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Molecular Image-Directed Biopsies: improving clinical biopsy selection in patients with multiple tumors

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Abstract

Site selection for image-guided biopsies in patients with multiple lesions is typically based on clinical feasibility and physician preference. This study outlines the development of a selection algorithm that, in addition to clinical requirements, incorporates quantitative imaging data for automatic identification of candidate lesions for biopsy. The algorithm is designed to rank potential targets by maximizing a lesion-specific score, incorporating various criteria separated into two categories: 1) physician-feasibility category including physician-preferred lesion location and absolute volume scores, and 2) imaging-based category including various modality and application-specific metrics. This platform was benchmarked in two clinical scenarios, a pre-treatment setting and response-based setting using imaging from metastatic prostate cancer patients with high disease burden (multiple lesions) undergoing conventional treatment and receiving whole-body [¹⁸F]NaF PET/CT scans pre- and mid-treatment. Targeting of metastatic lesions was robust to different weighting ratios and candidacy for biopsy was physician confirmed. Lesion ranked as top targets for biopsy remained so for all patients in pre-treatment and post-treatment biopsy selection after sensitivity testing was completed for physician-biased or imaging-biased scenarios. After identifying candidates, biopsy feasibility was evaluated by a physician and confirmed for 90% (32/36) of high-ranking lesions, of which all top choices were confirmed. The remaining cases represented lesions with high anatomical difficulty for targeting, such as proximity to sciatic nerve. This newly developed selection method was successfully used to quantitatively identify candidate lesions for biopsies in patients with multiple lesions. In a prospective study, we were able to successfully plan, develop, and implement this technique for the selection of a pre-treatment biopsy location.

Keywords

Positron Emission Tomography (PET); Musculoskeletal Biopsy; Bone Biopsy; Quantitative Imaging; Metastatic Prostate Cancer; Treatment Response; Image-Guided Biopsy

1. Introduction

Acquisition of diseased tissue is an essential diagnostic tool in oncology, most often performed via small needle biopsy. Given the vast spatial and temporal heterogeneity found in tumors, adequate sampling of diseased tissue is crucial to clinical treatment decisions (Gerlinger *et al.*, 2012; Shibata and Shen, 2013; Dexter and Leith, 1986). However, tissue acquisition techniques themselves do not reflect the level of biological sampling required to account for disease heterogeneity as its analysis only encompasses the small, localized area of a single tumor that is biopsied. (Dawood and Gonzalez-Angulo, 2012; Gogna *et al.*, 2008). Currently, biopsies are largely biologically non-discriminatory and acquired based on feasibility of the procedures, leading to random sampling (Larousserie *et al.*, 2012; Bombardieri *et al.*, 2010). Interpretation of the biologic significance of such samples can be misleading, especially in cases of patients with multiple lesions where investigation of all lesions is not reasonable.

In patients with multiple lesions, image-guided biopsies often aim to target the largest, most superficial lesion (Tehranzadeh *et al.*, 2007). While it is recommended that more than one biopsy sample is acquired in cases where lesions exhibit stark difference in appearance, this is not common practice (Amir *et al.*, 2008; Amir *et al.*, 2012; Simmons *et al.*, 2009; Kobayashi *et al.*, 2012; Hilton *et al.*, 2011; Bingham, 2002). This problem is further highlighted in aggressive metastatic diseases, such as prostate and breast cancers that often spread to bone. Recent works have shown discordance in the molecular profile between primary tumors and metastases, as well as across different metastases (Nakamura *et al.*, 2013; Vignot *et al.*, 2012; Vignot and Soria, 2013; Vignot *et al.*, 2013). Particularly of concern is successful targeting and sampling of these lesions. In a recent study, only 21 of 54 (39%) tissue samples produced high enough yield for subsequent genetic analysis in tissue obtained from bone biopsies, both planned and guided by anatomical imaging only, in metastatic prostate cancer patients (Spritzer *et al.*, 2013).

In conditions where there are multiple lesions that have a malignant potential, it can be very difficult to recognize cancerous lesions without the aid of an imaging agent (Bingham, 2002). While imaging can be used to survey total disease burden of the patient by capturing biological characteristics of all lesions, it does not match the breadth of biological characterization possible that can be obtained from specific analysis of biopsy samples from individual lesions (Morgan, 2011; Tan *et al.*, 2009). Combination of these two techniques could lead to a vast improvement in precision medicine initiatives (Hilton *et al.*, 2011). We have developed a novel technique for biopsy target selection in patients with multiple lesions, incorporating clinically relevant physician preferences and biologically relevant imaging data.

This work provides proof-of-principle analyses for our technique, guiding biopsy site selection using Positron Emission Tomography (PET) data from a population of metastatic prostate cancer patients. The high disease burden of metastatic prostate cancer leads to many biopsy targets, yet diagnostic yield accuracy is historically low (Wu *et al.*, 2008). PET/CT provides a molecular imaging platform from which biologically relevant information related to disease potential can be used to monitor patients with multiple lesions (Gambhir, 2002;

Weissleder, 1999; Morisson *et al.*, 2013). In a study of PET/CT guided bone biopsies in metastatic breast cancer, CT was useful in identifying precise location of bone lesions when used in conjunction with PET, but only revealed distinct characteristics of disease on CT in half of the highly PET-positive lesions (Lavayssière *et al.*, 2009; Nakamoto *et al.*, 2005). [^{18}F]NaF PET/CT is a strong candidate for not only imaging these metastatic lesions, but also for treatment response assessment of patients (Scher *et al.*, 2013; Klaeser *et al.*, 2010). We have identified two clinically relevant settings for biopsy target selection for this validation study. Single-timepoint NaF PET/CT imaging data was used to show feasibility in a pre-treatment biopsy setting. Lesion-specific response to treatment was quantified by longitudinal NaF PET/CT imaging data and was used to show feasibility in a post-treatment response-based biopsy setting.

2. Materials and Methods

2.1. Overview of methodology

The proposed biopsy site selection methodology is based on a patient-specific objective function, constructed as the weighted global sum of individual selection criteria (variables) separated into various clinically-relevant categories. These categories are assigned based on a variable's relative value within competing targets such as image-derived properties, physician-related procedural requirements, or risk functions for robotic-guided biopsies. Selection of biopsy targets for each patient can then be obtained based on inter-lesion score ranking. Separation of categories is critical for the assessment of selection performance, as well as minimizing bias between categories as they contain independent sets of variables.

Our goal is to guide selection by targeting highest scoring lesions within a patient. A weighted global sum is used for balancing quantitative and semi-quantitative variables without making assumptions of the underlying function for each (Marler and Arora, 2004). Scalar bias between variables was avoided by normalizing to the maximum value determined from all lesions for a given variable, $f_j^i(k)$, shown as

$$F_j^i(k) = \frac{f_j^i(k)}{\max_{x:1 \rightarrow k} f_j^i(k)} \quad (1)$$

Where $F_j^i(k)$ is the normalized value for each variable, j , contained in category, i , for lesion, k . Note the maximum in equation (1) is only taken from the population of lesions within a single patient. The generalized objective function for this technique is written as

$$S(k) = \frac{1}{\sum_{i=1}^N w_i} \cdot \sum_{i=1}^N \left[\frac{w_i}{n_i} \sum_{j=1}^{n_i} F_j^i(k) \right] \quad (2)$$

Where $S(k)$ is the lesion-specific selection score calculated across N total categories, each weighted by category-specific factor w_i and scaled according to the total number of variables within the category, n_i . By changing the weight, more preference to be placed on one selection category over another. Lesion-selection was defined by ranking $S(k)$ scores from highest to lowest within each patient.

2.2. Clinical Scenarios

This general technique was benchmarked in two clinical settings: first to select lesions for pre-treatment biopsy using baseline data from single timepoint NaF PET/CT imaging data and then used to select lesions for post-treatment biopsy, using response data from multi-timepoint NaF PET/CT imaging data. Aiming to increase sampling accuracy in routine clinical biopsies with the addition of imaging data, two such categories implemented in this work represent clinical feasibility metrics and quantitative imaging metrics. The workflow for this study is presented in Figure 1.

2.2.1. Clinical Feasibility Matrix—Clinical feasibility metrics represent quantifiable considerations of a biopsy procedure where a target is selected from multiple candidates such as tumor location, distance from organ/structure at risk, and size of lesion (Gogna *et al.*, 2008; Wu *et al.*, 2008). The clinical-feasibility category in this work includes two variables: physician-preferred lesion location and absolute volume scores. Physician-preferred lesion locations are selected by an interventional radiologist involved in the study. Regional preferences were determined by experience, safety/feasibility of the region (Tehranezhadeh *et al.*, 2007; Lis *et al.*, 2004), and historical success rate (Hau *et al.*, 2002; Altuntas *et al.*, 2005). A value of 1 is given to the highest preferred region (ilium), with lumbar spine, sacrum, posteroinferior ischium, femur, pubis, humerus, and anterior/posterior acetabular assigned decreasing decrements of -10% , -15% , -20% , -40% , -50% , -60% , and -90% , respectively. Absolute lesion volume is defined as the metabolically active volume determined from PET segmentation with a minimum acceptable volume criteria of 1.5 cm^3 needed for successful targeting and sampling of a lesion-of-interest.

2.2.2. Quantitative Imaging Matrix—The molecular imaging category will vary by clinical application of interest. Various metrics can be used to quantify tracer uptake within a tumor using standardized uptake values (SUV). Ideally imaging metrics incorporated in this selection algorithm would be those having shown prognostic or predictive behaviors in previous imaging studies of the disease of interest, for example SUV_{max} as a prognostic surrogate of disease aggressiveness in non-small cell lung cancer (Berghmans *et al.*, 2008). Alternative imaging features such as mean uptake (SUV_{mean}), total uptake burden ($\text{SUV}_{\text{total}}$), and mean uptake within a 1cm sphere surrounding SUV_{max} (SUV_{peak}) have shown promise as imaging biomarkers for various diseases (Chen *et al.*, 2012; Kim *et al.*, 2012; Kahraman *et al.*, 2012). These metrics have also been identified by Wahl *et al.* (2009) in recommendations for PET Response Criteria in Solid Tumors (PERCIST) for potential use as imaging response biomarkers. To date, no imaging biomarker or imaging response biomarker have been validated for metastatic lesions in bone (Scher *et al.*, 2013). Given the small tumor volumes typically found in bone, SUV_{peak} presents a challenge for robust quantitative definition and was not considered in this study. Remaining NaF PET/CT

imaging metrics evaluated for use in this study (SUV_{max} , SUV_{mean} , and SUV_{total}) have shown promising quantitative accuracy in characterizing metastatic disease (Lin *et al.*, 2016).

We additionally aim to condense the imaging category to only include metrics that provide independent quantitative information, thus ensuring the algorithm captures unique phenotypes within each lesion. To achieve this, associations between metrics and biopsy target selection were evaluated prior to final selection of imaging category for each clinical scenario. Previous works have reported on the heterogeneity of treatment response assessment when using changes from baseline of various SUV metrics (Vanderhoek *et al.*, 2013). To account for this heterogeneity, an overall response score (number of imaging metrics following similar response trends) was included to penalize lesions with mixed response across various metrics, thereby selectively targeting lesions with maximally increased or decreased imaging characteristics.

2.3. NaF PET/CT Imaging and Processing

This imaging study was performed as a pilot sub study of a multicenter clinical trial for metastatic Castrate Resistant Prostate Cancer (mCRPC) patients with bone metastases. The protocol was approved by the corresponding Institutional Review Board and the Radiation Safety Committee of each participating institution. All patients gave written informed consent to participate. 17 metastatic CRPC patients (age 47–88, mean 68.75 years) enrolled in the clinical trial received whole-body [^{18}F]NaF PET/CT scans pre-treatment (within 7 days prior to start of treatment, N=17) and mid-treatment (within 6–8 weeks after the start of treatment, N=16). All scans were acquired on a Discovery VCTTM (GE Healthcare, Waukesha, WI) scanner in 3D acquisition mode. A static PET scan was performed 60 minutes post-injection over 13 bed positions (scanned 3 minutes per bed position). Following CT attenuation-correction, all PET images were reconstructed following same standard-of-care procedure. Standardized uptake values (SUV) were calculated for each voxel as signal normalized to the ratio of injected activity and patient mass.

Bone lesion segmentation and response quantification was completed using a novel, semi-automatic technique, Quantitative Total Bone Imaging (QTBI). In short, metastatic lesions are localized and identified based on PET uptake, assisted with an anatomical CT mask applied to exclude soft tissue uptake followed by a $SUV > 15$ threshold (Lin *et al.*, 2016). The PET threshold was selected to exclude additional activity with low statistical likelihood of being abnormal bone based on previous studies (Kurdziel *et al.*, 2012; Rohren *et al.*, 2015). An experienced nuclear medicine physician reviewed lesion contours on the PET/CT images to confirm all non-disease uptake was excluded from analysis. Employing hybrid PET/CT segmentation and articulated skeletal-registration (Yip *et al.*, 2014), bone lesions were identified and tracked across scans to allow for response assessment (Yip and Jeraj, 2014). Articulated registration employs a piece-wise rigid registration of skeletal bones from CT and applies transformations to bones and lesions from PET/CT.

For both applications, location score was quantified by applying the lesion ROIs to the CT to determine bone location (Figure 1 (A)). From each scan timepoint, three SUV metrics (SUV_{max} , SUV_{mean} , and SUV_{total}) and the PET-positive (PET+) volume were reported for

each lesion. For the pre-treatment biopsy application, SUV statistics from baseline scans were considered for use in the molecular imaging category and the PET+ volume for the clinical-feasibility category (Figure 1 (B)). For the response-based biopsy application, percent change from baseline to mid-treatment scans was calculated for each SUV metric and reported for each lesion for inclusion in the molecular imaging category. The PET+ volume from the mid-treatment scan (time of biopsy) was reported for the clinical-feasibility category (Figure 1 (C)).

2.4. Algorithm Performance Analysis

Proof-of-principle for this algorithm's utility were established in a series of analyses to test the robustness of target selection, comparison to simulated clinical benchmarks, and performance in a prospective case study.

2.4.1. Metric Selection and Weighting Sensitivity Analysis—Metric selection analysis was completed to identify independent imaging metrics for use in the selection algorithm. Pairwise Pearson's R correlation coefficients were calculated between all metrics considered for use in the imaging category. $S(k)$ output was first calculated incorporating all possible metrics in the imaging category (SUV_{max} , SUV_{mean} , and SUV_{total}). Performance leverage of individual imaging metrics on selection scores was evaluated by varying the imaging category to only include each metric individually. Additionally, selection scores were calculated by varying the algorithm to include all multivariate combinations of uncorrelated metrics in the imaging category. Linear regression was performed on all variations of the algorithm and statistical differences in output were evaluated using ANCOVA analysis. All statistical analysis was performed in MATLAB (MATLAB 2015).

After determination of optimal metrics, sensitivity analysis was performed across categories. The relative weights between the two categories are largely arbitrary, and needs to be evaluated carefully. Weighting ratios between clinical and imaging categories were varied from 10:1 to 1:10. Sensitivity of the ranking and $S(k)$ scores were evaluated using Pearson's R correlation. Percent change in lesion-selection $S(k)$ scores relative to 1:1 weighting scheme was calculated for each lesion individually.

2.4.2. Simulated Clinical Benchmarks—Clinical biopsy targets were simulated to performed for benchmark analysis of the algorithm in each of the two settings. Currently, the clinical standard for core needle biopsies of the skeleton is by CT guidance (Welker *et al.*, 2000). Multiple studies have asserted the added benefit of using PET during planning or in-suit as a reference to identify active malignancies (Cerci *et al.*, 2013; Pezeshk *et al.*, 2006). As outlined by Pezeshk et al (2006), in the event of multiple PET-positive (PET+) lesions for targeting the final biopsy site is selected by interventional radiologist based on safety and accessibility. Also taken into clinical consideration is lesion size, which has been shown to play an important role in successful diagnostic targeting (Wu *et al.*, 2008). To adopt these methodologies and simulate a clinical benchmark of lesion rankings in the pre-treatment biopsy case, all PET+ lesions that met the minimum volume criteria for targeting were ranked first according to their anatomical location, and then by lesion volume for cases of

multiple lesions in a single region. Lesion rankings and PET characteristics were compared between the simulated clinical standard and our algorithm.

To our knowledge, there is no literature on techniques or recommendations for response-based biopsies in patients with multiple tumors. To evaluate the clinical utility of the algorithm to identify proper biopsy targets, 2–3 biopsy sites per patient were relayed to the interventional radiologist who simulated a clinical planning workflow for biopsy target evaluation by reviewing both anatomical (CT) and PET/CT imaging together to determine the feasibility of targeting each lesion. Each lesion was then marked “suitable” or “non-suitable”, with description for why the target failed to be feasible in the case of non-targetable lesions.

2.4.3. Case Study—A single, prospective case study was completed for pre-treatment identification of target lesions for biopsy. A 63 year old Caucasian male with a history of prostate cancer presented with suspected metastases using convention imaging techniques (planar ^{99m}Tc bone scintigraphy). For enrollment in a therapeutic clinical trial at our institution, a tumor biopsy confirming metastatic adenocarcinoma of the prostate was required. Written informed consent for NaF PET/CT imaging study and an image-guided interventional procedure was obtained. NaF PET/CT imaging was acquired under the same protocol as described in section 2.3. Image analysis and biopsy target selection using this algorithm was completed the same day as NaF PET/CT was acquired. Interventional radiologists reviewed proposed biopsy targets, NaF PET/CT and diagnostic CT imaging to confirm appropriateness of target. The biopsy was performed by experienced interventional radiologists using standard core-needle biopsy techniques.

3. Results

3.1. Pre-treatment biopsy selection

Of 800+ total lesions identified in 17 patients, 136 lesions (average 8.1 lesions/patient, range 3 – 15) met minimum criteria to qualify for biopsy in the pre-treatment biopsy application (volume $> 1.5 \text{ cm}^3$ and contained in target skeletal regions). Of imaging metrics considered, SUV_{max} and SUV_{mean} were found to be highly correlated ($R = 0.92$) while $\text{SUV}_{\text{total}}$ did not show strong correlation to either ($R=0.37$ and $R=0.24$, respectively). Based on these associations, the imaging category was varied to include each metric individually, uncorrelated combinations of metrics ($\text{SUV}_{\text{max}} + \text{SUV}_{\text{total}}$ and $\text{SUV}_{\text{mean}} + \text{SUV}_{\text{total}}$), and all metrics ($\text{SUV}_{\text{max}} + \text{SUV}_{\text{mean}} + \text{SUV}_{\text{total}}$). Using ANCOVA analysis to determine which metric(s) had the highest overall impact on lesion selection, $\text{SUV}_{\text{total}}$ was shown to be the dominate univariate metric influencing selection when regressed against method using all metrics. However, $\text{SUV}_{\text{max}} + \text{SUV}_{\text{total}}$ significantly outperformed all other tested methods with a slope closest to 1, indicating near identical agreement in selection scores with the all-metrics method (Table 1). All further algorithm sensitivity testing and piloting was completed using only $\text{SUV}_{\text{total}}$ and SUV_{max} .

Assessing sensitivity of selections scores for each lesion, $S(k)$ changed variably as weighting was altered from 1:1 to either clinically-biased (10:1) or imaging-biased (1:10). 18 lesions showing $<10\%$ change over the entire domain and 26 lesions showing more extreme

variation of >50% (Figure 2). Overall, the majority of this variation in $S(k)$ was noted as weighting of each individual category doubled (i.e. 1:2 or 2:1) before converging thereafter. Evaluating the impact of this variability on within-patient rankings, Pearson's R Correlation coefficient was used to compare absolute $S(k)$ values. Within-patient lesion rankings based on $S(k)$ values were stable 1:2-to-2:1 ($R=0.95$) for all lesions (Figure 3). Additionally, the lesion identified as having the maximum $S(k)$, ($S_{\max}$), remained top-seeded in 14/17 patients and in the top three highest ranking lesions in 17/17 patients over the domain ranging from 1:2 to 2:1 clinical:imaging.

The selection algorithm using even-weighting between physician and imaging-based scores were used to rank lesions from most-to-least preferred. The frequency of skeletal regions containing the top ranking lesions based on the selection algorithm is summarized in Table 2. The ilium and lumbar spine had the highest frequency of selected lesions regardless of burden. For each patient, the top three ranked lesions based on simulated clinical benchmark were identified and rankings compared to selection algorithm (Table 3). In 13/17 patients, the most preferred target lesion based on the algorithm was concurrent with the most preferred clinical target lesion. In one patient, the most preferred lesion based on the selection algorithm was the 3rd-rated lesion from clinical benchmark. Upon further investigation of 15 lesions meeting clinical requirements for biopsy in this patient, 7 were located within the most preferred region (ilium or iliac crest) indicating all are sufficient for targeting.

3.2. Response-based biopsy selection

In the 16 patients receiving a second mid-treatment scan, a total of 129 lesions were identified as meeting minimum requirements for biopsy (volume > 1.5 cm³ and contained in target skeletal regions). Similar to pairwise analysis of imaging metrics in pre-treatment scenario, response values of $SUV_{\max}$ and SUV_{mean} were found to be highly correlated ($R = 0.88$). While slightly higher correlation was found between response values in SUV_{total} with $SUV_{\max}$ and SUV_{mean} ($R = 0.43$ and $R = 0.31$, respectively), overall correlation was low regardless of favorable or unfavorable changes (Figure 4). In contrast to pre-treatment scenario, no single imaging metric was shown to outperform the others in univariate regression. All combinations of uncorrelated multivariate methods showed significantly higher slope values than any individual metrics when regressed to method incorporating all imaging response metrics (Figure 5). No statistically significant differences were found between the multivariate methods, SUV_{total} and $SUV_{\max}$ response metrics ($b = 0.74$, $SE = 0.02$) were used in the imaging category for the remainder of this validation study, as in the absence of statistical superiority, they had slope closest to 1.

Comparing sensitivity of lesion-specific selection scores to the pre-treatment biopsy selection, $S(k)$ scores generally showed higher variability but no major differences in trends were noted (results not shown). Within-patient lesion rankings based on $S(k)$ values were stable from 1:1-to-2:1 ($R=0.94$), 1:1-to-1:2 ($R=0.95$), and from 1:2-to-2:1 ($R=0.80$) for all lesions (Figure 6).

As weighting was varied from even clinical:imaging to clinically-biased (2:1) and imaging-biased (1:2), the lesion identified as the top-candidate by $S_{\max}$ remained top-seeded in 11/16

patients. In one example patient, $S_{\max}$ was inconsistent amongst lesions in clinical-biased rankings due to small variation in $S(k)$ between top three lesions (Figure 7). Lesion #1 has the highest ranking for even-weighting and imaging-biased ranking due to extreme increase in both imaging metrics throughout therapy. Lesion #2 and Lesion #3 each saw substantial decreases in both imaging metrics throughout therapy. Selection scores for Lesion #2 and Lesion #3 are within 0.05% of each other for even-weighting, with each showing marginal favorability in physician-biased and imaging-biased selection, respectively. Discretion of the physician would be used for final selection in this case. Across all patients, $S_{\max}$ ranged from <1%–27% greater than the next highest scoring lesion. Evaluating this further, the top-candidate lesion remained in the three highest ranking lesions in all patients over the 1:2-to-2:1 domain.

A clinical benchmark was simulated by physician review/planning of selected lesions. Two or three optimal candidates per patient were sent to the physician. Biopsy feasibility was confirmed by the physician for 32/36 high-ranking lesions, of which all top choices were confirmed as feasible targets. Four lesions were deemed inappropriate for sampling due to complications stemming from proximity to either degenerative bone disease or sciatic nerve.

3.3. Case Study

This selection technique was applied in a single prospective case study. Six of the patient's 100+ lesions identified the baseline NaF PET/CT scan met minimum physician-based criteria for biopsy, they are shown in Figure 8(a). $S(k)$ values were stable over entire weighting domain for all lesions with a single optimal candidate consistently outperforming remaining lesions (Figure 8(b)). This analysis along with NaF PET/CT scan was delivered to interventional radiologists performing the biopsy procedure and target lesion feasibility was initially confirmed. However, prior to the procedure, a second diagnostic CT revealed angle of approach originally determined to be feasible was in close proximity to sciatic nerve bundle, as shown in Figure 8(c).

Following procedure, the next highest ranking lesion was selected, shown in Figure 8(d). Visual matching of NaF-avid region to sclerotic abnormality on CT was confirmed by radiology team. Eight core-needle biopsy samples taken along a 4 cm track were obtained. In this case, the bone lesion was considered a successful clinical target as it was able to provide histopathological validation of adenocarcinoma of the prostate.

4. Discussion

Using advanced image analysis techniques and a survey of clinical biopsy feasibility requirements, we were able to develop and validate a novel selection algorithm for biopsy site selection in patients with multiple lesions. Sensitivity analysis of this technique in two applications was able to show robust lesion-selection that was both feasible for physician to sample, yet also highly relevant from an imaging point of view. In a prospective study, clinical implementation of this technique was used in a case of a metastatic prostate cancer patient imaged with NaF PET/CT and was successfully able to identify an appropriate biopsy target. Patients used in this validation study, i.e. those whose cancer has metastasized to bone, present a large clinical problem by presenting with potentially hundreds of feasible

biopsy targets. This population represents one of the lowest in terms of successful tissue acquisition and sampling, primarily due to high prevalence of small, deep seeded lesions occurring in the pelvis, spine, and ribs (Wu *et al.*, 2008; Rimondi *et al.*, 2011; Altuntas *et al.*, 2005). Implementation of this methodology has the potential to increase tissue yield from these patients, particularly due to the inclusion of quantitative imaging parameters. True clinical validation was not presented in this study, but is currently being investigated in as part of clinical trial (NCT02677376).

In a simulation study comparing selection choices for clinical benchmark (i.e. based on location and size) and our algorithm, good concordance was observed in top-ranking lesions in both methods. The worst case of discordance was seen in a single patient when the selection algorithm identified the clinical benchmarks 3rd-ranked lesion at its top preference. Here, all lesions top ranking lesions were contained within the ilium, an optimal location for bone biopsy in these patients (Hau *et al.*, 2002). In this scenario, quantitative imaging was able to identify top choices automatically, providing a useful tool for clinicians to streamline clinical decision-making. Such was the case in the prospective pre-treatment biopsy case study. The selection algorithm was able to provide a number of preferred targets, leading to a successful biopsy after review and ultimate consensus from the interventional radiology team. Computation time for the pre-treatment case study was 3 hours from completion of scan to transmission of information to radiology. Image-directed bone lesion biopsies sites are currently selected based on safety and efficacy of zone targeting (Pezeshk *et al.*, 2006). Providing a number of preferred targets, as was done here, could ease the timely clinical burden of lesion identification and selection.

In this study, the top-ranking lesion for response-based targeting showed multiple high-scoring targets, indicating some patients have multiple lesions desirable for biopsy. In the case of one patient, clinically-biased sampling revealed three potential lesion-targets all scoring within 5% of one another (Figure 5). The rankings successfully dichotomized lesions demonstrating substantial changes in imaging characteristics throughout therapy from those with moderate change and/or mixed response across the two imaging response metrics, indicating this technique can successfully identify a biologically relevant target. Following this protocol could potentially decrease inherent bias from random sampling of lesions and provide direct sampling of acquired resistance to anti-cancer initiatives in these patients. Techniques such as the one presented here are crucial for development of imaging biomarkers and identification of pathways of disease resistance, particularly in cases of metastatic prostate cancer where the AR mutation rates of sampled tissue can range from 5–50% between primary and metastatic sites (Scher *et al.*, 2013). Development of tools for accurate selection of relapsing tissue is not currently part of routine clinical management of these patients, but given the different known biological response of these patients and the various targeted treatment options, an algorithmic selection platform based on molecular imaging is a natural choice for adequate sampling (Scher *et al.*, 2011).

Advantages of PET-guidance are well established for directing successful biopsies in various cancer sites and dedicated PET-guided platforms have been developed for clinical use (Klaeser *et al.*, 2010; Kalinyak *et al.*, 2011; Tatli *et al.*, 2011; Govindarajan *et al.*, 2009). As PET/CT image quantification both improves and expands beyond traditional imaging

metrics, imaging biomarker identification has the potential to significantly impact the quantitative prediction of patient's responsiveness and outcomes to therapy (Lambin *et al.*, 2012). NaF PET/CT biomarker assessment of mCRPC is still in its infancy. All molecular-imaging metrics identified for use in this application have shown favorable quantification repeatability (Lin *et al.*, 2016) and have been identified as potential imaging response biomarkers for solid tumors (Scher *et al.*, 2013; Vanderhoek *et al.*, 2013). In this study, we elected to only include independent imaging metrics that would likely represent unique phenotypic information on imaging. This selection was done by indentifying uncorrelated imaging metrics that were drivers of selection scores when regressed against a methodology using all imaging metrics. The resulting imaging metrics, SUV_{total} and SUV_{max} , are attractive surrogate measures of tumor burden and tumor aggressiveness, respectively. Until further validation of NaF PET/CT imaging biomarkers is completed, selection of imaging metrics will remain a potential limitation of this algorithm.

Adaptive forms of this algorithm could enhance its clinical utility. While NaF PET/CT was the focus of this validation framework other PET tracers could provide a disease-specific target depending on the patient population, such as ^{18}F -DCFPyL targeting prostate specific membrane antigen (PSMA) in metastatic prostate cancer (Rowe *et al.*, 2016). Other adaptations to this framework could provide updated site preferences for biopsy based on history of physician-based procedures, increasing the likelihood of successful lesion targeting and decreasing risk to the patient. In the case of multi-institution studies, this methodology could help ensure similar selection across multiple physicians for precise localization. This study used a stepwise decreasing selection bias over seven generalized regions of the skeleton that have historically provided safe/feasible targets with higher accuracy than other skeletal regions not included in this algorithm. Imperfections in this preference maps led to lesion selection in close proximity to the sciatic nerve for the response-based clinical simulation. Future work could consider automated methods to determine distance from sensitive structures, like the sciatic nerve, for inclusion in physician feasibility matrix.

The selection algorithm described here can also be modified beyond NaF PET/CT and bone biopsies, extending into general imaging biomarker identification and validation for other disease sites and imaging targets. For example, patients with advanced melanoma often present with multiple metastatic lymph lesions, the location and involvement of which is an important prognostic indicator for this population (Howlader *et al.*, 2016). ^{18}F -Fluoro-deoxyglucose (FDG) PET/CT imaging is standard-of-care throughout treatment of metastatic melanoma, which has been shown to predict treatment response in these patients (Sachpekidis *et al.*, 2015). Similar examples can be found with other quantitative imaging technologies for assessing glioblastoma (Thomas *et al.*, 2013) and even outside of oncology, in multiple sclerosis (Metz *et al.*, 2014). Implementation of this biologically-directed site selection should be considered in software development of future uses of these dedicated systems to aid physicians in site selection of the most biologically relevant tissue of interest. Additionally, this platform could be adapted for use of sampling across a single tumor where substantial heterogeneity is has been noted both on macroscopic (imaging) and microscopic (genetic) levels for various solid cancers (Miller *et al.*, 2003).

5. Conclusions

This work outlines the development and testing of a novel algorithm to guide clinical biopsies. By incorporating molecular imaging information and quantitatively combining with physician feasibility and preference requirements, robust biopsy site selection in patients with multiple lesions was achieved. This methodology was tested in 17 metastatic prostate cancer patients receiving up to two NaF PET/CT scans. The validation of its use was successfully shown in a prospective study, resulting in eight adequate biological samples acquired from what is typically a few low-yield procedure. As an increasing number of clinical trials demand tissue collection as part of translational substudies of targeted drug agents this novel algorithm can serve as a basis for automatic site selection in any case of patients with multiple lesions, not limited to metastatic bone diseases.

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Figure 1.

Molecular Image-Directed Biopsy Methodology. **(A)** A single baseline NaF PET/CT scan is acquired and lesions automatically segmented (A-1), followed by automatic identification of lesions in anatomical regions feasible for biopsy targeting (A-2). **(B) For pre-treatment biopsy application**, data is extracted from lesions identified in step A that also meet minimum size criteria for biopsy (B-1). Target selection is determined by ranking scores derived from objective function based on various physician and imaging metrics (B-2). **(C) For response-based biopsy application**, a second mid-treatment NaF PET/CT scan is acquired and lesions automatically segmented (C-1). Response for each lesion is calculated as relative change from baseline to mid-treatment (A-1 to C-1) for lesions that meet biopsy criteria at mid-treatment (C-2). Target selection is determined given response-based objectives (C-3)

Figure 2.

Change (%) in $S(k)$ relative to 1:1 weighting for all lesions from all 17 patients. Category weights were changed such that bias in a particular category was introduced, ranging from physician-biased (red) to imaging-biased (blue). Across all patients, lesions demonstrate wide variability in sensitivity to weighting, ranging from <1% change in score over the entire domain, to maximum 103% change in score (as seen in P13). Overall, average maximum variability over the entire domain for all lesions was <20% in either direction.

Figure 3.

Within-patient lesion $S(k)$ values for weighting schemes (clinical:imaging) from 1:2-to-2:1 ($R=0.95$) with colormap representing 1:1 weighting, highlighting stable rankings pre-treatment biopsy application. Despite changes in selection scores across different weighting schemes, the relative distribution of ranking within a patient remained highly stable indicating consistent biopsy-site location.

Figure 4.

Response in SUV_{total} and response in SUV_{max} across all targetable lesions, inconsistent correlation is shown across all lesions, regardless of favorable (bottom left quadrant, decrease in both metrics) or unfavorable changes in imaging (top right quadrant, increase in both metrics). Several lesions had discordance in SUV_{max} and SUV_{total} response, with majority showing decrease in SUV_{max} despite increase in SUV_{total}.

Figure 5.

$S(k)$ for selection methods varied by imaging category (individual and uncorrelated pairs) vs. $S(k)$ for selection method using all imaging metrics. Slope close to 1 indicating $S(k)$ scores closely mirror selection based on all metrics. Combination of uncorrelated imaging metrics (SUV_{mean} & SUV_{total} ; SUV_{max} & SUV_{total}) show significantly improved modeling compared to all singular imaging methods, but no significant improvements across these multivariate methods. No differences were found between singular imaging metric methods.

Figure 6.

Within-patient lesion $S(k)$ values for various weighting schemes (clinical:imaging). Shown from 1:1 vs. 1:2 ($R=0.93$, left), from 1:1 vs. 2:1 ($R=0.95$, middle), and from 1:2-to-2:1 ($R=0.79$, right). Rankings post-treatment biopsy application were less stable in general, but still show good agreement in the relative distribution of ranking within a patient remained highly stable indicating consistent biopsy-site location.

Figure 7.

Within-patient $S(k)$ values for various weighting schemes (clinical:imaging) of example patient showing variable top biopsy candidate lesion selected by S_{max} for different weighting scenarios (left) and their SUV_{total} response characteristics overlaid on CT (right). Rankings obtained from evenly weighting the categories are numerically labeled. In this scenario, selection scores from the top three lesions are within 2% of one another and consistently outperform three other technically feasible but undesirable lesions.

Figure 8. Case Study

Five NaF PET/CT-defined lesions overlaid on projection CT (a), with red and blue outlines representing the 1st and 2nd choice lesions, respectively, from evenly weighting the categories. Lesion ranking characteristics are shown over 1:2-to-2:1 clinical:imaging domain in (b). Upon further review, the top biopsy candidate was located too near to the patient's sciatic nerve bundle and joint (c), the next highest ranking lesion was selected and successfully sampled (d).

Table 1

Slope of all methods defined by varied imaging categories from ANCOVA analysis. F-statistic shows differences between each method and the highest performing multivariate combination (SUV_{max} and SUV_{total})

Imaging Category	Slope	Standard Error	F-statistic*
SUV_{max}	0.54	0.01	$\ll 0.0001$
SUV_{mean}	0.43	0.01	$\ll 0.0001$
SUV_{total}	0.70	0.02	$\ll 0.0001$
SUV_{mean} & SUV_{total}	0.85	0.01	$\ll 0.0001$
SUV_{max} & SUV_{total}	0.93	0.01	--

Table 2

Frequency of skeletal location of lesions identified as most-preferred for biopsy based on selection algorithm. For all patients, the single top-ranked lesion was included. For patients with 5 or more lesions (N = 13), the top three lesions from each patient were included (39 total lesions) and the top 5 ranked targets for patients with 10 or more lesions (N = 6, 30 total lesions).

Skeletal Region	Top-ranked lesion from all patients (N = 17)	Top 3 Ranked Lesions from patients with 5+ lesions (N = 13)	Top 5 Ranked Lesions from patients with 10+ lesions (N = 6)
Ilium	41.2%	38.5%	50.0%
Lumbar Spine	41.2%	41.0%	33.3%
Sacrum	0.0%	2.6%	0.0%
Posteroinferior Ischium	11.8%	5.1%	10.0%
Femur	5.9%	10.3%	3.3%
Pubis	0.0%	2.6%	3.3%
Humerus	0.0%	0.0%	0.0%
Acetabular	0.0%	0.0%	0.0%

Table 3

Concurrency matrix for all patients comparing ranking lesions from simulated benchmark to rankings from selection algorithm

		Simulated Clinical Benchmark Rank		
		1	2	3
Selection Algorithm [S(k)] Rank	1	13	3	1
	2	4	9	0
	3+	0	3	10