoring may be even more critical for 21st century therapies, including immunotherapies and so-called “living drugs” that entail gene and cell therapies. “Living drugs” are uniquely challenging to monitor because of their dynamic behavior *in vivo*. For example, a cell therapy may undergo autonomous regulation with dynamic expansion upon target recognition which includes orders of magnitude increases in the number of therapeutic cells and related contraction in cell number following target clearance. This type of therapy does not follow the traditional pharmacokinetics principles developed around conventional chemotherapeutic and biologic drugs (26). Furthermore, the dynamic and self-regulating underpinnings of a living drug approach magnifies the concern for on-target, off-tumor toxicities, such as a case of pulmonary toxicity and subsequent death of a CAR T treated patient thought to be related to the native expression of ERBB2 on normal lung tissue (27). Therefore, developing therapeutic biomarkers for cell-based therapies to better understand the dose and dynamic behaviors of the “living drugs” is a key milestone.

There are two main strategies for tracking “living drugs” over time: direct and indirect labeling methods (28). For direct labeling methods, cells are labeled with imaging agents such as [^{89}Zr]-oxine and monitored over a time course of hours to days. Weist et al. recently demonstrated [^{89}Zr]-oxine labeling of IL13Rα2-targeted and prostate stem cell antigen (PSCA)-targeted CAR T cells to monitor *in vivo* trafficking to glioblastoma tumors and subcutaneous prostate tumors, respectively (29). The oxine-labeled CAR T cells were detectable for at least 6 days following labeling, and the labeling did not result in a significant reduction in functionality of the CAR T cells.

While relatively straightforward and inexpensive, direct labeling strategies are hampered by dilution of signal upon cell division and death, and is therefore not amenable to medium or long-term monitoring of therapy (28). Two alternative approaches include using cell surface labels (e.g. ICOS or CD8) coupled with immunoPET radioprobes or reporter gene imaging platforms. In the latter, living drugs such as CAR T cells are transduced with a reporter gene of interest that can be specifically targeted via a complementary radioprobe, allowing for imaging over the entire lifetime of the cell with the faithful signal amplification with each cell division (28). One of the most extensively studied PET reporter genes is HSV1-tk. HSV1-tk-engineered cytotoxic T lymphocytes expressing IL-13 CAR to target human gliomas post surgical resection, were monitored using a radiolabeled analog of the anti-herpes drug penciclovir [^{18}F]-FHBG. The PET imaging of [^{18}F]-FHBG demonstrated CTL accumulation in the areas of the tumor, noting that [^{18}F]-FHBG does not naturally cross the intact blood brain barrier (30).

FOLH1 encodes for prostate-specific membrane antigen (PSMA) and is example of a PET reporter gene that has gained a lot of attention given its human origin. It has been engineered into CD19 CAR T cells to assess its trafficking to CD19-expressing tumor cells in mice with high sensitivity (31). An elegant finding from this work was the demonstration that the number of intratumoral CAR T cells derived from the PET images did not correlate with the T cell counts in the blood, suggesting that the peripheral blood may not reflect the degree to which tumors are infiltrated with CAR T cells, which is therapeutically relevant information.

We recently described *E. coli* dihydrofolate reductase (eDHFR) and radiotracer derivatives of its highly specific small-molecule inhibitor trimethoprim (TMP) as a promising reporter gene-probe pair for monitoring CAR T cells. [^{18}F]-TMP imaging showed early residence of eDHFR-expressing GD2-targeted CAR T cells in the spleen by day 7, followed by on-target accumulation in GD2+ tumor by day 13 (32). *Ex vivo* anti-human CD8 immunohistochemistry (IHC) showed that as few as 11,000 CD8 eDHFR-expressing CAR T cells per mm^3 of tumor tissue could be detected.

Beyond CAR T cells, reporter gene imaging approaches have been established as a common platform to monitor other 21st century therapies. Notably, gene therapy / gene replacement with adeno-associated virus (AAVs) and cancer therapies using oncolytic viruses (OVs) are important fields where the penetrance and durability of the viral vector often has impact on the therapeutic outcome (33, 34).

Section 4: Pharmacodynamic (PD) Biomarkers

As the above paradigms are geared for making proper clinical management choices from the beginning of a therapeutic intervention (predictive) and measuring whether the drug reaches its target (therapeutic), crucial downstream measures of efficacy can be assessed with pharmacodynamic (PD) biomarkers. These markers are well known to the nuclear medicine community and have been studied for decades. Two key classes of PD markers are metabolic and proliferative measures. The most widely adopted metabolic radiotracer is [^{18}F]-FDG, which functions as a measure of glycolysis (35). Many clinical patient streams rely on PET/CT imaging with [^{18}F]-FDG for diagnosis, including lymphoma, head and neck tumors, high-risk skin cancers (e.g. melanoma), and breast cancer patients (36). These patients are treated with diverse chemotherapeutic, biologic, immunotherapeutic, and now cell therapy approaches, and [^{18}F]-FDG remains a crucial tool to understand the glycolytic response. Not only have National Comprehensive Cancer Network (NCCN) guidelines been developed to include PET/CT with input from referring clinicians, but also routine response criteria such as PERCIST have been developed to standardize results communication (37). On the horizon, metabolic markers biomarkers such as amino acid derivatives related to acetate and glutamine are in development. These may be applied in certain clinical situations as surrogates for understanding specific intracellular processes such as fatty acid synthesis or transport of amino acids, particularly in non-[^{18}F]-FDG avid tumors and for metabolically targeted therapies (38, 39).

Alternatively, a therapy may impact the phenotype of the disease process, a feature for which quantitative molecular imaging is ideally suited. An exciting recent example of imaging a pharmacodynamic response to estrogen challenge was illustrated by Dehdashti and colleagues (40). This approach leverages the observation that stimulation of pathways downstream of the estrogen receptor in breast cancer lead to increased expression of the closely related progesterone receptor (PgR). They showed that an increase in the uptake of the PgR radiotracer, ^{18}F-fluorofuranylnorprogesterone (FFNP), in response to estradiol challenge is a potent way to assess for ER receptor function that can predict breast cancer response to endocrine therapy. PD biomarkers for biologics and living drugs also have great potential, especially with respect to immune-oncology.

The focus of imaging PD markers is no longer solely on the tumor itself but rather on the immune system. For example, CDT8 minibodies image immune cells in inflammatory conditions and cancer, and can be used in conjunction with a baseline image to understand how the immune system responds to immunotherapy such as a checkpoint inhibitor (anti-CTLA4 or anti-PD1) (41). Another notable example that is nearing clinical translation is the use of ICOS to monitor STING agonist immune activation or CAR T cell trafficking (42, 43). Xiao et al. showed that immunoPET imaging of ICOS with [^{89}Zr]-DFO-ICOS enabled specific detection of activated T cells and their coordinated immune response in response to STING and PD-1 checkpoint blockade in the setting of Lewis lung cancer models, highlighting the promising potential of ICOS imaging as a way to monitor T-cell-mediated immune response to various immunotherapies (Fig. 2C**).** They also demonstrated that ICOS signal is detectable prior to changes in tumor volume, suggesting that ICOS imaging will allow for highly sensitive, early detection of response compared to traditional anatomical imaging approaches.

Despite the success of biologic therapies and antibodies (including bi-specifics), integration of similar such protein-based imaging tools into the clinical paradigm has been challenging. This is in part due to the long circulation time of many of these therapies themselves and the practical challenges of imaging full-length antibodies for example. For 21st century therapy imaging, small molecules and/or immune molecules engineered specifically for imaging will continue to play a large role and efforts to make smaller biologic probes using shorter half-life radioisotopes have shown promise (41).

Conclusion:

The use of imaging biomarkers, in their diverse capacities, can impact and improve upon a one-size-fits-all approach to medical diagnosis and treatment. Precision medicine promises that, with a deep understanding of the molecular mechanisms and pathology heterogeneity, tailored therapies can be prescribed for the improved treatment and health of patients. This molecular imaging biomarker paradigm for both cancer clinical trials and future clinical applications serves as a reference for basic scientific developments in the field of cancer molecular imaging and a formulaic approach to guide clinical trials. Imaging serves to complement diagnostics based on *in vitro* assays and tissue sampling, especially in terms of predictive biomarkers. Optimizing dose regimen and the whether the drug reaches the pathology is a key component of therapeutic biomarkers, and finally pharmacodynamic biomarkers are used to assess the downstream processes that are affected by the drug and ultimately entail the tumor response to therapy.

The future outlook for imaging biomarkers continue to be bright. As there has been an acceleration in the development of living drugs and new 21st century therapeutics, the field of molecular imaging should be positioned to meet the needs of pharmaceutical development efforts in terms of companion diagnostics and therapeutic biomarker drug assessment, with an eye toward clinical applications and integration.

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MAS is an IP inventor and equity holder in Vellum Biosciences, a company supporting the commercialization of TMP radiotracers. DAM is an inventor on TMP radiotracers and also has financial conflict of interest related to licensing of a PET agent for PARP imaging.

FIGURES

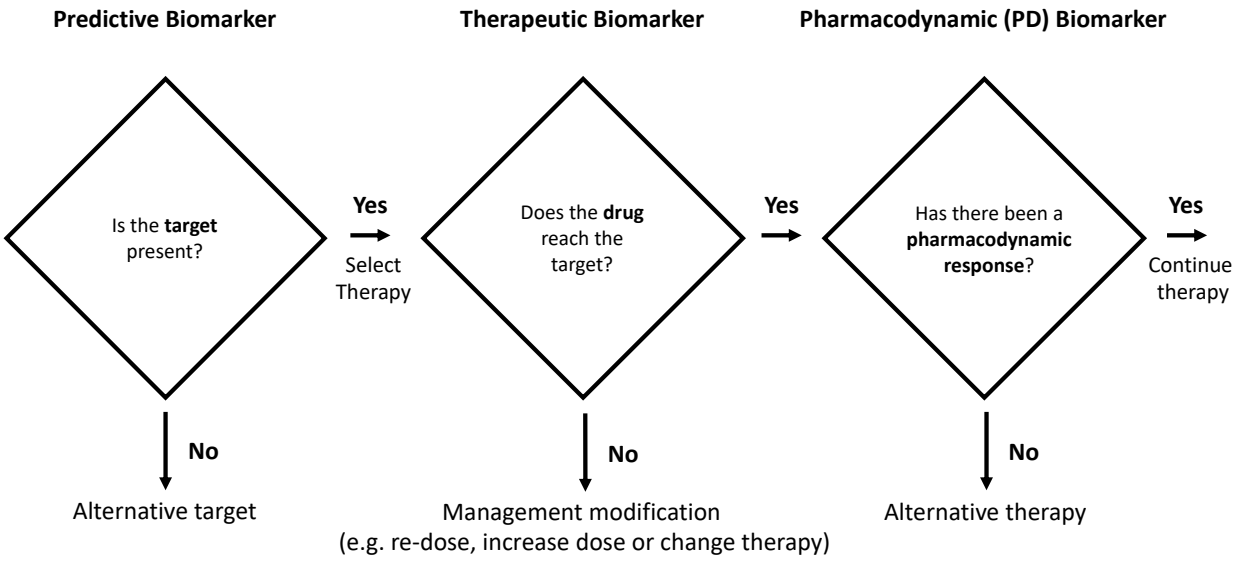

Figure 1: **A clinical decision pathway incorporating imaging biomarkers.** This path highlights three different potential roles of molecular imaging as a predictive, therapeutic, and pharmacodynamic (PD) biomarker. Updated from (2).

Figure 2

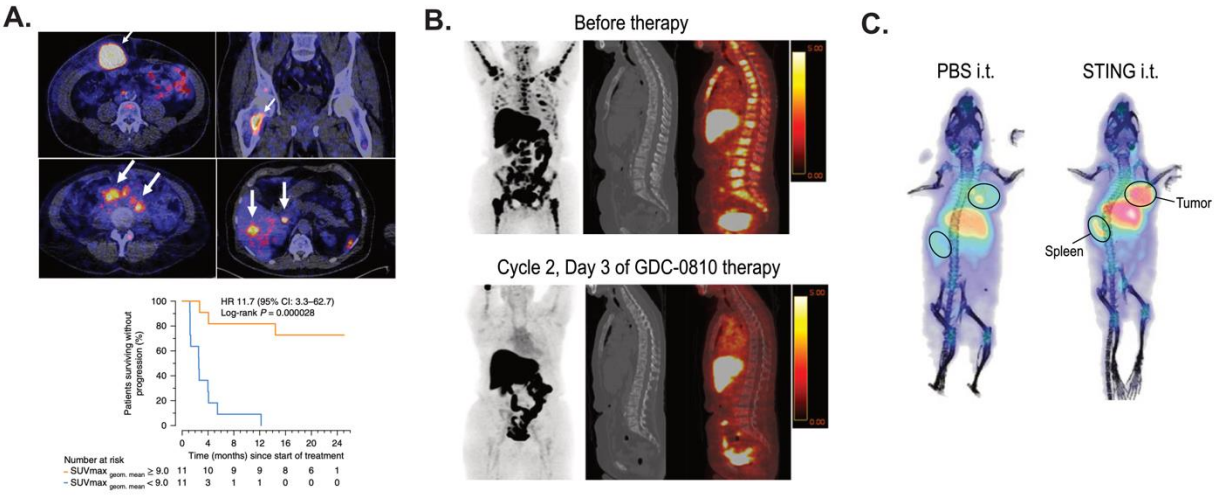

Figure 2: **Examples of predictive (A), therapeutic (B), and pharmacodynamic (PD) (C) markers for 21st century therapies**. **A)** [^{89}Zr]-atezolizumab tumor uptake predicts clinical response to PD-L1 blockade therapy and the overall progression-free survival. Originally published in (20). **B)** [^{18}F]-fluroestradiol (FES) PET/CT images demonstrate complete suppression of FES-avid lesions with ER-targeting therapeutic GDC-0810, highlighting the potential role of [^{18}F]-fluoroestradiol (FES) PET/CT as a biomarker of estrogen receptor (ER) occupancy and/or downregulation for determining dosages of various ER-targeted therapeutics**.** Originally published in (25). **C)** ImmunoPET imaging of [^{89}Zr]-DFO-ICOS on Day 2 post tracer administration shows increased uptake of the tracer by ICOS-positive, activated T cells in the tumor, TDLN, and spleen of mouse treated with STING i.t. compared to PBS i.t.. Originally published in (43).

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