A Clinical Model for Identifying Radiosensitive Tumor Genotypes 

in Non-Small Cell Lung Cancer

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TRANSLATIONAL RELEVANCE

Radiation therapy is an integral component of treatment for non-small cell lung cancer (NSCLC). However the molecular determinants of NSCLC radiosensitivity remain largely unknown, in part due to the limitations of current in vitro and clinical models. We have devised a novel clinical model that utilizes local control of metastatic brain lesions after stereotactic Gamma Knife (GK) radiotherapy to define radiosensitive and radioresistant genotypes in NSCLC. Our work suggests that tyrosine kinase activating mutations of EGFR or ALK are radiosensitive NSCLC genotypes, while KRAS mutant tumors do not exhibit a radiosensitive phenotype. These findings underscore the utility of the GK clinical model for determining tumor radiosensitivity, and provide a foundation for further exploration into the possibility of tailoring radiation therapy to the underlying radiosensitivity of specific tumor genotypes.
ABSTRACT

Purpose: 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 (GK) radiotherapy for NSCLC intracranial metastases to evaluate the utility of this model for determining radiosensitive tumor genotypes.

Experimental Design: Between 2005 and 2012, 239 NSCLC patients were enrolled in a prospective GK data repository. Molecular pathology regarding 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 GK 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 to other NSCLC patients (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 GK.

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.
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). Radiation therapy (RT) 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 RT.

A major barrier to investigating tumor RT responses is that validated clinical models of radiosensitivity are virtually non-existent. 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 CT-based target definition, and the interactive effects of systemically delivered therapy. Furthermore CT based assessments of RT response in NSCLC are discordant with pathologic findings (13), and demonstrate 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 radiation therapy.

_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 NCI collection has been estimated to be approximately 12% (15) and more recent NSCLC specific studies using modern techniques have estimated this rate to be 25-30% (16, 17), demonstrating that the majority of patient tumors do not grow well in culture and therefore are not represented by _in vitro_ 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 tumors 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 utility of a brain metastases model for determining NSCLC radiosensitivity. This model employs clinical data derived from the prospectively collected Yale Gamma Knife (GK) Data Repository. Gamma Knife radiotherapy (or radiosurgery) is a stereotactic technique for delivering RT that employs uniform doses (20) and uniform treatment planning based on MRI imaging (21). Thus both the patient population
and therapeutic intervention are relatively homogeneous for GK 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 performed 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 GK brain metastasis model is a novel approach for defining radiosensitive and radioresistant genotypes in NSCLC.

**MATERIALS AND METHODS**

*Patients*

239 patients with NSCLC and brain metastases treated with Gamma Knife radiosurgery (GKRS) were prospectively enrolled in an IRB 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 radiation therapy, 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 (KD) 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 fluorescence in-situ hybridization (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, eleven tumors had exon 19 deletions (two with a T790M secondary mutations), nine tumors had exon 21 L858R or L861Q missense mutations (one with a T790M secondary mutation), and one tumor had both an exon 20 duplication and point mutation in exon 21. The KRAS mutant tumors included fifteen G12 mutations in exon 1 (6 G12C, 4 G12V, 3 G12S, 1 G12R, and 1 G12A), one Q61 mutation in exon 2 (Q61H), and one 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, 61 ALK, 105 KRAS mutant, and 139 without detected mutations). For the Cox proportional hazards models to evaluate local control, patients who did not have follow up 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 GK 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 <1 cm 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 prior to treatment, such that no patient received concurrent GK with chemotherapy or a tyrosine kinase inhibitor (TKI).

Response Evaluation

GK treatment plans and all post-treatment surveillance brain magnetic resonance imaging (MRI) studies were reviewed to assess local control. In-field recurrence was defined by 1) a greater than or equal to 20% increase in the longest diameter of the lesion over nadir, measured on post-contrast T1 axial images, 2) consensus amongst a multi-disciplinary team consisting of a neurosurgeon, radiation oncologist, and radiologist, or 3) 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 GK treatment-planning MRIs.

Statistical Analysis

Statistical analysis was performed using SAS 9.2 (SAS Institute, Cary, North Carolina) and Stata Version 11 (StataCorp LP, College Station, Texas). The follow-up and local control times were calculated from the date of GK treatment to last follow-up or recurrence as documented by MRI. The GK 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 chi-square 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 GK 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 GK sessions, and for multiple lesions treated per GK session (22, 23). Two analyses were performed for the recurrence data. First, patients with EGFR KD mutation or ALK translocation were analyzed together to compare TK activated patient populations to those with non-TK activated tumors. Chi-square tests or Fisher’s exact test were also used to compare the absolute recurrence rates in these groups. A second analysis was performed for individual genotypes (EGFR KD 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 TK activated vs. 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 performed 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:*
79 patients were included in this analysis, and the median follow-up time for the entire cohort was 6.2 months. For the local control model, median follow-up 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 GK 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 standard deviation = 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 GK session was 2 overall (5 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 GK treatment, and 32% of patients received whole brain radiation therapy. 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 GK. The distribution of patients ≤ 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-GK gadolinium enhanced MRI images are presented in Figure 1 and demonstrate examples of both local control (top panels) and local failure (bottom panels) following GKRS. Absolute recurrence rates for the 79 patients with 469 GKRS treated brain metastases are shown in Table 2 (upper panel) 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 1cm in diameter at time of GKRS, and the largest lesion that recurred was 2.4 cm.

**Analysis of Local and Elsewhere Brain Recurrence for TK Activated Tumors:**

The local control of metastases with EGFR tyrosine kinase (TK) 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 TK mutation, in-field
recurrence was observed in 18% of patients (13 of 244 treated lesions) compared with no recurrences in patients with TK activated NSCLC tumors (0 of 225 treated lesions). Absolute in-field recurrence rates were therefore significantly different for TK activated vs. non-activated tumors at both the patient (p = 0.01) and lesion (p < 0.001) levels. In contrast, examination of distant brain recurrence demonstrated essentially equivalent rates of failure in the TK activated and non-activated populations (53% vs. 47% respectively, p = 0.58; Table 2, lower panel).

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

**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 (Figure 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 performed. The data in Table 1 demonstrated that metastasis diameter was significantly smaller while 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 TK-mutation or an ALK translocation as independent predictors of improved local control \( (p<0.0001; \text{Table 3}) \). In contrast, both EGFR mutation status and ALK translocation status did not independently predict for distant brain failure on multivariate analysis \( (p = 0.67 \text{ and } 0.17, \text{respectively}) \). 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 \( (p = 0.05) \).

*Tyrosine Kinase Inhibitor (TKI) Use in EGFR KD Mutant Patients:*  
EGFR tyrosine kinase inhibitors have weak CNS penetrance \( (29-31) \), and strategies for pulsed high dose therapy have been employed 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 prior to GK in approximately half of the EGFR mutant patients, and we investigated whether this was a potential confounder for local control.
(Table 4). Of the 164 GK 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 prior to or after GK radiotherapy, cannot sufficiently explain the observed increase in local control.

**DISCUSSION**

Approximately 30% of advanced stage NSCLC patients develop brain metastases (34) and radiation therapy 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 GK treatments. The three main findings from this work are: (1) 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; (2) tumors with EML4-ALK translocation also have superior control rates with radiation therapy, a novel finding generated from this analysis; and (3) 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 to analysis of thoracic radiation therapy 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 – 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 post-operative setting) of variable size that are prescribed doses that range from 41.4 – 74 Gy (37). The third advantage of this model is the number of lesions treated per patient, compared to the single primary site of thoracic RT. 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 radiation therapy. 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-TK driven tumors, strongly suggesting that the improved local control was the consequence of radiation therapy. 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 prior to GK 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 under estimate local control rates and that the model is generally applicable for patients treated with GK radiotherapy. In comparison to thoracic RT, the use of MRI
imaging may be superior, as major limitations for CT assessment of lung tumors have been well demonstrated. An example of the inaccuracies of CT 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 CT scan after RT, 14 had a pathologic complete response to therapy, 10 had minimal microscopic disease, and 16 had gross residual disease present, demonstrating 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 performed on the primary tumors and presumed to be representative of each metastatic lesion. Though 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 TK driven tumors have a discordant mutation status compared to the matched primary tumors. A meta-analysis including 598 matched primary NSCLC tumors and metastatic lesions (357 of which were positive for EGFR mutations) demonstrated that EGFR mutation status was concordant between primary and metastatic tumors in 83% of the pairs (RR = 0.86, p = 0.31) (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). This data suggests 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 demonstrated 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 RT 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 demonstrated to be radiosensitive compared to 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 WBRT 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 radiation therapy. Due to 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 GK 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 radiation therapy. 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 et al. showed that not all KRAS mutant
tumors are dependent upon KRAS to drive cell proliferation and survival (53), and Lim et al.
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 due to the clinical nature of our study, oncogene 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 to be exploited as a means to personalize the delivery of radiation therapy. 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 radiation therapy. In the setting of brain metastases, where radionecrosis of the brain is an infrequent but problematic risk of therapy, we propose that it may 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 demonstrate that these patients have a 25% probability of local recurrence at six months, and thus may benefit from escalated 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 demonstrates that the unique biology of EGFR TK-mutant and ALK translocation tumors results not only in an improved response to targeted systemic therapies, but also inherent radiosensitivity compared to TK-wild type tumors. Moreover, we have established and validated a statistical model to use local control of metastatic brain lesions after GK as a platform for determining the radiosensitivity of molecular subtypes of tumors.
REFERENCES


Table 1: Patient and Treatment Characteristics

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<td>1 (6%)</td>
<td>1 (3%)</td>
<td>5 (6%)</td>
<td></td>
</tr>
<tr>
<td># of Lesions Treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0.05</td>
</tr>
<tr>
<td>Range</td>
<td>1-23</td>
<td>1-11</td>
<td>1-13</td>
<td>1-21</td>
<td>1-23</td>
<td></td>
</tr>
<tr>
<td>Lesion Diameter (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.71</td>
<td>0.68</td>
<td>0.79</td>
<td>0.88</td>
<td>0.78</td>
<td>0.03</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.38</td>
<td>0.46</td>
<td>0.61</td>
<td>0.48</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Dose Prescribed (Gy)</td>
<td></td>
<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>
<tr>
<td>Range</td>
<td>18-24</td>
<td>18-22</td>
<td>16-24</td>
<td>16-24</td>
<td>16-24</td>
<td></td>
</tr>
<tr>
<td>History of Craniotomy</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>

Abbreviations: WBRT = whole brain radiation therapy
Table 2: Absolute Recurrence Rates by Molecular Subtype and for Tyrosine Kinase-Activated Tumors vs. 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>By Patient</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</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</td>
<td>39/79 (49%)</td>
</tr>
<tr>
<td>By Lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<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</td>
<td>13/469 (3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>TK-Activated</th>
<th>Other</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>By Patient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-Field</td>
<td>0/30 (0%)</td>
<td>9/49</td>
<td>0.01</td>
</tr>
<tr>
<td>Distant Brain</td>
<td>16/30 (53%)</td>
<td>23/49</td>
<td>0.58</td>
</tr>
<tr>
<td>By Lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-Field</td>
<td>0/225 (0%)</td>
<td>13/244</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
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-value</th>
<th>Distant Brain Control: p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR Mutation</td>
<td>&lt;0.0001</td>
<td>0.67</td>
</tr>
<tr>
<td>ALK Translocation</td>
<td>&lt;0.0001</td>
<td>0.17</td>
</tr>
<tr>
<td>KRAS Mutation</td>
<td>0.66</td>
<td>0.21</td>
</tr>
<tr>
<td>Mean Lesion Diameter</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td># of Lesions Treated</td>
<td>0.31</td>
<td>0.29</td>
</tr>
</tbody>
</table>
**Table 4: Prior Tyrosine Kinase Inhibitor Use for EGFR Mutant Tumors**

<table>
<thead>
<tr>
<th>TKI Status</th>
<th>EGFR Mutant Patients</th>
<th>EGFR Mutant Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKI Resistant</td>
<td>10/21 (48%)</td>
<td>85/164 (52%)</td>
</tr>
<tr>
<td>TKI Naive</td>
<td>2/21 (9%)</td>
<td>10/164 (6%)</td>
</tr>
</tbody>
</table>

*Abbreviations: TKI = tyrosine kinase inhibitor*
FIGURE LEGENDS

FIGURE 1: Representative pre- (A and C) and post-GK (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 demonstrated 15 months after GK for the EGFR mutant lesion (B), whereas the lesion without detected TK mutations recurred by 6 months after GK (D). Ultimately viable tumor cells were found when craniotomy and resection of this lesion was performed at 11 months.

FIGURE 2: Survival curves generated by the Cox proportional hazards model to estimate in-field and distant brain local control are shown, stratified by presence or absence of an activating TK (tyrosine kinase) mutation (A), or by specific molecular subtype [EGFR mutant, ALK translocation, KRAS mutant, or other tumors with no mutation detected] (B). One minus the model predicted risk of recurrence is graphed on the y-axis, versus time in months on the x-axis.
Figure 1: Representative MRI Images of Local Control and Local Failure after Gamma Knife Radiotherapy
Figure 2: Cox Proportional Hazards Model-Predicted In-Field and Distant Brain Local Control by Molecular Subtype

A

In-Field Local Control

Distant Brain Local Control

B

Median recurrence time
TK-Activated: not reached
Other: 18.4 months
p < 0.0001

Median recurrence time
TK-Activated: 7.7 months
Other: 7.7 months
p = 0.97

Median recurrence time
EGFR: 5.9 months
ALK: 6.3 months
KRAS: 4.6 months
Other: 3.9 months
p = 0.22

p < 0.0001
Clinical Cancer Research

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

Kimberly L Johung, Xiaopan Yao, Fangyong Li, et al.

Clin Cancer Res Published OnlineFirst July 29, 2013.

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