A Clinical Model for Identifying Radiosensitive Tumor Genotypes in Non–Small Cell Lung Cancer

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Abstract

Purpose: Non–small cell lung cancer (NSCLC) includes a spectrum of radiosensitive and radioresistant tumors. However, little is known about the molecular determinants of cellular radiation responses. We examined clinical outcomes after gamma knife radiotherapy for NSCLC intracranial metastases to evaluate the use of this model for determining radiosensitive tumor genotypes.

Experimental Design: Between 2005 and 2012, 239 patients with NSCLC were enrolled in a prospective gamma knife data repository. Molecular pathology regarding EGF receptor (EGFR), ALK, and KRAS mutation status was available for 81 patients. Local and distant brain control was determined for 79 patients with 469 brain metastases. Modified Cox proportional hazards models were established to evaluate local control for treated lesions after serial gamma knife treatments.

Results: In total, 11% of patients developed in-field recurrence. No patients with metastases from tumors with EGFR mutations (0/164 lesions) or EML4-ALK translocations (0/61 lesions) recurred in-field. In contrast, 19% of patients without these mutations and 18% of patients with KRAS mutations recurred in-field (10/139 and 3/105 lesions, respectively). Rates of distant brain recurrence did not significantly differ across tumor genotypes. The predicted median in-field local control was significantly longer for EGFR-mutant and ALK-translocated tumors compared with other patients with NSCLC (P < 0.001), whereas distant brain recurrence time was equivalent (P = 0.97). On multivariate analysis, EGFR mutation, ALK translocation, and metastasis size were independent predictors for superior local control after gamma knife treatment.

Conclusions: This study suggests that EGFR kinase domain mutations and EML4-ALK translocations are radiosensitive NSCLC genotypes, and proposes a novel model to identify radiosensitive subtypes of NSCLC.

Clin Cancer Res; 19(19); 5523–32. ©2013 AACR.

Introduction

Radiation is an effective therapeutic modality for non–small cell lung cancer (NSCLC) and is indicated in the treatment of both early and advanced stage disease confined to the thorax (1–4), as well as for brain metastases (5, 6). Radiotherapy has been incorporated into standard NSCLC therapeutic regimens over the past three decades; however, little is known about the molecular determinants of tumor radiation responses. Although several studies have used candidate gene approaches to evaluate radiation responses in NSCLC (7–10), none have been validated or are commonly used to guide clinical decision making. The inability to understand the fundamental basis for NSCLC sensitivity or resistance to radiation prevents a risk-based stratification for clinical trials as well as target driven therapeutic strategies for improving the efficacy of radiotherapy.

A major barrier to investigating tumor radiotherapy responses is that validated clinical models of radiosensitivity are virtually nonexistent. Multiple gene signatures associated with radiosensitivity have been described (11, 12), but clinical models able to detect single gene variants associated with radiation response have not been validated. In the case of NSCLC, determination of local control rates for thoracic tumors are confounded by variations in multiple treatment factors including dose, tumor size, planning techniques, respiratory motion, the uncertainty of computed tomography–based target definition, and the interactive effects of systemically delivered therapy. Furthermore, computer tomography–based assessments of radiotherapy response in NSCLC are discordant with pathologic findings (13), and show the difficulty in verifying response or recurrence in the irradiated lung. Together, these diverse factors lead to heterogeneity within a treated population of...
patients and preclude a method for confidently identifying tumors that are either sensitive or resistant to radiotherapy.

In vitro laboratory models of clonogenic survival have served as the gold standard for determining radiation sensitivity for more than 50 years (14), but also have significant limitations. First, there is a substantial selection bias for the establishment and propagation of cell lines from human patient samples. The success rate for generation of viable cell lines from the National Cancer Institute collection has been estimated to be approximately 12% (15) and more recent studies. Second, in vitro clonogenic studies are limited by cell growth characteristics that preclude colony formation. Although protocol manipulations such as optimization of cell culture media or plating cell suspensions in agar may improve colony formation, many cell lines remain challenging for clonogenic survival analyses. Third, the complex interactions of tumor cells with surrounding stroma and normal tissues which can affect radiation responses (18, 19) are not recapitulated with in vitro models.

In our efforts to identify an unbiased and robust clinical model with the statistical power to evaluate NSCLC radiation responses, we explored the use of a brain metastases model for determining NSCLC radiosensitivity. This model uses clinical data derived from the prospectively collected Yale Gamma Knife Data Repository. Gamma knife radiotherapy (or radiosurgery) is a stereotactic technique for delivering radiotherapy that uses uniform doses (20) and uniform treatment planning based on MRI imaging (21). Thus, both the patient population and therapeutic intervention are relatively homogeneous for gamma knife treatment. Furthermore, because of the blood brain barrier and the tendency for systemic therapy to be discontinued at the time of treatment, a study of brain metastasis outcomes reduces the potentially confounding effects of concurrent or adjuvant systemic therapies on local control. We therefore carried out an analysis of in-field recurrence and out-of-field recurrence for NSCLC brain metastases treated with gamma knife radiosurgery (GKRS), and investigated outcomes for tumors with known gene mutations (EGFR, KRAS, ALK) with the goal of identifying molecular markers that predict radiosensitivity. Our findings support the concept that a gamma knife brain metastasis model is a novel approach for defining radiosensitive and radioresistant genotypes in NSCLC.

Materials and Methods

Patients

Two hundred and thirty-nine patients with NSCLC and brain metastases treated with GKRS were prospectively enrolled in an Institutional Review Board and Human Investigations Committee approved database between 2005 and 2012. Informed consent or a waiver of consent was obtained for each participant. Age, gender, race, craniotomy history, history of whole brain radiotherapy, and prior and concurrent use of systemic agents were recorded for each patient, as well as treatment parameters including number of lesions treated, tumor location, volume, and margin dose.

Molecular pathology results for EGFR kinase domain mutations, EML4-ALK translocations, or KRAS mutations were available for 81 patients. EGFR mutation status was determined by PCR amplification and sequence analysis of exons 18, 19, 20, and 21 comprising the tyrosine kinase domain. KRAS genotyping was assessed by PCR amplification and sequence analysis of exon 1 (codons 12 and 13) and exon 2 (codon 61). ALK rearrangement was assessed by FISH. In total, molecular analysis of the primary tumors identified 21 tumors with EGFR mutations, 17 with KRAS mutations, 9 with ALK translocations, and 34 without these mutations. Of the EGFR-mutant tumors, 11 tumors had exon 19 deletions (2 with a T790M secondary mutation), 9 tumors had exon 1 L858R or L861Q missense mutations (1 with a T790M secondary mutation), and 1 tumor had both an exon 20 duplication and point mutation in exon 21. The KRAS-mutant tumors included 15 G12 mutations in exon 1 (6 G12C, 4 G12V, 3 G12S, 1 G12R, and 1 G12A), one Q61 mutation in exon 2 (Q61H), and 1 tumor in which the specific mutation was not documented. Patients who underwent GKRS for consolidation of resection cavities were excluded, leaving 21 EGFR-mutant, 17 KRAS-mutant, 9 ALK-rearranged, and 32 patients without detected mutations, corresponding to 469 metastatic brain lesions (164 EGFR-mutant, 1 ALK, 105 KRAS-mutant, and 139 without detected mutations). For the Cox proportional hazards models to evaluate local control, patients who did not have followup brain MRI assessment were excluded from analysis because of inability to accurately assess local control.

Treatment

Patients were treated with a Leksell gamma knife Model C or Leksell gamma knife Perfexion, and GammaPlan...
software was used for treatment planning (Elekta Inc). Dose selection for gamma knife therapy was based on institutional modifications of the RTOG 90-05 (20) and 95-08 (5) trial doses. In general, marginal doses of 22–24 Gy were prescribed for metastases less than 1 cm in diameter, 20 Gy for metastases 1–2 cm, 18 Gy for metastases 2–3 cm, and 16 Gy for metastases 3–4 cm. For patients on systemic therapies, standard protocol was for discontinuation of systemic agents several days before treatment such that no patient received concurrent gamma knife therapy with chemotherapy or a tyrosine kinase inhibitor (TKI).

Response evaluation

Gamma knife treatment plans and all posttreatment surveillance brain MRI studies were reviewed to assess local control. In-field recurrence was defined by (i) a greater than or equal to 20% increase in the longest diameter of the lesion over nadir, measured on postcontrast T1 axial images, (ii) consensus amongst a multi-disciplinary team consisting of a neurosurgeon, radiation oncologist, and radiologist, or (iii) pathology at the time of resection. Gamma knife treatment plans were reviewed for all local failures to confirm that all recurrences occurred within the 50% isodose line, excluding marginal failure as a factor in this analysis.

Distant brain failure was defined as a new brain lesion detected on surveillance MRI imaging that was not previously treated or present on prior gamma knife treatment-planning MRIs.

### Statistical analysis

Statistical analysis was performed using SAS 9.2 (SAS Institute) and Stata Version 11 (StataCorp LP). The follow-up and local control times were calculated from the date of gamma knife treatment to last followup or recurrence as documented by MRI. The gamma knife treatment planning software was used to calculate lesion volume in cubic centimeters, and lesions were assumed to be a sphere in order to estimate lesion diameter for Table 1. Patient and treatment characteristics were compared among molecular subtypes of tumors at the patient level using χ² tests for categorical variables. Linear mixed models were built to compare continuous variables for both per-treatment and per-lesion analysis of different genotypes, with patients included as a random variable to account for within-subject correlation. The standard Kaplan–Meier method excludes events for patients who undergo subsequent gamma knife treatment or have multiple sequential recurrences. Thus, for survival analysis, a modified Cox proportional hazards model was built with a robust sandwich covariance matrix estimate to account for the dependence of recurrence events within a single patient after serial gamma knife sessions, and for multiple lesions treated per gamma knife session (22, 23). Two analyses were carried out for the recurrence data. First, patients with EGFR kinase domain mutation or ALK translocation were analyzed together to compare tyrosine kinase–activated patient populations to those with non–tyrosine kinase activated tumors. χ² tests or the Fisher exact test were also used to compare the absolute recurrence rates

<table>
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<tr>
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<th>ALK translocation</th>
<th>KRAS mutant</th>
<th>Other</th>
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<th>P</th>
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<td>5.9</td>
<td>6.2</td>
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<td></td>
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<td>5 (56%)</td>
<td>7 (41%)</td>
<td>15 (47%)</td>
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<td>4 (44%)</td>
<td>10 (59%)</td>
<td>17 (53%)</td>
<td>43 (54%)</td>
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</tr>
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<td></td>
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<tr>
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<td>3 (33%)</td>
<td>8 (47%)</td>
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<td>14 (44%)</td>
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<td>2 (6%)</td>
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<tr>
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<td>1 (6%)</td>
<td>1 (3%)</td>
<td>5 (6%)</td>
<td></td>
</tr>
<tr>
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<td>3</td>
<td>1</td>
<td>2</td>
<td>0.05</td>
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<tr>
<td>Mean</td>
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<td>0.68</td>
<td>0.79</td>
<td>0.88</td>
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<td>Dose prescribed (Gy)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>18</td>
<td>20</td>
<td>18</td>
<td>20</td>
<td>20</td>
<td>0.08</td>
</tr>
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<td>18–22</td>
<td>16–24</td>
<td>16–24</td>
<td>16–24</td>
<td></td>
</tr>
<tr>
<td>History of cranietomy</td>
<td>3 (14%)</td>
<td>4 (44%)</td>
<td>3 (18%)</td>
<td>6 (19%)</td>
<td>16 (20%)</td>
<td>0.28</td>
</tr>
<tr>
<td>History of WBRT</td>
<td>6 (29%)</td>
<td>4 (44%)</td>
<td>6 (35%)</td>
<td>9 (28%)</td>
<td>25 (32%)</td>
<td>0.79</td>
</tr>
</tbody>
</table>
in these groups. A second analysis was carried out for individual genotypes (EGFR kinase domain mutation, ALK translocation, KRAS mutation, and other) to explore differences in outcome by these molecular subtypes. Recurrence probability curves for both local and distant-brain sites were generated from the Cox proportional hazards model for tyrosine kinase–activated versus non-activated groups, as well as by mutant genotype, to compare these risks between patient groups. The median recurrence time for each NSCLC molecular subgroup was calculated according to the predicted survival function. A bivariate Cox proportional hazards model followed by multivariate analysis was then carried out including the specific genotypes, age, number of lesions treated, lesion diameter, and dose prescribed in the model. A two-sided $P$ value of $\leq 0.05$ was considered statistically significant.

Results

Patient and treatment characteristics

Seventy nine patients were included in this analysis, and the median followup time for the entire cohort was 6.2 months. For the local control model, median followup was 9.5 months (range 1.5–45.1 months) for EGFR-mutant patients, 8.0 months (range 4.7–36.6 months) for ALK patients, 5.2 months (range 2.2–38.9 months) for KRAS patients, and 7.4 months (range 1.3–84.5 months) for patients without these mutations. In-field local control of gamma knife–treated lesions and distant brain control were determined for each patient. The characteristics of these patients and their treatment are summarized in Table 1. The mean lesion diameter was 0.78 cm (0.71 cm for EGFR-mutant, 0.68 cm for ALK, 0.79 cm for KRAS-mutant, and 0.88 cm for tumors without detected mutations; overall SD = 0.49 cm). The mean lesion diameter was significantly different across mutation types ($P = 0.03$), and this significance was attributed to the EGFR kinase domain–mutant metastases having a slightly smaller size than those without detected mutations. The median prescribed dose for all patients was similar (18 Gy for EGFR-mutant, 20 Gy for ALK, 18 Gy for KRAS-mutant, and 20 Gy for tumors without detected mutations; overall range 16–24 Gy), with a borderline difference for lower prescription dose for EGFR-mutant tumors ($P = 0.08$). This difference could be attributed to an increased number of lesions treated per patient with EGFR mutation and an institutional tendency to decrease prescription dose as lesion number increases. The median number of lesions treated per gamma knife session was 2 overall (3 in the EGFR-mutant patients, 2 in the ALK patients, 3 in the KRAS-mutant patients, and 1 in the patients without detected mutations), and this difference was significant across the groups ($P = 0.05$). 20% of patients underwent a craniotomy and resection of a brain metastasis before or after gamma knife treatment, and 32% of patients received whole-brain radiotherapy. There was no significant difference across the groups in the fraction of patients receiving craniotomy for tumor resection or whole-brain radiotherapy either before or after gamma knife. The distribution of patients $\leq 60$ years of age or $>60$ years also did not differ significantly among tumor subtypes; however, the EGFR-mutant and ALK translocation-positive populations had increased proportions of Asian race and female gender, consistent with other published reports (24–26).

Evaluation of brain recurrence after GKRS

Representative pre and post gamma knife gadolinium-enhanced MRI images are presented in Fig. 1 and show examples of both local control (top) and local failure (bottom) following GKRS. Absolute recurrence rates for the 79 patients with 469 GKRS-treated brain metastases are shown in Table 2 (top) and subdivided by the tumor molecular subtype. In 21 patients with EGFR-mutant tumors (164 treated lesions), no lesion recurred in-field after GKRS. Similarly, in 9 ALK translocation-positive patients (61 treated lesions), there were no in-field recurrences. In contrast, 19% of patients without a detected mutation (10/139, 7.2% of treated lesions) developed an in-field recurrence and a similar recurrence rate of 18% (3/105, 2.9% of treated lesions) was observed for patients with a KRAS mutation. Of the 13 lesions that recurred, 9 were less than 1 cm in diameter at time of GKRS, and the largest lesion that recurred was 2.4 cm.

Analysis of local and elsewhere brain recurrence for tyrosine kinase–activated tumors

The local control of metastases with EGFR tyrosine kinase domain mutations or ALK translocation was superior, and because both genetic events are mechanistically analogous and cause tyrosine kinase-dependent tumor growth (27, 28), we combined these molecular subtypes for further statistical analysis. In patients without molecular evidence of tyrosine kinase mutation, in-field recurrence was observed in 18% of patients (13 of 244 treated lesions) compared with no recurrences in patients with tyrosine kinase–activated NSCLC tumors (0 of 225 treated lesions). Absolute in-field recurrence rates were therefore significantly different for tyrosine kinase–activated versus nonactivated tumors at both the patient ($P = 0.01$) and lesion ($P < 0.001$) levels. In contrast, examination of distant brain recurrence showed essentially equivalent rates of failure in the tyrosine kinase–activated and nonactivated populations (53% vs. 47% respectively, $P = 0.58$; Table 2, bottom).

We next sought to generate probabilities for local and distant control of brain metastases for these 2 patient subgroups using the Cox proportional hazards model. The estimated in-field local control was significantly different for the patients with tyrosine kinase–activated tumors compared with patients with either no detected mutation or a KRAS mutation ($P < 0.0001$; Fig. 2A left). Median in-field recurrence time was 18.4 months for patients without tyrosine kinase mutations, but was not reached for tyrosine kinase–activated tumors. Otherwise stated, the predicted in-field local control for tyrosine kinase–activated tumors within this time frame is 100%, while tyrosine kinase wild-type patients have a 50% predicted risk of in-field recurrence. For those patients with non–tyrosine kinase activated tumors, the estimated rate of in-field recurrence...
at 6 months (a clinically relevant time point for patients with brain metastases) was 25%. In contrast with the in-field local control data, the median distant-brain-recurrence time was 7.7 months and equivalent for both tyrosine kinase-activated and tyrosine kinase wild-type tumors ($P = 0.97$, Fig. 2A right panel).

### Table 2. Absolute recurrence rates by molecular subtype and for tyrosine kinase-activated tumors versus other tumors

<table>
<thead>
<tr>
<th></th>
<th>EGFR mutant</th>
<th>ALK translocation</th>
<th>KRAS mutant</th>
<th>Other</th>
<th>ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>By patient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-field</td>
<td>0/21 (0%)</td>
<td>0/9 (0%)</td>
<td>3/17 (18%)</td>
<td>6/32 (19%)</td>
<td>9/79 (11%)</td>
</tr>
<tr>
<td>Distant brain</td>
<td>9/21 (43%)</td>
<td>7/9 (78%)</td>
<td>10/17 (59%)</td>
<td>13/32 (41%)</td>
<td>39/79 (49%)</td>
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<td><strong>By lesion</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>In-field</td>
<td>0/164 (0%)</td>
<td>0/61 (0%)</td>
<td>3/105 (3%)</td>
<td>10/139 (7%)</td>
<td>13/469 (3%)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Tyrosine kinase-activated</th>
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<th>$P$</th>
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</thead>
<tbody>
<tr>
<td><strong>By patient</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>In-field</td>
<td>0/30 (0%)</td>
<td>9/49 (18%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Distant-brain</td>
<td>16/30 (53%)</td>
<td>23/49 (47%)</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>By lesion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-field</td>
<td>0/225 (0%)</td>
<td>13/244 (5%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 1. Representative pre- (A and C) and post–gamma knife (B and D) T1 contrast-enhanced MRI images are shown for a patient with an EGFR-mutant tumor (A and B), and a patient without mutations detected (C and D). Sustained local control is shown 15 months after gamma knife for the EGFR-mutant lesion (B), whereas the lesion without detected tyrosine kinase mutations recurred by 6 months after gamma knife (C). Ultimately viable tumor cells were found when craniotomy and resection of this lesion were carried out at 11 months.
Analysis of local and elsewhere brain recurrence by mutant genotype

The generated Cox proportional hazards model was also used to analyze patient outcomes by mutant genotypes for both in-field and distant-brain control (Fig. 2B). In-field local control was superior for EGFR-mutant and ALK translocation-positive tumors, whereas distant-brain recurrence rates were not significantly different among the molecular subtypes \((P = 0.22)\), although ALK- and KRAS-mutant patients trended towards having increased probabilities of distant-brain recurrences.

Using this model, multivariate analysis for independent predictors of local recurrence was then carried out. The data in Table 1 showed that metastasis diameter was significantly smaller, whereas the number of lesions treated was significantly greater in the EGFR-mutant population. However, despite these significant correlations, these factors were not predictors of local control in bivariate Cox regression analysis. Moreover, multivariate analysis including these covariates with tumor genotype identified EGFR tyrosine kinase–mutation or an ALK translocation as independent predictors of improved local control \((P < 0.0001)\); Table 3).

In contrast, both EGFR mutation status and ALK translocation status did not independently predict for distant-brain failure \((P = 0.67 \text{ and } 0.17, \text{ respectively})\). KRAS mutation status did not correlate with in-field local control \((P = 0.66)\) or distant-brain failure \((P = 0.21)\). As expected, mean lesion diameter was also independently associated with in-field local control \((P = 0.05)\).

TKI use in EGFR kinase domain–mutant patients

EGFR tyrosine kinase inhibitors have weak central nervous system (CNS) penetration \((29–31)\), and strategies for pulsed high-dose therapy have been used to obtain CNS disease stability \((32, 33)\). Although patients with EGFR-mutant tumors were not treated with concurrent tyrosine kinase inhibitors in our cohort, EGFR TKI therapy was initiated before gamma knife in approximately half of the EGFR-mutant patients, and we investigated whether this

Table 3. Multivariate analysis to determine independent predictors of in-field local control and distant brain control

<table>
<thead>
<tr>
<th></th>
<th>In-field local control: (P)</th>
<th>Distant brain control: (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR mutation</td>
<td>&lt;0.0001</td>
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</tr>
<tr>
<td>ALK translocation</td>
<td>&lt;0.0001</td>
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<td>KRAS mutation</td>
<td>0.66</td>
<td>0.21</td>
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<tr>
<td>Mean lesion diameter</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td>Number of lesions treated</td>
<td>0.31</td>
<td>0.29</td>
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</table>
was a potential confounder for local control (Table 4). Of the 164 gamma knife–treated EGFR-mutant lesions, 85 (52%) had progressed after initiation of EGFR TKI therapy, suggesting that these patients had developed acquired-resistance. The high incidence of progression on EGFR TKI therapy in this population as well as the high rate of distant-brain–failure strongly suggest that TKI therapy alone, received before or after gamma knife radiotherapy, cannot sufficiently explain the observed increase in local control.

**Discussion**

Approximately 30% of the patients with advanced stage NSCLC develop brain metastases (34) and radiotherapy delivered with either whole-brain radiation or stereotactic techniques (such as gamma knife) is a central therapeutic component of patient management. We proposed that an analysis of outcomes after GKRS in combination with tumor molecular characterization would provide a unique clinical scenario to further our understanding of the relationships between tumor genotype and radiation response. We therefore developed a clinical model to estimate local disease control for patients with NSCLC in the setting of serial gamma knife treatments. The three main findings from this work are: (i) tumors with EGFR kinase domain mutations treated with GKRS have superior control rates as determined by imaging, a finding that provides a clinical validation for previous in vitro observations (35, 36) using NSCLC cell lines; (ii) tumors with EML4-ALK–translocation also have superior control rates with radiotherapy, a novel finding generated from this analysis; and (iii) tumors with G12 or Q61 KRAS mutations have a similar radiosensitivity profile to that of other NSCLC tumors without EGFR kinase domain mutation or EML4-ALK–translocation. Together, the results of this study support the concept that the GKRS brain metastasis model can be used to identify radiosensitive subtypes of NSCLC.

There are several major advantages of the brain metastasis model in comparison with analysis of thoracic radiotherapy outcomes. First, there is reduced uncertainty and variability of treatment parameters for GKRS, due to the use of a stereotactic head frame and high resolution MR imaging, which obviates the need for approximations of microscopic disease-invasion and thus CTV and PTV radiation planning expansions. Second, the NSCLC metastases treated with GKRS have similar clinical characteristics with regard to size and therefore receive similar dose-prescriptions. In this cohort, the diameter of metastases ranged from 0.23 to 2.95 cm, and all molecular subtypes were treated with similar doses of radiation. In contrast, studies of thoracic radiation can include T1-T4 tumors (treated definitively or in the postoperative setting) of variable size that are prescribed doses that range from 41.4 to 74 Gy (37). The third advantage of this model is the number of lesions treated per patient, compared with the single primary site of thoracic radiotherapy. Independent surveillance of each brain metastasis increases the statistical power of the analysis and allows for the identification of significant differences between groups in the setting of a limited number of patient treatment events.

Another potential advantage of the CNS-metastasis model is the ability to separate the effects of systemic therapies from radiotherapy. Thoracic radiation is delivered with concurrent or adjuvant systemic therapy which contributes to local control of the tumor (4). The investigation of brain metastases substantially reduces, although does not completely eliminate, the contributions of this confounding variable. The comparison of local and distant control in the brain also provides a method to further understand the contributions of systemic therapy to local CNS disease control. In this study we found that tumors with EGFR kinase domain mutation and EML4-ALK–translocation had 100% local control after radiation but failed in non-irradiated brain sites at the same rate as non–tyrosine kinase driven tumors, strongly suggesting that the improved local control was the consequence of radiotherapy. Furthermore, the independent effect of radiation on local control is reinforced for EGFR kinase domain–mutant tumors by the fact that approximately half of these patients had received a TKI before gamma knife radiotherapy and had progressed on treatment, indicating that acquired resistance and loss of sensitivity to the inhibitor had likely already developed. Prospective studies using EGFR-specific TKIs as first-line or salvage therapy for brain metastases have reported partial responses in the brain, particularly in EGFR TKI naïve patients, with limited durable responses. This is likely due to the low CNS drug penetration for erlotinib which is in the range of 1%–5% of plasma concentrations and the incomplete understanding of drug pharmacokinetics within a CNS metastasis (31–33, 38–45). Although there is clear merit to these clinical reports, in the context of decreased CNS activity and our detailed review of both CNS failure and tumor characteristics in our cohort, there is insufficient data to imply that the improvements in local control are entirely secondary to the use of TKIs.

This CNS metastasis model is limited by the heavy reliance on imaging based identification of local failure. Although MR imaging was used to judge recurrence, this modality cannot definitively discriminate between recurrence and radionecrosis in all cases. We judged local lesion control with a multidisciplinary team based on serial imaging, the presence of symptomatic progression, and neurosurgical intervention when indicated. Overall, the local control rate in our cohort was the same as that of other published series (46, 47), suggesting that we did not over or
underestimate local control rates and that the model is generally applicable for patients treated with gamma knife radiotherapy. In comparison with thoracic radiotherapy, the use of MRI may be superior, as major limitations for computer tomography assessment of lung tumors have been well shown. An example of the inaccuracies of computer tomography is provided by the INT0160 trial of neoadjuvant chemo-radiation for superior sulcus tumors (13). Of the 40 patients in that study with stable disease by computer tomography-scan after radiotherapy, 14 had a pathologic complete response to therapy, 10 had minimal microscopic disease, and 16 had gross residual disease present, showing that reliable prediction of thoracic radiation treatment response is problematic. Regardless, the difficulty in obtaining pathologic confirmation of local control remains a limitation of this CNS model.

A further limitation of this CNS model is the fact that molecular analysis was carried out on the primary tumors and presumed to be representative of each metastatic lesion. Although it is known that the genotype of primary tumors may not reflect that of each metastatic lesion, analyses of EGFR and ALK mutation status indicate that only a minority of metastatic lesions for tyrosine kinase–driven tumors have a discordant mutation status compared with the matched primary tumors. A meta-analysis including 598 matched primary NSCLC tumors and metastatic lesions (357 of which were positive for EGFR mutations) showed that EGFR mutation status was concordant between primary and metastatic tumors in 83% of the pairs (RR = 0.86, P = 0.31; ref. 48). A study of ALK translocation status in 67 primary NSCLCs and their corresponding metastatic lesions detected ALK rearrangement in 7.5% of the primary tumors and 9.0% of the metastases (49). These data suggest that EGFR mutations and ALK translocations are primarily conserved in primary tumors and metastatic lesions.

The enhanced radiosensitivity of tumors harboring EGFR kinase domain mutations shown in this clinical cohort validates previously published in vitro data, which suggests that NSCLC tumors with somatic activating mutations in the tyrosine kinase domain of EGFR may be more sensitive to ionizing radiation (35, 36). Although there are differences in the biologic response of tumor cells to high dose per fraction radiation, we suggest that the findings from our model may still be relevant for clinical settings of fractionated radiotherapy regimens. In vitro, NSCLC cell lines with somatic activating mutations in the tyrosine kinase domain of the EGFR, including those with secondary mutations that confer TKI resistance, have been previously shown to be radiosensitive compared with wild-type cell lines and also exhibit impaired radiation-induced cell-cycle checkpoints, delayed repair of radiation-induced DNA double-strand breaks, and increased apoptosis (35, 36). Our results provide clinical validation of this in vitro finding, and also add to the clinical observations of improved clinical response (50) and improved survival (51) following whole-brain radiotherapy for patients with EGFR kinase domain–mutations, as well as reduced thoracic recurrence after combined modality therapy (37). The second interesting finding from our clinical model is that tumors with EML4-ALK–translocations could also have relatively increased sensitivity to radiotherapy. Because of the low incidence of this translocation in the NSCLC population and the absence of multiple established NSCLC cell lines with EML4-ALK translocation, this association cannot yet be tested in vitro. This brain metastasis model also provides insight regarding the association of KRAS mutation with radioresistance. There is considerable debate as to whether endogenous KRAS mutations confer radioresistance (52) and our data suggests that tumors with these mutations are at least of intermediate radiosensitivity; more resistant than tumors with EGFR- or ALK-mutation but no more resistant than other NSCLC tumors. Given the prevalence of EGFR-, ALK-, and KRAS mutations, this analysis suggests that for lung adenocarcinoma specifically, mutant genotypes identify a radiosensitive phenotype in approximately 15% (EGFR-mutant and ALK translocation) and intermediate sensitivity in approximately 25% (KRAS-mutant) of tumors. We anticipate that further genotype–phenotype correlations for the remaining 60% of adenocarcinomas will be made in the future to refine our understanding of NSCLC radiosensitivity.

The clinical outcomes from gamma knife radiotherapy suggest that tyrosine kinase–driven tumors (EGFR and ALK) could be a relatively more radiosensitive subtype of NSCLC. The results of this analysis, however, cannot suggest a mechanistic basis for altered radiosensitivity; rather the mutant genotypes identified in this study can currently only be interpreted as biomarkers that are associated with tumor radiation responses. Thus the observed increase in local control may only be a characteristic of oncogene-driven tumors and not a phenotype caused by oncogenic signaling. Yet it is tempting to speculate that oncogene-driven tumors such as EGFR and ALK are more sensitive to radiotherapy. The finding that KRAS-mutant tumors are not more radiosensitive is not inconsistent with this hypothesis, as the relationship between KRAS mutation and oncogene addiction is complex. Singh and colleagues showed that not all KRAS-mutant tumors are dependent upon KRAS to drive cell proliferation and survival (53), and Lim and colleagues have shown in animal models that while a KRAS mutation can drive tumor growth at initiation, it is dispensable as the tumor progresses (54). Thus, the KRAS findings from this metastasis model may reflect the more heterogeneous nature of tumors that harbor mutations of this gene. Ultimately, because of the clinical nature of our study, oncogenic addiction cannot be definitively associated with a radiosensitive phenotype, and further work will be required to adequately test this hypothesis.

Cancer therapeutic regimens are becoming increasingly tailored by the detection of specific molecular and genetic alterations; however, no tumor genotype has yet been exploited as a means to personalize the delivery of radiotherapy. Our results suggest that EGFR kinase domain mutations, and perhaps ALK translocations, are candidate tumor biomarkers that could be used to modify prescribed doses for radiotherapy. In the setting of brain metastases, where radionecrosis of the brain is an infrequent but
problematic risk of therapy, we propose that it might be possible to safely reduce stereotactic doses without significant compromise to tumor control. We plan to test this hypothesis in a prospective trial at our institution. The second group that may derive benefit from this analysis are the patients without EGFR kinase domain mutation or ALK translocation. Despite a high rate of local control for these tumors, we show that these patients have a 25% probability of local recurrence at 6 months, and thus may benefit from escalating GKRS dose or intensification of therapy such as through the addition of a systemic agent at the time of stereotactic radiation. Thus, our findings not only provide insights into radiation responses associated with NSCLC genotypes, but also suggest methods for patient stratification and optimization of therapeutic regimens.

Conclusion
This study shows that the unique biology of EGFR tyrosine kinase–mutant and ALK-translocation–tumors results not only in an improved response to targeted systemic therapies, but also inherent radiosensitivity compared with tyrosine kinase wild-type tumors. Moreover, we have established and validated a statistical model to use local control of metastatic brain lesions after gamma knife as a platform for determining the radiosensitivity of molecular subtypes of tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.L. Johung, X. Yao, F. Li, S.N. Gettinger, S. Goldberg, R.H. Decker, V.L. Chiang, J.N. Contessa
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Received March 25, 2013; revised June 6, 2013; accepted July 14, 2013; published OnlineFirst July 29, 2013.

References


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