PREDICTING PATIENT RESPONSE TO CANCER IMMUNOTHERAPY USING QUANTITATIVE COMPUTED TOMOGRAPHY BASED TEXTURE ANALYSIS

A thesis submitted to the University of Arizona College of Medicine – Phoenix
in partial fulfillment of the requirements for the Degree of Doctor of Medicine

Joshua Gordon
Class of 2017

Ronald Korn, MD, PhD

Abstract:

Background/Significance: Cancer therapies have evolved continuously, with the newest class being immunotherapies targeting the PD-L1/PD-1 pathway. This pathway is often overexpressed in malignancies, which allow the aberrant cells to evade the body's natural immune response that would normally eliminate them. The novel therapies currently being investigated are monoclonal antibodies that target either the PD-L1 on the tumor cell or the PD-1 on the lymphocyte. Considering there are significant toxicities with these therapies, namely gastrointestinal and endocrine adverse effects, a predictive tool that could allow physicians which patients are likely to respond to these immunotherapies could spare patients unnecessary therapy and potential economic harm. Since repetitive imaging of patients with cancer is necessary to monitor treatment response, advanced imaging analysis techniques on standard of care images, such as CT scans may provide insights into tumor patterns that could help to predict treatment response. Quantitative texture analysis (QTA) of computed tomography scans has been used in various settings to examine tissue heterogeneity as a predictive biomarker of response; we hypothesized that QTA may have potential value in predicting tumor response to immunotherapy.

Objective: We performed a QTA on standard of care CT scans from patients to determine if a unique textural imaging signature could be identified that would serve as a predictive biomarker for response to PD-L1/PD-1 therapies in subjects with solid tumor malignancies in the lungs, liver, and lymph nodes.

Design: This study examined the diagnostic standard of care CT scans of the chest, abdomen, and pelvis (CT CAP) at baseline and follow-up, which were acquired as part of routine clinical care for tumor staging and treatment response in 20 subjects whose personal health care information was removed prior to analysis. Regions of interest (ROI) were drawn around all identifiable tumor lesions on baseline CT scans provided that tumors were of reasonable size (>10 mm in diameter) and conspicuity. CT texture analysis was performed on these lesions to obtain a histogram readout of tumor texture based upon tissue densities on a per pixel bases. The output values from the QTA platform provided an estimate of tumor signal properties as

expressed as the mean pixel density, standard deviation, entropy, kurtosis, skewness, and mean positive pixel values. Each subject was designated as achieving either a RECIST based treatment response or not. Statistical modeling was then conducted using regression techniques.

Results: There was no identifiable signature when examining all of the lesions together, but there were statistically significant correlations noted between QTA and RECIST responses for lung-based lesions. The QTA derived mean pixel density parameter was a major component of separating out responders from non-response. Of the 14 lung lesions (8 responder vs. 6 non-responder) there was a significant difference in the mean density with a threshold cutoff of 11.91 ($p < 0.0001$). A Mann-Whitney U-test was performed on the total data set yielding a Z statistic of 2.6 ($p=0.0092$).

Conclusions: Despite the relatively small number of patients in this initial study, there were promising findings regarding the mean density of lesions, suggesting that texture analysis can be used to predict if patients respond to PD-L1/PD-1 inhibitors. Further investigation is warranted in a larger population that can be differentiated by tumor type to validate these results.

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Introduction/Significance:

Cancer therapy has evolved tremendously in the twentieth and twenty-first centuries. Chemotherapy and radiation have given way to small molecules or monoclonal proteins targeting a specific aberrant cellular feature. One of the next frontiers in the field of oncology is the use of a patient's immune system to attack cancerous cells.

Focusing on the patient's immune system is truly one of the new, promising frontiers in the field of oncologic treatment. In particular, the PD-L1/PD-1 (programmed death ligand, programmed death) pathway has been studied extensively with several promising new drugs. PD-L1 is expressed on tumor cells and serves to inactivate cytotoxic T-lymphocytes (CTLs) from recognizing and attacking the abnormal-appearing tumor cells. PD-1 is expressed on CTLs surface; the binding of the two produces an inhibitor effect on the immune response (2). The PD-L1 response is possibly an evolved mechanism to prevent chronic inflammation in various viral infections (2). PD-L1 is often highly expressed in melanoma, non-small cell lung cancer (NSLC), nasopharyngeal cancer, along with many other tumor types. Therefore, targeted therapies with monoclonal antibodies have been formulated to target both proteins since they can blunt the anti-immune effect on CTLs attacking tumor cells.

A secondary, but still vital objective of developing these novel treatments is to minimize the adverse effects associated with them. Researchers and physicians are met with the same difficult balancing act when developing therapies: Many cancer treatments often yield unpredictable patient response rates, as well as variable and sometimes significant toxicities. The latter can make treatment completion a barrier for patients. As a result, it is increasingly important to prescribe therapies, if possible, that have a significant chance of attaining a response, while simultaneously reducing potential toxicities. General toxicities for the PD-L1/PD-1 inhibitors include fatigue, rash, diarrhea, colitis, hypophysitis, thyroid dysfunction, and adrenal insufficiency. There has also been documented hepatoxicity, most commonly with asymptomatic elevations of transaminases (23). Pneumonitis has also been reported adverse effect in less than 10% of patients. Rarer side effects include neurotoxicity, ocular toxicity, and renal toxicity.

In order to optimize the treatment that patients are given, physicians assess the pre-test probability of a given therapy prior to administering drug. Given the need to maintain a risk-benefit balance to follow the "do no harm" principle, it would be advantageous to have an accurate prediction as to whether a patient would respond to some of the novel immunotherapies being used, specifically those targeting PDL-1/PD-1. Even more beneficial would be a test that could be performed non-invasively, sparing a patient from a biopsy or blood draw (25).

The role of imaging cannot be understated in the overall management of patients with cancer. Routine imaging can be used in initial diagnosis of disease, to monitor response of tumor lesions to treatment, and to identify progression of disease. For instance, the National Comprehensive Cancer Network's (NCCN) guidelines for patients with non-small cell lung cancer who have no evidence of disease following treatment advise CT scans to monitor for new lesions every 6 months for 2-3 years and then annual CT scans after that period.

Computed tomography, much like conventional radiographs, creates an image based on the density of the tissue that a beam of x-rays travels through (18). The quantification and subsequent representation of that tissue density is the data that is extracted and manipulated to provide QTA values.

Quantitative computed tomography based texture analysis (QTA) has been previously used to provide an objective quantitative profile of the distribution of signal within a lesion or tissue (20, 24, 27-29). Such work has begun to provide insights into the hallmarks of cancer, prognostic information, predictive biomarkers and mutational status of tumor biology (20). For example, QTA has been shown to differentiate K-ras mutated non small cell lung cancer (NSCLC) from pan-wild-type NSCLC on standard of care CT scans obtained from patients undergoing percutaneous biopsies for cancer treatment decisions (27). The unique features of the QTA software provides an examination of tumor texture on manually selected regions of interest by creating a quantitative analysis of image-based histogram parameters. It is thought that the underlying biology in the tissue manifests as unique imaging features on CT scans. If the tumor microenvironment is a key factor in tumor behavior and response to treatment, QTA may provide a means of predicting response. Among these quantitative parameters are the mean

(average brightness of the image) pixel value, kurtosis (measure of peakedness and tailedness), skewness (measure of asymmetry), and standard deviation (measure of deviation from the mean) (6). By analyzing, retrospectively, the pre-treatment computed tomography (CT) scans of patients who both responded and did not respond to PDL-1/PD-1 therapies, we hope to identify a texture analysis signature that would allow physicians to accurately determine whether a patient would benefit from PD-L1/PD-1 therapy.

Materials and Methods

Patient and Scan Selection:

This study was designated as not requiring IRB approval based on the study design and methods, which were evaluated by the University of Arizona College of Medicine Phoenix. Patients were selected based on their initial response to an immunotherapy by the outside treating oncologist. There were a total of 10 patients from each group (total n = 20) that were included in the initial investigation. The patients had a varying number of lung, liver, and lymph node lesions that were examined with QTA based on lesion size. The type of malignancy was intentionally variable, as this was a broad investigation of the utility of QTA. CT imaging was obtained from Honor Health and Scottsdale Medical Imaging. There were a total of 20 studies acquired and de-identified consisting of 10 responders and 10 non-responders. The scans were performed prior to patients receiving immunotherapy and were all done between 2012-2014. The scans used were a combination of contrast and non-contrast CT scans of the chest, abdomen, and pelvis. These scans were downloaded from the hospital PACS, de-identified of all identifying personal information, and transferred to TexRAD database for analysis. TexRAD is a proprietary software algorithm developed by Ganeshan et al for the analysis of medical images.

Quantitative Texture Analysis (QTA):

Patient scans from the 20 subjects were imported into TexRAD. A medical student placed regions of interest (ROI) on baseline CT scans around malignant lesions; these ROI were then reviewed and modified by a board-certified radiologist to independently verify the accuracy and presence of a malignant lesion. These ROIs were placed on axial slices at least 2 cm in diameter.

QTA is a multistep process. The first step of texture analysis software operations is the manipulation of the original image to accentuate any macroscopic features while attenuating and filtering out random photon noise. The TexRAD platform uses a Laplacian of Gaussian band-pass filter that creates spatial scale factors (SSF) of varying sizes to perform this maneuver. The SSF of 2-6 pixels can be used as filter parameters. A smaller SSF is considered to be a finer filter, compared to a coarser filter, like SSF6. The SSF examined were SSF0 (no filtration), SSF2 (2 mm radius), SSF3 (3 mm radius), SSF4 (4 mm radius), SSF6 (6 mm radius) (6).

Once the images have been filtered, histograms are developed of the pixel signal intensity data in Hounsfield units. Hounsfield units are a standardized unit of measurement for density of different substances on CT scans. Hounsfield units are obtained from a linear transformation of attenuation coefficients, which reflect how easily the beam travels through a given substance. This is a reflection of the density of the tissue and the units are based on water and air at standard temperature and pressure. These histograms are generated for each ROI at each of the filtering densities. These parameters include mean, entropy, kurtosis, skewness, and standard deviation (6).

The histograms developed from the pixel signal intensity data were stored in CSV formats. They were then combined into an excel database, where they underwent statistical modeling using MedCalc. For the purposes of our study, results that demonstrated a p-value of <0.05 were considered significant.

Results:

Table 1: General Patient Characteristics

		Sex	Age at time of Scan	Primary Malignancy	Previous Treatment
Non-responders	1	F	44	Ovarian	Y
	2	F	60	Pancreas	Y
	3	M	84	NSCLC	Y
	4	M	59	NSCLC	Y
	5	M	57	NSCLC	Y
	6	M	70	NSCLC	Y
	7	F	51	Urothelial	Y
	8	F	49	Cervical	Y
	9	F	45	NSCLC	Y
	10	F	67	Urothelial	Y
Responders	1	M	73	NSCLC	Y
	2	M	76	RCC	Y
	3	M	56	RCC	Y
	4	F	64	Breast	Y
	5	F	72	NSCLC	Y
	6	M	59	RCC	Y
	7	M	65	Urothelial	Y
	8	M	65	Urothelial	Y
	9	M	79	Urothelial	Y
	10	M	58	NSCLC	Y

This table demonstrates an even initial distribution of patients in both the responder and non-responder group (n = 10). The primary malignancy varied within the groups, as did the number of lesions and the age at which this baseline scan was acquired. All of these patients underwent some form of treatment prior to the immunotherapy, according to their oncologist.

Table 2: Texture Analysis of Patients that Responded to Immunotherapy

		Parameter					
		Mean	SD	Entropy	MPP	Skewness	Kurtosis
Responders	SSF 0	56.378	26.992	4.478	59.449	-0.471	1.024
	SSF 2	1.489	45.718	4.656	37.624	0.039	0.511
	SSF 3	-0.916	31.821	4.157	26.888	0.003	0.099
	SSF 4	8.481	28.017	4.142	28.792	-0.074	0.466
	SSF 5	24.785	22.296	3.688	35.260	-0.182	0.190
	SSF 6	32.061	21.312	3.631	43.001	-0.217	-0.036

This table examines the compiled histogram values from all malignant lesions for those patients who obtained a RECIST response to immunotherapy.

Table 3: Texture Analysis of Non-Responders to Immunotherapy

		Parameter					
		Mean	SD	Entropy	MPP	Skewness	Kurtosis
Non-Responders	SSF 0	48.884	29.520	4.486	53.535	0.312	4.097
	SSF 2	-13.273	41.692	4.471	28.694	0.143	0.801
	SSF 3	-11.214	31.332	4.138	22.894	0.174	0.410
	SSF 4	-14.566	25.409	3.924	20.467	0.257	0.751
	SSF 5	-14.547	22.078	3.509	19.658	-14.547	0.043
	SSF 6	-8.938	25.431	3.727	21.566	0.268	-0.187

This table examines the compiled histogram values from all malignant lesions for those patients who did not obtain a RECIST response to immunotherapy.

Table 4: Texture Analysis of Lung Lesions From Responders and Non-Responders

		Parameters					
		Mean	SD	Entropy	MPP	Skewness	Kurtosis
Lung Lesions	SSF 0	49.128	37.004	4.672	58.161	0.302	6.851
	SSF 2	-4.056	58.177	4.746	41.513	-0.032	2.244
	SSF 3	3.089	43.283	4.476	36.301	-0.097	1.011
	SSF 4	8.140	37.960	4.454	33.772	-0.186	1.121
	SSF 5	7.614	34.741	4.272	35.187	-0.083	0.340
	SSF 6	11.533	37.453	4.490	38.634	-0.103	0.111

This table shows the histogram readouts from the lung lesions examined from both responders and non-responders.

Table 5: Texture Analysis of Lymph Node Lesions From Responders and Non-Responders

		Parameters					
		Mean	SD	Entropy	MPP	Skewness	Kurtosis
Lymph Node Lesions	SSF 0	56.265	26.670	4.447	58.787	-0.334	1.771
	SSF 2	-6.582	39.863	4.447	31.337	0.189	0.254
	SSF 3	-6.654	28.329	3.965	22.397	0.135	-0.029
	SSF 4	2.838	22.867	3.908	22.871	0.013	0.405
	SSF 5	16.534	16.330	3.174	27.289	-0.051	0.106
	SSF 6	20.566	15.764	3.024	35.539	-0.089	-0.291

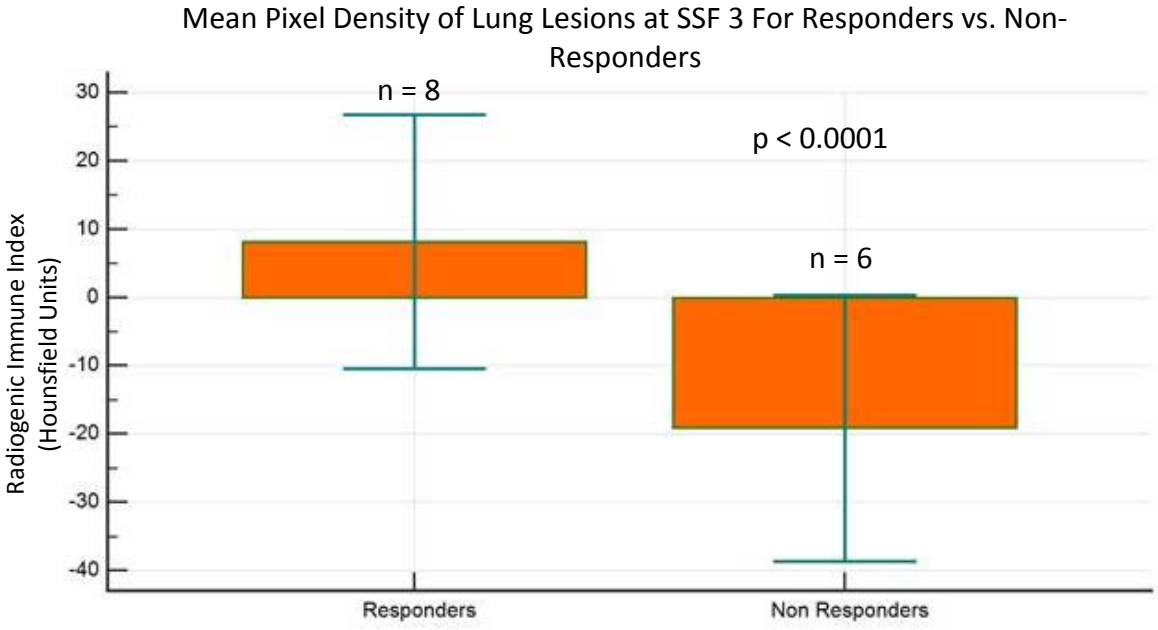
This table shows the histogram readouts from the lymph node lesions examined from both responders and non-responders.

Table 6: Texture Analysis of Liver Lesions From Responders and Non-Responders

		Parameters					
		Mean	SD	Entropy	MPP	Skewness	Kurtosis
Liver Lesions	SSF 0	47.186	22.446	4.347	48.478	0.209	0.141
	SSF 2	-9.758	34.842	4.568	25.737	0.044	-0.196
	SSF 3	-17.412	24.594	4.125	16.036	0.246	0.019
	SSF 4	-30.941	19.658	3.709	15.122	0.615	0.432
	SSF 5	-30.073	15.171	3.336	12.217	0.675	-0.206
	SSF 6	-15.098	14.250	3.457	10.483	0.560	-0.226

This table shows the histogram readouts from the liver lesions examined from both responders and non-responders.

Figure 1: Mean Density of Lung Lesions at SSF3



This graph depicts the mean pixel density for the 14 lung lesions that were evaluated in this study. There were 8 from patients that responded to immunotherapy and 6 from patients that did not respond. The density was measured in Hounsfield units.

Discussion:

In this study of various oncologic patients undergoing immunotherapy with PDL-1/PD-1 inhibitors, we sought to identify a unique signature via CT textural analysis of pre-treatment CT scans that would serve as a predictive indicator for treatment response. We found that using a medium-sized filter to examine lung lesions and their mean density of the lesion was correlated with responders vs. non-responders ($p = < 0.0001$, $n = 14$, $ssf = 3$). A Mann-Whitney (MW) U-Test of this data was performed because of the uneven distribution in this subset of data (responders = 8, non-responders = 6). This test ranks data in an ordinal fashion as opposed to a continuous one (15). The MW U-Test revealed a Z statistic of 2.6, which allowed us to reject the null hypothesis that there was no difference in the texture analysis between responders and non-responders.

Immunotherapy and Tumor Microenvironment:

The relatively novel development of immunotherapy in the treatment of various malignancies has provided promising results, but is not perfect in that cancer is a series of adaptive mutations that allow malignant cells to survive indefinitely. The recently developed drugs that target the PD-L1/PD-1 pathway aim to disable the malignant cells adaptive "mask" that prevents lymphocytes from recognizing the severely mutated and distorted cell surface (2). In doing so, the host immune system would be better able to eliminate the malignancy.

It has been suspected that the surrounding microenvironment may play a role in determining the likelihood of a favorable response. The microenvironment of the malignant cells influences the tumor in multiple fashions, most significantly the increased access to nutrients that provides a survival advantage for the malignant cells compared to normal ones (2).

QTA Serving as a Response Indicator:

Texture analysis has shown utility in a number of settings including detecting K-Ras mutations in non-small cell lung cancer, staging and survival of esophageal cancer, and response to treatment in renal cell carcinoma (10-13, 19, 27). One of the main advantages of using QTA as a pre-treatment assessment tool is that it provides a non-invasive option for physicians to assess the disease burden or qualities of a given malignancy. Other options to

tailor treatment plans include biopsies and molecular testing, which also play a vital role in the management of oncologic patients. In the world of continuously striving towards personalized medicine, effective tests such as texture analysis and molecular analysis all play complimentary roles in ensuring that patients are prescribed the best, most effective treatment plan, minimizing toxicity and risk of disease progression.

Limitations:

There are several limitations of this study, including the small number of patients and lesions that we acquired for texture analysis. While the results that were obtained in this study were promising, additional validation of these results would be important. Because we hoped to investigate the utility of texture analysis as an overall predictor, we cast a wide net and examined many different types of lesions, which included lymph nodes, liver lesions, and lung lesions. While this allowed us to examine broadly how texture analysis could prove useful in the initial management of oncologic patients, it does present the question of how the technology can be applied to specific tumor types, locations of lesions, and size of the lesions.

Despite the fact that there was a relatively even distribution of responders vs. non-responders (10 vs. 11) during the initial patient selection, the type and number of lesions that were analyzed was variable from patient to patient. Given the small number of patients, this makes it possible that one patient's lesions dominated the data set and influenced the data.

Because this study was exploratory, as texture analysis has not been investigated in this setting, there was no data or literature to compare our results to.

Future Directions:

The results that suggest that texture analysis could be used to predict whether patients respond to PDL-1/PD-1 inhibitors are promising and intriguing enough to warrant further investigation. Our next steps in this area will include replicating the initial analysis with a much larger data set in which we will separate data based on tumor type. This will hopefully provide more information as to how useful texture analysis can be.

Once the topic of initial response has been addressed, a longer analysis of patients that have responded can be conducted that would possibly provide information as to whether the tumor microenvironment may influence the duration of response. Similarly, it would be interesting to have a comparison of tumor heterogeneity prior to the treatment compared to afterwards and to evaluate whether there is a difference in the texture analysis signature that may provide information as to the extent and/or duration of response to treatment.

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