Outcomes by Tumor Histology and KRAS Mutation Status After Lung Stereotactic Body Radiation Therapy for Early-Stage Non–Small-Cell Lung Cancer

Outcomes by Tumor Histology and *KRAS* Mutation Status after Lung Stereotactic Body Radiation Therapy for Early Stage Non-Small Cell Lung Cancer

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Conflicts of Interest Statement:

We would like to make the following disclosures:

- A.B. Chen reports grant support from the American Cancer Society and American Society of Radiation Oncology, unrelated to the current study
- J.H. Lewis reports grant support from Varian Medical Systems, unrelated to the current study
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- All other authors have no conflicts to report

MICROABSTRACT:

We analyzed outcomes after lung stereotactic body radiation therapy (SBRT) for early stage non-small cell lung carcinoma (NSCLC) in patients by histology and *KRAS* mutation status. Histology was not associated with outcomes, but *KRAS* mutation was associated with lower freedom from recurrence on univariable analysis, and decreased cancer-specific survival on multivariable analysis. Given the small sample sizes, these results are hypothesis-generating and further study of SBRT outcomes by tumor genotype in larger datasets is needed.

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ABSTRACT:

BACKGROUND:

We analyzed outcomes after lung stereotactic body radiation therapy (SBRT) for early stage non-small cell lung carcinoma (NSCLC) by histology and *KRAS genotype*

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PATIENTS AND METHODS:

We included 75 patients with 79 peripheral tumors treated with SBRT (18 Gray x 3 or 10-12 Gray x 5) at our institution from 2009-2012. Genotyping for *KRAS* mutations was performed in 10 patients. Outcomes were analyzed using the Kaplan-Meier (KM) method/Cox regression, or cumulative incidence method/Fine-Gray analysis.

RESULTS:

The median patient age was 74 (range, 46-93) and ECOG performance status was 0-1 in 63%. Tumor histology included adenocarcinoma (44%), squamous cell carcinoma (25%), NSCLC (18%). Most tumors were T1a (54%). Seven patients had *KRAS*-mutant tumors (9%).

With a median follow-up of 18.8 months among survivors, the 1-year estimate of overall survival (OS) was 88%, cancer-specific survival (CSS) 92%, primary tumor control (TC) 94%, and freedom from recurrence (FFR) 67%. In patients with *KRAS*-mutant tumors, there was a significantly lower TC (67% vs. 96%; p=0.04), FFR (48% vs. 69%; p=0.03), and CSS (75% vs. 93%; p=0.05). On multivariable analysis, histology was not associated with outcomes, but *KRAS* mutation (HR: 10.3, 95% CI: 2.3-45.6; p=0.0022) was associated with decreased CSS after adjusting for age.

CONCLUSION:

In this SBRT series, histology was not associated with outcomes, but *KRAS* mutation was associated with lower FFR on univariable analysis, and decreased CSS on multivariable analysis. Due to small sample size, these hypothesis-generating results need to be studied in larger datasets.

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Keywords: Stereotactic body radiation therapy; Non-small cell lung cancer; Early stage; KRAS

Short Title: SBRT outcomes in KRAS-mutant NSCLC

INTRODUCTION:

Stereotactic body radiation therapy (SBRT) has emerged as the treatment of choice for medically inoperable stage I non-small cell lung cancer (NSCLC) in the past decade. Multiple prospective[1-3] and retrospective[4-6] series of different SBRT regimens have demonstrated very high local control (80-90% at 2-3 years), high overall survival (50-60%) and cancer-specific survival rates (60-70%) compared to historical series of patients treated with conventionally fractionated radiation therapy (RT). However, in these series, both regional and distant recurrences remain an issue with reported incidences of 5-13% and 14-25%, respectively.[1, 2, 4, 6] Thus, developing prognostic markers that identify patients at highest risk for recurrence after SBRT remains an important area for further research.

Alongside the emergence of SBRT technology, advances in cancer genomics in the last decade have identified genetically distinct sub-groups of lung adenocarcinoma defined by mutations in oncogenes such as *v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) and *epidermal growth factor receptor* (*EGFR*).[7] The unique biology of each genotypic sub-group has led to the development of personalized, "genotype-directed" therapy in the stage IV setting resulting in the widespread adoption of clinical *EGFR* mutation testing and evidence that first line EGFR tyrosine kinase inhibitor therapy results in improved outcomes.[8-10]

However, the role of tumor genotype in earlier stages of disease remains under-studied and controversial. *KRAS* mutation status has been studied extensively as both a prognostic factor and predictor of response to chemotherapy in stage I NSCLC patients with conflicting, inconclusive results.[11-17] Furthermore, the radiation responsiveness and clinical outcomes after RT for the genotypic subgroups of NSCLC have not been well

elucidated. Prior studies have shown possible associations between NSCLC genotype and response to RT. For example, retrospective series have demonstrated that patients with locally advanced *EGFR* mutant NSCLC had lower risk of locoregional failure compared to *EGFR*-wild-type patients after chemotherapy and conventional RT,[18-20] while patients with *KRAS*-mutant LA-NSCLC had decreased overall survival compared to those with *KRAS*-wild-type tumors.[19] However, it remains unclear if differences in radiation response by genotype is relevant at the higher doses delivered with SBRT.

Since patients receiving SBRT for stage I NSCLC typically have substantial medical co-morbidities that often preclude adjuvant chemotherapy, this patient subset provides a unique population to study *KRAS*-genotype as a potential prognostic marker. In this retrospective study, we build on these prior studies by performing an analysis of patients with stage I NSCLC treated with SBRT, and analyzing outcomes after SBRT by tumor histology and *KRAS*-genotype.

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MATERIALS AND METHODS:

Patients:

With Institutional Review Board approval under a waiver of consent, we reviewed the records of 75 consecutive patients with newly diagnosed early stage NSCLC treated with SBRT from 2009 to 2012 at our institution. Patients who received SBRT for locally recurrent disease, local progression of advanced stage disease or metastases to the lung from other sites of primary disease were excluded.

Tumor Genotyping

Ten patients had their tumors genotyped for activating *KRAS* mutations as part of routine clinical care at the discretion of the treating physician, either in our institution's

pathology department or in a commercial laboratory. Briefly, in all cases, DNA was isolated from tumor in paraffin-embedded tissue specimens and polymerase chain reaction using primers specific for codon 12, 13 and 61 of the *KRAS* gene was performed.[21] The primer extension products were then analyzed by capillary gel electrophoresis. Only one patient's tumor sample underwent testing for EGFR mutation status and none were tested for ALK mutation status. Thus, these tumor characteristics were not assessed.

Covariates:

Pre-treatment patient characteristics were collected, including age, gender, race, ECOG performance status (PS), and smoking history. Smoking status was categorized as: 1) never smokers; < 100 cigarettes in their lifetime; 2) former smokers; quit smoking >1 year prior to diagnosis, and 3) current smokers; smoking at the time of diagnosis or had quit < 1 year prior. Tumor characteristics were noted, including histology and TNM stage according to 7th edition of the AJCC Staging Manual. Treatment characteristics including SBRT prescribed dose, SBRT technique (conformal or volumetric modulated arc therapy), and biologically effective dose delivered (BED) were collected.

SBRT Treatment and Follow-up:

All patients were treated with SBRT per institutional norms, which included 1) restriction of SBRT to peripheral tumors as defined in RTOG 0236;[2] 2) use of abdominal compression to restrict tumor motion < 1 cm; 3) 4D-CT planning to create an internal target volume (ITV); 4) a 5 mm planning target volume (PTV) margin with no clinical target volume (CTV) margin; 5) dose of 10-12 Gy x 5 fractions for tumors close to the chest wall and 18 Gy x 3 fractions for all other tumors; 6) daily setup and image-guided

treatment with Exac Trac®, cone-beam CT, and portal imaging using a linear accelerator.

Patients were followed every 3-4 months after treatment for the first two years with a chest CT, then every 6 months for the next three years, and annually thereafter.

Outcomes

Overall survival (OS), cancer-specific survival (CSS) and patterns of failure, including local tumor control (TC; absence of tumor recurrence in-field or within 1 cm of PTV), lobar control (LC; including local tumor control and absence of recurrence in the same lobe), regional control (RC; absence of hilar and mediastinal recurrences), local-regional control (LRC; composite endpoint of lobar and regional control), freedom from distant metastases (FFDM), freedom from any recurrence (FFR), and recurrence-free survival (RFS; survival with absence of LRR or DM) were calculated from the date of completion of SBRT treatment to the time of first failure.

CSS was defined as absence of death from NSCLC, and cause of death was ascertained by death certificates when available. In cases where death certificates were not available, death with active, progressing NSCLC and/or enrollment on hospice for NSCLC prior to death was considered death from NSCLC. TC and LC were defined based on the definitions outlined in RTOG 0236. In brief, primary tumor failure was defined as (1) local enlargement defined as at least a 20% increase in the longest diameter of the gross tumor volume per CT scan and (2) evidence of tumor viability (either PET-CT demonstrating FDG-uptake of similar intensity as the pretreatment staging PET, or with pathologic confirmation via biopsy). Primary tumor failure included marginal failures occurring within 1 cm of the planning target volume (1.5-2.0 cm from

the gross tumor volume). Failure beyond the primary tumor but within the involved lobe was also ascertained and lobar control was defined as absence of primary tumor and/or involved lobe failure. Censoring for patients without disease progression was performed at the date of the last re-staging study (any chest CT or PET-CT) without evidence of progression.

Statistical Analyses

Descriptive statistics were used to characterize patients at study entry. Differences in the distribution of categorical variables and continuous variables by *KRAS* mutation status were analyzed using Fisher's exact and Wilcoxon rank sum tests, respectively.

OS, and all patterns of failure outcomes were analyzed using the Kaplan-Meier method and log-rank test. For the TC and LC endpoints, all tumors treated were included in the analysis, whereas the other endpoints were analyzed on a per patient basis.

Cox regression analysis was performed to identify predictors of each outcome. As the risk of failures was changed due to the administration of systemic treatment during the follow-up period, a Cox proportional hazards regression model with systemic treatment as a time-dependent covariate was used to evaluate the associations between patient/tumor characteristics and all patterns of failures. Gray's method was used to analyze time to first recurrence with death as a competing risk, and separately CSS with death of other causes as a competing risk. Univariable analysis was performed, and covariates with a p-value less than 0.10 were included in the multivariable analysis. Backward selection was performed to select significant predictors of outcome on multivariable analysis. Competing risk analysis was performed using R 2.10.0 while all the other analyses were performed using SAS version 9.2 (Carey, NC).

RESULTS:

Patients

A total of 75 patients with 79 early stage NSCLC tumors treated by SBRT were included in the analysis. The pre-treatment patient characteristics are shown in Table 1. Tumor histology included adenocarcinoma (44.3%), squamous cell carcinoma (25.3%), and NSCLC, not otherwise specified (NOS; 17.7%), with 12.7% of patients treated based on a radiographic diagnosis. Of ten patients who had tumor genotyping, seven had *KRAS-mutant* tumors, and three were *KRAS* WT including one with an *EGFR* mutation. The *KRAS* mutations were all in Codon 12 including four c.34G>T (p.Gly12Cys), two c.35G>A (Gly12Asp) and one c.35G>T (p.Gly12Val).

Treatment:

As shown in Table 1, all patients were treated with SBRT to a BED of at least 100 Gy_{10}. Only three patients received chemotherapy, and all of these received induction chemotherapy with platinum doublets (1 *KRAS*-mutant and 2 *KRAS*-WT/unknown).

Outcomes

With a median follow-up of 18.8 months among survivors, the 1-year estimates of survival are shown in Table 2. The median survival was 26.6 months in all patients, and there was no significant difference in patients with *KRAS*-mutant tumors (median not reached) versus with *KRAS*-WT/unknown tumors (median 26.6 months; p=0.51). CSS was significantly lower in patients with *KRAS*-mutant tumors versus WT/unknown on a competing risk analysis with death due to other causes as a competing risk (Figure 1; HR: 4.6; 95% CI: 1.1-19.; p=0.04).

Patterns of Recurrence

The 1-year estimates of patterns of recurrence are shown in Table 2. There were three primary tumor recurrences and an additional three intralobar recurrences with 1-year TC estimate of 94.2% and 1-year LC estimate of 88.9%. There were 12 nodal recurrences with a 1-year RC estimate of 81.2%, and the 1-year LRC estimate was 74.3%. Seventeen patients had a distant recurrence with a 1-year FFDM estimate of 72.8%. Sites of distant recurrence included brain (n=3), bone (n=5), liver (n=2), multifocal lung (n=5), pleural effusion (n=1) and abdominal lymph nodes (n=1). There were a total of 22 local and/or distant recurrences with 1-year estimate of FFR of 66.7% and median time to any recurrence of 27.2 months. Three patients were diagnosed with a second primary lung tumor, with a 1-year estimate of 3.7%.

Outcomes and Patterns of Recurrence by Histology

Comparing the patterns of recurrence in tumors treated without a biopsy (i.e. treated with a radiographic/clinical diagnosis alone; n=10) versus those with biopsy-proven NSCLC (n=69), there was no statistically significant difference in TC (100% vs. 93.3%; p=0.51), LC (100% vs. 87.3%; p=0.35), RC (77.1% vs. 82.0%; p=0.74), LRC (77.1% vs. 72.4%; p=0.83), FFDM (61.7% vs. 74.4%; p=0.66), nor FFR (61.7% vs. 67.3%; p=0.94).

There was also no significant difference in patterns of recurrence when comparing by adenocarcinoma (n=35) versus squamous cell carcinoma (n=20), versus NSCLC NOS (n=14), histology with 1-year estimates as follows: TC (85.4% vs. 100% vs. 100%; p=0.15), LC (82.6% vs. 85.1% vs. 100%; p=0.36), RC (79.4% vs. 77.3% vs.

92.3%; p=0.51), LRC (67.5% vs. 64.9% vs. 92.3%; p=0.25), FFDM (61.5% vs. 84.6% vs. 84.6%; p=0.32), or FFR (59.2% vs. 64.9% vs. 84.6%; p=0.35). Nor was there a significant difference in the pair-wise comparisons between the three histological groups (p-values not shown).

However, in comparing tumors with adenocarcinoma histology (n=35) versus those with either SCC or NSCLC NOS histology (n=34), there was a trend toward decreased TC in adenocarcinomas (85.4% vs. 100%; p=0.053**)** and all primary tumor failures occurred in tumors of adenocarcinoma histology. However, there was no significant difference in LC (82.6% vs. 91.5%; p=0.28), RC (79.4% vs. 84.2%; p=0.34), LRC (67.5% vs. 76.7%; p=0.22), FFDM (61.5% vs. 85.6%; p=0.13), nor FFR (59.2% vs. 74.1%; p=0.23) for adenocarcinoma versus other histologies.

Outcomes and Patterns of Recurrence by KRAS Mutation Status

There was no statistically significant difference in OS or RFS when comparing *KRAS-mutant* versus *KRAS*-WT/unknown status (Table 2), but there was a difference in CSS on competing risk analysis with more deaths due to cancer among patients with *KRAS* mutations (1-year estimate: 75.0% vs. 93.3%; p=0.05). As shown in Table 2, there was both decreased TC (p=0.04) and FFR (p=0.03) for *KRAS*-mutant tumors versus those with *KRAS*-WT/unknown status. The patterns of recurrence including sites of first recurrence are shown in Table 3.

Univariable and Multivariable Analysis

On univariable analysis, there were no clinical, pathologic nor treatment features associated with primary TC, LC, RC, nor LRC. *KRAS* mutation status was not significantly associated with LC, RC, or LRC, but there was a trend for decreased

primary TC (HR: 8.0; 95% CI: 0.82-78.4; p=0.07). On multivariable analysis with backward selection, no variables were associated with any of these local and/or regional recurrence endpoints. Neither univariable nor multivariable analysis with backward selection identified any clinical variables associated with risk of distant recurrence.

However, univariable analysis of any recurrence demonstrated that *KRAS* mutation status was associated with increased incidence of any recurrence (HR: 3.2; 95% CI 1.1-9.6; p=0.04; Table 4). However, no variables were associated with FFR on multivariable analysis with backward selection.

On univariable and multivariable analysis of CSS with death of other causes as a competing risk, presence of *KRAS* mutation was associated with increased risk of death from lung cancer (HR: 10.3, 95% CI: 2.3-45.6; p=0.0022), after adjusting for age (Table 5; Figure 1).

DISCUSSION:

In this study of patients treated with SBRT for early stage NSCLC, we demonstrate high primary TC and OS with the predominant sites of failure in regional nodes or distant sites, which is comparable to previously published series.[1, 2, 6] We performed sub-group analyses to determine whether tumor biology as reflected by tumor histology or genotype was associated with outcomes after SBRT. We demonstrated that tumor histology was not associated with local, regional or distant recurrence, but *KRAS* mutation status was associated with decreased TC and FFR on univariable analysis, and decreased CSS on multivariable analysis.

Prior SBRT series have shown an association between adenocarcinoma histology and increased risk of distant metastases,[2] but few have studied association between NSCLC histology and TC. In our series, there was not a clear association between TC and histology, but of note, primary tumor recurrences occurred in only patients with adenocarcinoma histology. The high biologically equivalent dose delivered with SBRT and low incidence of local failure events likely minimizes the likelihood of detecting a histological difference in radiosensitivity, particularly in small series. Further study of TC by tumor histology will likely require pooled analyses of larger SBRT datasets.

The most interesting finding of this study was an association between *KRAS* mutation status with FFR and CSS. The role of *KRAS* mutation status as a potential prognostic and predictive marker for early stage NSCLC remains controversial. A recent pooled analysis of multiple adjuvant chemotherapy trials demonstrated that *KRAS* mutation status was not prognostic, but codon 13 *KRAS* mutation was possibly predictive of decreased response to chemotherapy (HR = 5.78; 95% CI, 2.06 to 16.2; P<0.001; interaction P =0.002).[15] In our study, there was no clear association between primary TC and *KRAS* mutations status, but the high dose delivered with SBRT may obscure any underlying variability in radiation responsiveness that may be imparted by tumor genotype, and the low number of primary tumor recurrence events with SBRT also reduces the power to detect any such association. However, our study demonstrated an association between *KRAS* mutation status and both CSS and risk of any recurrence. However, given the small sample size, this hypothesis generating results must be further studied in a larger dataset before *KRAS* genotype can be utilized as a prognostic biomarker among patients treated with SBRT. Since our study and other published SBRT series demonstrate that distant metastases and regional nodes are the

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predominant sites of failure,[1-3, 6] potential biomarkers such as *KRAS* mutation status that identify patients at high risk for such recurrence may help guide the use of adjuvant therapy, and may be particularly important in the medically ill subset of patients treated with SBRT.

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One of the main limitations of this study was incomplete genotyping. There is a possibility that incomplete genotyping may introduce bias, but since *KRAS* mutations are present in approximately 20% of adenocarcinomas, it is likely that the incomplete genotyping would bias the results toward the null, since there would be patients with undetected *KRAS* in the control group. Another unusual finding was the high incidence of *KRAS* mutations (70%; n=7) in the subset of patients (n=10) who had tumor genotyping, which may have been due to chance or possibly due to the heavy smoking history in this patient population (>95% were smokers with median 50 pack-years history). Thus, the association between outcomes and *KRAS* mutation status must be interpreted with caution due the potential for confounding given the unexpectedly high incidence of *KRAS* mutation in the genotyped cohort, and the association between *KRAS* mutation and larger tumor size in this study.

Additionally, less common genotypic subgroups such as patients with *EGFR*-mutant or *ALK*-translocated tumors could not be analyzed in this cohort due to small numbers and lack of testing, which was not clinically-indicated due to the low incidence of alterations of these genes in a group of patients with heavy smoking . Similarly, this study was conducted in an era where clinical genotyping involved only a limited panel of genes (*KRAS, EGFR,* and *ALK*), and thus, co-mutations in genes that are known to alter the underlying biology of *KRAS* mutant tumors such as *LKB1* and *p53* were not genotyped.[22] Clearly, further studies analyzing outcomes after SBRT by genotyping

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KRAS and a more comprehensive set of associated genes are needed, but may be limited by the difficulty of obtaining sufficient tumor samples in the medically inoperable subset of patients with NSCLC. For instance, in this study, the majority of patients underwent fine-needle aspiration which precluded additional genetic analyses. Additionally, due to concerns for significant potential biopsy-related morbidity (e.g. pneumothorax) among these medically ill patients, many patients treated with SBRT do not have a pathological diagnosis, but are treated with a radiographic diagnosis only.[6] This underscores the need for cooperation and coordination between multiple centers to comprehensively genotype patients undergoing SBRT who have biopsy specimens available.

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CONCLUSIONS:

In this series of patients with medically inoperable early stage NSCLC who were treated with SBRT, there was no significant difference in outcomes by histology. A small *KRAS-mutant* sub-group had a significantly higher risk of recurrence on univariable analysis and cancer-specific mortality on multivariable analysis compared to patients with wild-type or unknown *KRAS* status. Differences in outcomes after SBRT by *KRAS* genotype is worthy of further study, but, further study may be limited by the difficulty of obtaining sufficient tumor samples in the medically inoperable subset of patients with NSCLC.

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CLINICAL PRACTICE POINTS:

SBRT remains an important treatment for early stage NSCLC. Tumor genotyping of patients with NSCLC may yield further insight into radiation response of molecular sub-types of NSCLC and provide information for future trials of adjuvant or salvage targeted therapies in high risk patients.

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FIGURE LEGENDS:

Figure 1: Outcomes by KRAS mutation status including: (A) Kaplan-Meier plot of overall survival; (B) Kaplan-Meier plot of freedom from any recurrence; (C) Cumulative incidence plot of death due to cancer with death due to other causes as a competing risk.