Response to ERBB3-Directed Targeted Therapy in NRG1-Rearranged Cancers


ABSTRACT
NRG1 rearrangements are oncogenic drivers that are enriched in invasive mucinous adenocarcinomas (IMA) of the lung. The oncoprotein binds ERBB3–ERBB2 heterodimers and activates downstream signaling, supporting a therapeutic paradigm of ERBB3/ERBB2 inhibition. As proof of concept, a durable response was achieved with anti-ERBB3 mAb therapy (GSK2849330) in an exceptional responder with an NRG1-rearranged IMA on a phase I trial (NCT01966445). In contrast, response was not achieved with anti-ERBB2 therapy (afatinib) in four patients with NRG1-rearranged IMA (including the index patient post-GSK2849330). Although in vitro data supported the use of either ERBB3 or ERBB2 inhibition, these clinical results were consistent with more profound antitumor activity and downstream signaling inhibition with anti-ERBB3 versus anti-ERBB2 therapy in an NRG1-rearranged patient-derived xenograft model. Analysis of 8,984 and 17,485 tumors in The Cancer Genome Atlas and MSK-IMPACT datasets, respectively, identified NRG1 rearrangements with novel fusion partners in multiple histologies, including breast, head and neck, renal, lung, ovarian, pancreatic, prostate, and uterine cancers.

SIGNIFICANCE: This series highlights the utility of ERBB3 inhibition as a novel treatment paradigm for NRG1-rearranged cancers. In addition, it provides preliminary evidence that ERBB3 inhibition may be more optimal than ERBB2 inhibition. The identification of NRG1 rearrangements across various solid tumors supports a basket trial approach to drug development. Cancer Discov; 8(6); 686–95. © 2018 AACR.

INTRODUCTION
Oncogenic gene fusions or rearrangements are identified across a wide range of solid tumors (1). Many of these events, such as fusions involving ALK, ROS1, RET, NTRK1/2/3, FGFR1/2/3, and PDGFB, are clinically actionable. In lung adenocarcinomas, targeted therapy for patients with ALK- or ROS1-rearranged tumors is highly effective, and several tyrosine kinase inhibitors (TKI) are currently approved or being investigated for the treatment of these patients. Similarly, dramatic and durable responses have been reported in NTRK1/2/3-rearranged solid tumors regardless of histology (2), and in select cancers with FGFR1/2/3 or RET rearrangements (1).

Fusions involving the neuregulin-1 gene (NRG1) were identified by Fernandez-Cuesta and colleagues in non-small cell
lung cancers (NSCLC) with an initially approximated frequency of 1.7% in lung adenocarcinomas (3). Transcriptome sequencing revealed fusion of CD74 to NRG1 exons encoding the NRG1 III-B3 isoform. Other fusions, such as SLC3A2–NRG1 and SDC4–NRG1, have also been identified (4, 5). NRG1 fusions are particularly enriched in invasive mucinous adenocarcinomas (IMA) of the lung, where they are found in 27% to 31% of cases. These are often mutually exclusive with KRAS mutations, a driver known to be enriched in IMAs (4, 6–8).

NRG1 rearrangements are drivers of cancer growth. NRG1 fusions encode several isoforms that contain an EGF-like domain that serves as the ligand of the receptor ERBB3 (9). Chimeric transmembrane proteins encoded by NRG1 fusions are predicted to maintain this extracellular domain, thus activating ERBB3 in a para/juxtacrine or autocrine fashion (10). NRG1 binding to ERBB3 results in heterodimerization of the latter with ERBB2, activation of downstream signaling including the ERK, PI3K–AKT, and NF-κB pathways, and increased tumor cell proliferation and growth (9, 11). Therefore, rational targeting of ERBB3 or ERBB2 in NRG1-rearranged tumors could be efficacious (3).

In this article, we provide proof of principle that targeting ERBB3 in NRG1-rearranged cancers represents a novel therapeutically efficacious paradigm. In contrast, we demonstrate that targeting ERBB2 is not as effective in patients in this series despite preclinical data supporting its use and previously published clinical reports. Finally, we present large-scale genomic profiling data supporting a basket trial drug-development approach for NRG1 rearrangements that extends beyond NSCLCs to other cancers.

RESULTS
Outcomes with Targeted Therapy in Patients with NRG1-Rearranged Lung Cancers

An 86-year-old man presented with recurrent, unresectable, advanced invasive mucinous adenocarcinoma 9 months after an initial resection and radiation for stage IIA (pT2bN0M0) disease. He was treated with carboplatin and pemetrexed, followed by paclitaxel and bevacizumab (with a best objective response of stable disease with both regimens), and finally nivolumab (with primary progressive disease). Broad, hybrid capture-based next-generation sequencing (NGS) using MSK-IMPACT (12) identified an in-frame CD74–NRG1 translocation (Fig. 1A), resulting in the fusion of exons 1 to 6 of CD74 with exons 6 to 13 of NRG1; the only other alteration identified was an ARID2 c.592A>T nonsense mutation. The patient was enrolled in an NSCLC expansion cohort of a phase I trial (NCT01966445) of GSK2849330, an anti-ERBB3 mAb. GSK2849330 is a humanized mAb that binds with high affinity to the ERBB3 domain III, where it blocks the binding of NRG1 and inhibits receptor heterodimerization (13).

GSK2849330 was administered at the recommended phase II dose of 30 mg/kg weekly during induction, followed by maintenance therapy of 30 mg/kg every 2 weeks. A confirmed partial response to therapy was achieved, with substantial shrinkage of a right lower lobe mass (Fig. 1B) accompanied by resolution of the patient’s dyspnea. Although the maximal decrease in measurable tumor burden by RECIST v1.1 was 32%, this underestimated total tumor shrinkage; an exploratory volumetric analysis revealed a 90% reduction in tumor volume at the nadir. An on-treatment tumor biopsy was performed on day 14 of therapy, but little tumor tissue was present, likely due to the robust response observed; pharmacodynamic changes thus could not be assessed. The patient tolerated the drug with no major issues. The response was durable and lasted 1 year and 7 months, exceeding the duration, in aggregate, of the prior systemic therapies he received.

Although GSK2849330 bears antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) enhancements (13), no other responses were noted in the same trial (NCT01966445, n = 29), which enrolled several patients with similar and higher ERBB3 expression. None of these other tumors were known to harbor an NRG1 fusion. These data strongly suggest that ADCC, CDC, or ERBB3 expression alone do not predict responses, and that our index NRG1-rearranged patient was an exceptional responder to therapy.

In contrast to these outcomes, treatment with afatinib did not achieve a response in 4 patients with NRG1-rearranged lung cancers. After progression of disease, the same patient with a CD74–NRG1-rearranged lung cancer described above was immediately transitioned to afatinib (40 mg daily). Repeat imaging after 8 weeks of therapy revealed progressive disease, with an increase in the right basilar mass and paratracheal lymph node, and new satellite nodules. In addition to this case, three other targeted therapy-naïve patients with NRG1-rearranged lung cancers received afatinib at 40 mg daily, none of whom responded to therapy. The clinicopathologic and molecular details of these three additional patients are summarized in Supplementary Table S1. The first patient with a CD74–NRG1 fusion had a best response of stable disease at 5 weeks (7% disease shrinkage), but frank disease progression at 13 weeks. The second patient with an SDC4–NRG1 fusion had frank disease progression at 6 weeks, similar to the third patient with a CD74–NRG1 fusion who had primary disease progression at 8 weeks; both patients died of disease progression shortly after discontinuing afatinib.

Targeted Inhibition of ERBB3/ERBB2 Results in Decreased Growth of Cell Lines with Aberrant NRG1 Expression

Published preclinical data on ERBB3/ERBB2 inhibition in NRG1-altered cell lines is limited (4), and the activity of ERBB2 inhibition with select clinically available TKIs has largely been examined in cells with ectopic expression of CD74–NRG1 cDNA (3). We thus explored the effect of ERBB3/ERBB2 inhibition by genetic and pharmacologic approaches in cell lines with endogenous NRG1 alterations.

We first examined the breast cancer cell line MDA-MB-175-VII, previously published to harbor a DOCA–NRG1 fusion (10, 14), and confirmed the finding via RT-PCR (Fig. 2A). Orthogonal sequencing of the PCR amplicon revealed that exon 12 of DOCA is fused in frame with exon 2 of NRG1 [confirmed by targeted RNA sequencing (RNA-seq); Supplementary Fig. S1A]. To identify a lung cancer cell line with aberrant expression of NRG1, 67 lung cancer cell lines were screened using microarray expression data. The HCC-95 cell line had the highest level of NRG1 mRNA expression (3-fold higher than the next highest cell line, Supplementary Fig. S1B). Although an NRG1 fusion was not detected in this cell line using the
Figure 1. Clinical response to anti-ERBB3 mAb therapy in a patient with an advanced NRG1-rearranged non-small cell lung cancer. A. A never-smoker with advanced invasive mucinous adenocarcinoma was treated with several lines of systemic therapy (chemotherapy and immune therapy) with no response to any of these treatments. His tumor was subsequently found to harbor an in-frame CD74–NRG1 fusion that includes the EGF-like extracellular domain that serves a binding site for ERBB3. Targeted therapy with GSK2849330, an anti-ERBB3 monoclonal therapeutic antibody, was initiated on a phase I clinical trial. B. A durable (19 months) and confirmed partial response (RECIST version 1.1) was achieved that exceeded the duration of disease control achieved on all prior systemic therapy regimens in aggregate. Substantial disease shrinkage of a right lower lobe mass was noted early (by week 6 as shown, circled) in the course of therapy. SD, stable disease; PD, progressive disease.
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Figure 2. Cell lines with aberrant expression of NRG1 are exquisitely sensitive to downregulation of ERBB3 signaling. A, Expression of the DOC4–NRG1 fusion was confirmed by RT-PCR (left) and by DNA sequencing of the PCR amplicon (right) in MDA-MB-175-VII cells. B, Copy-number breakpoints affecting NRG1 in HCC-95, a human lung cancer cell line, are depicted using normal human DNA as reference. The outer circle depicts human autosomes (1–22), chromosomes X and Y, and the mitochondrial genome (M). Chromosome 8, zooming into the amplified NRG1 locus (inset), is shown. Genome-wide distribution of log2 ratios is represented in concentric circles as gains (red) and losses (blue), each data point representing a probe from the array according to the locations in the human genome (NCBI 36). C, Quantitative RT-PCR was used to determine the levels of NRG1 mRNA in MDA-MB-175-VII and HCC-95 using MCF-7 and HCC827 as control cell lines for comparison. D, Cells were treated with GSK2849330 for 1 hour and then stimulated with heregulin (HRGB1) for 30 minutes before preparation of whole-cell extracts for western blotting with the antibodies described. A representative blot is shown. E, Cells were left untreated, or treated with 10 μg/mL GSK2849330 or nonspecific IgG and then measured for growth by CTG assays at the indicated time points. The y-axis represents the mean ± SD of relative luciferase units (RLU) measured in triplicate. F, Cells were infected with lentivirus containing nontargeting (NT) or shRNAs targeting the genes shown and then examined for relative number of cells remaining after 7 days of infection (top) or relative caspase 3/7 enzymatic activity (bottom). Results represent the mean ± SD of two experiments in which each condition was assayed in duplicate. *, Significantly different from NT-shRNA, P < 0.05, two tailed t test.

A

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MSK Solid Fusion Assay, NRG1 was subsequently found to be amplified on the basis of array comparative genomic hybridization analysis (Fig. 2B). Both MDA-MB-175-VII and HCC-95 significantly overexpressed NRG1 relative to tissue-type-matched control cell lines (Fig. 2C). In addition, using a phospho-receptor tyrosine kinase (RTK) array that allows for profiling the activation state of 49 RTKs (15), we observed that the MDA-MB-175-VII cell line had high levels of phosphorylation of EGFR, ERBB2, and ERBB3, as expected, but little activation of any of the remaining 46 RTKs (Supplementary Fig. S1C).

To further investigate these cell lines with aberrant NRG1 expression, we examined the effect of the ERBB3 mAb GSK2849330 on proliferation and activation of the ERBB3 pathway. MDA-MB-175-VII and HCC-95 cells showed higher basal levels of phospho-ERBB3 compared with control cell lines (MCF-7 and H292) with low NRG1 expression (Fig. 2D). Addition of exogenous heregulin did not further stimulate ERBB3, as evidenced by little effect on phospho-ERBB3 or phospho-AKT levels in the MDA-MB-175-VII or HCC-95 cell lines, unlike the effects observed in control cell lines. MDA-MB-175-VII and HCC-95 cells treated with GSK2849330 showed suppression of ERBB2, ERBB3, and AKT phosphorylation compared with treatment with a nonspecific IgG control. Moreover, relative to the control lines, both cell lines exhibited significant growth inhibition upon GSK2849330 treatment over the IgG control (Fig. 2E). We observed similar results repeating the same experiments with a variety of ERBB2/EGFR inhibitors, such as afatinib, erlotinib, and neratinib (Supplementary Figs. S2 and S3), complementing prior experience with trastuzumab, pertuzumab, or T-DM1 treatment in vitro (16). These data collectively show that these models, despite their molecular differences in terms of NRG1 alterations, are similar in their constitutive activation, dependence on the pathway, and sensitivity to inhibitors.

To dissect the roles of ERBB family members on in vitro growth and survival, we tested shRNAs targeting EGFR, ERBB2, or ERBB3 in the NRG1-fusion–positive cell line MDA-MB-175-VII (Supplementary Fig. S4). ERBB2 or ERBB3 knockdown reduced cell growth significantly (Fig. 2F, top) and induced >8-fold increase in caspase-3/7 activity (Fig. 2F, bottom). However, these cells were less sensitive to EGFR inhibition, phenocopying the reduced cell viability observed with neratinib and afatinib relative to erlotinib (Supplementary Fig. S3). Collectively, these results support that NRG1 alteration drives basal activation and dependence on the ERBB2/ERBB3 signaling pathway and confers sensitivity to ERBB2/ERBB3 inhibitors such as GSK2849330 and afatinib in vitro, reflecting the clinical experience, here and elsewhere (5, 17, 18).

**Differential Growth Inhibition Is Observed with ERBB3 versus ERBB2 Targeting in OV-10-0050, a PDX Model Harboring an NRG1 Fusion**

Profiling a variety of patient-derived xenograft (PDX) models by RNA-seq identified OV-10-0050, an ovarian model with outlier expression of NRG1 mRNA (Fig. 3A). Further analysis of this sample revealed a novel CLU–NRG1 fusion resulting from the intragenic fusion of exon 2 of CLU with exon 6 of NRG1, retaining the EGF-like extracellular domain. Mice bearing OV-10-0050 tumors implanted subcutaneously were treated with vehicle, afatinib (15 mg/kg daily), GSK2849330 (25 mg/kg, biweekly), or control IgG (25 mg/kg, biweekly). Afatinib treatment caused a significant reduction in tumor growth compared with vehicle (Fig. 3B; Supplementary Fig. SSA); however, no tumor regression was observed.

In contrast, GSK2849330 elicited a more profound antitumor response as evidenced by durable and complete tumor regression (Fig. 3B; Supplementary Fig. SSA) that persisted even after treatment was discontinued after 35 days of dosing (Supplementary Fig. S5B). Pharmacodynamic analysis of samples collected 4 hours after a single dose treatment of GSK2849330 demonstrated near-complete inhibition of phospho-ERBB3 and phospho-AKT relative to IgG control (Fig. 3C). Afatinib-treated samples, however, showed relatively lower inhibition of phospho-ERBB3 and little to no change in phospho-AKT.

Taken together, these in vivo results were analogous to the differential clinical responses observed with ERBB3- versus ERBB2-directed targeted therapy as described earlier, where a deep and durable objective response to therapy was noted with GSK2849330, as opposed to afatinib therapy, where no responses were observed in patients with NRG1-rearranged tumors.

**NRG1 Fusions Are Identified Across a Variety of Cancers**

Recognizing the clinical activity of targeted therapy in NRG1-rearranged lung cancers, we proceeded to determine the prevalence of NRG1 fusions in advanced solid tumors of both lung and non-lung origin. Using MSK-IMPACT alone, among 17,485 patients with a variety of advanced solid tumors, NRG1 rearrangements were detected in NSCLCs [3/2,079 patients (0.14%) with lung adenocarcinomas], pancreatic adenocarcinoma [1/791 patients (0.13%) with pancreatic adenocarcinoma], and ER+/HER2– breast cancer [1/2,703 patients (0.04%) with breast carcinoma].

Knowing that NRG1 fusions are more common in IMAs of the lung, and that the NRG1 fusions of 2 patients who received targeted therapy in this series (Supplementary Table S1) were detected by targeted RNA-seq (MSK Solid Fusion Assay, Archer FusionPlex) and not NGS of DNA (MSK-IMPACT), we performed additional targeted RNA-seq of IMAs of the lung that did not harbor KRAS mutations using the MSK Solid Fusion Assay. In patients with KRAS wild-type IMAs, NRG1 fusions were detected in 4 of 36 patients (11%). Targeted RNA-seq also detected an NRG1 fusion in a pancreatic adenocarcinoma not previously detected by MSK-IMPACT alone.

Collectively, MSK-IMPACT and the MSK Solid Fusion Assay detected 10 NRG1 fusions in total: 7 in lung adenocarcinomas, 2 in pancreatic adenocarcinomas, and 1 in a breast carcinoma. All fusions included exon 6 that encodes the EGF-like domain of NRG1 that likely binds to ERBB3 to trigger downstream signaling. CD74 was the most common upstream partner (Fig. 4A). Additional fusions included the known SDC4–NRG1 and the novel ROCK1–NRG1 and FOXA1–NRG1 fusions (Fig. 4B).

We then performed an analysis of RNA-seq data for 8,984 tumors from The Cancer Genome Atlas (TCGA). NRG1 rearrangements with a wide variety of novel 5′ and 3′ partners were found across several tumor histologies. These included breast, lung, and pancreatic cancers, matching the results
**Figure 3.** In vivo growth inhibition elicited by afatinib and GSK2849330 in a CLU-NRG1-rearranged PDX mouse model. **A,** A panel of PDX models was profiled by RNA-seq. An ovarian cancer PDX model, OV-10-0050, showed a high level of NRG1 mRNA expression, resulting from a novel CLU-NRG1 intragenic rearrangement (inset) where exon 2 of CLU (chromosome 8) was fused with exon 6 of NRG1 (chromosome 8) with retention of the extracellular EGF-like domain in the fusion product, as shown. **B,** Female BALB/c nude mice were subcutaneously implanted with CLU-NRG1-rearranged tumors and treated with afatinib at 15 mg/kg daily (QD) for 28 days, resulting in a significant inhibition of tumor growth compared with the vehicle arm (n = 6, top). The same PDX mouse model was treated with the anti-ERBB3 mAb GSK2849330 at 25 mg/kg biweekly (BIW) for 35 days, resulting in a dramatic response of complete tumor regression compared with vehicle- or IgG-treated controls (n = 10, bottom). Changes from baseline tumor volume (TV), as measured by \((\text{TV (final)} - \text{TV (initial)}) \times 100 / \text{TV (initial)}\), are shown in the waterfall plots. **C,** Pharmacodynamic changes in tumor tissues as measured 4 hours after a single dose of treatment with afatinib, vehicle, GSK2849330, or IgG control (n = 3) are shown.
**Figure 4.** NRG1 rearrangements are found in multiple solid tumors. **A,** A schematic of CD74–NRG1, the most commonly identified genomic rearrangement identified in lung cancers in this series, is shown. The CD74 gene (chromosome 5q) is disrupted and inverted downstream of exon 5 and subsequently ligated to a position upstream of exon 6 of the NRG1 gene. **B,** Structural features of fusions, involving the indicated 5′ and 3′ chromosomal partners, identified by next-generation DNA sequencing (MSK-IMPACT; \( n = 17,485 \) tumors profiled) or targeted RNA-seq (MSK Solid Fusion Assay) are shown. The EGF-like domain is maintained in all fusions identified. **C,** Fusion junction exons, the associated chromosomal partners, and corresponding transcripts (Ensembl database version 75) are shown for all fusions detected by analyzing the TCGA dataset (\( n = 8,984 \) tumors analyzed), with the EGF-like domain indicated, wherever applicable. NRG1 fusion partners are not drawn to scale. The asterisk indicates that two distinct fusions were found in the same tumor sample. **D,** NRG1 RNA-seq expression is plotted across fusion-positive TCGA cancer types (x-axis) with fusion-positive samples indicated in red. Note that only one NRG1 expression value is plotted for the lung squamous cell carcinoma (LUCC) sample with two distinct fusions. BRCA, breast-invasive carcinoma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; UCS, uterine carcinosarcoma.
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DISCUSSION

In this article, we provide early clinical proof-of-principle data supporting the use of ERBB3-directed targeted therapy in patients with advanced NRG1-rearranged cancers. GSK2849330, an anti-ERBB3 mAb, elicited a dramatic and durable response in an exceptional responder with an advanced CD74–NRG1-rearranged IMA on a phase I trial. This activity is supported by durable tumor regression observed in a PDX mouse model and antiproliferative activity noted in MDA-MB-175-VII with anti-ERBB3 mAb therapy. On a molecular level, these NRG1-rearranged models demonstrate functional similarity in their dependence on ERBB3 by basal pathway activation and near-complete suppression of downstream signaling upon ERBB3 inhibition. NRG1 fusions can be added to a growing list of therapeutically actionable driver alterations, including ALK, ROS1, RET, NTRK1/2/3, FGFR1/2/3, and PDGFB fusions (1, 19).

In contrast, ERBB3-directed targeted therapy was not as effective in this population. None of the 4 patients with NRG1-rearranged tumors in this report responded to afatinib (two of whom developed rapidly progressive disease resulting in death), although significant responses to afatinib were recently reported in other patients with NRG1-rearranged tumors (5, 17, 18). Our cases temper the previous observations of responses to afatinib and underscore the potential for enhanced antitumor activity with ERBB3 inhibition. It is noteworthy that our index case demonstrated a much longer response to GSK2849330 (19 months) relative to all other published cases of afatinib responses (≤12 months; refs. 5, 17, 18). This was consistent with the substantially reduced antitumor activity and suboptimal pathway inhibition noted in our NRG1-rearranged PDX model treated with afatinib relative to GSK2849330. Although genomic context and heterogeneity may play a role, we speculate that these differences could be explained by NRG1-mediated activation of ERBB3 limiting responses to and/or offering an escape from ERBB2 inhibition (9).

Targeted therapy trials should evaluate dedicated cohorts of NRG1-rearranged tumors. Several ERBB3 inhibitors, besides GSK2849330, have been explored in the clinic, such as patritumab (U3-1287, AMG-888), seribantumab (MM-121), lumretuzumab, AV-203, and LJM716 (20–24), but early testing did not focus on molecular enrichment for NRG1 fusions. A case could also be made for the use of ERBB2 inhibitors, such as afatinib, lapatinib, neratinib, trastuzumab, T-DM1, or pertuzumab, in patients with NRG1-rearranged cancers; however, our study and previous data (16) suggest that agents such as afatinib, trastuzumab, pertuzumumab, or T-DM1 may provide more limited benefits. Finally, combinations of one or more of these agents may maximize antitumor activity in NRG1-rearranged tumors as previously explored preclinically (16).

We recommend that screening for NRG1-rearranged tumors be performed. In this article, NRG1 rearrangements were more optimally detected when a combination of DNA-based targeted hybrid capture–based NGS and targeted RNA-seq with anchored multiplex PCR was performed. Considering the diversity of NRG1 fusion partners, the latter offers the distinct advantage of detecting unknown fusion partners using a unidirectional targeted approach. Consistent with previous data (3, 6, 25), the NRG1 rearrangements we identified occurred predominantly in lung cancers. These cancers thus represent a target population to screen in the absence of other actionable drivers, especially in driver-negative lung IMAs.

Finally, using two large genomic datasets, we show that NRG1 rearrangements can be detected across a variety of other cancers, including breast, head and neck, renal, ovarian, pancreatic, prostate, and uterine cancers. This supports the utility of a basket trial approach to drug development for this genomic subset. We previously demonstrated the clinical utility of this approach with NTRK1/2/3-rearranged tumors, where age- and histology-agnostic responses were observed with TRK-directed targeted therapy across a wide variety of adult and pediatric solid tumors (2). Should NRG1 fusions be similarly amenable to targeted therapy regardless of histology, tumor-agnostic development should be adopted to explore the activity of ERBB3/ERBB2 inhibition.

METHODS

Molecular Profiling

Targeted hybrid capture NGS was performed using the MSK-IMPACT (Integrated Mutational Profiling of Actionable Cancer Targets; data accessible on www.cbioportal.org), designed to capture base substitutions, small indels, copy-number alterations, and select rearrangements in more than 340 cancer-related genes as described previously (12, 19). Targeted RNA-seq was performed using the MSK Solid Fusion Assay, an NGS-based assay designed to detect fusions which utilizes Anchored Multiplex PCR technology (Archer FusionPlex, Illumina MiSeq). Unidirectional gene-specific primers targeted specific exons in more than 35 genes known to be involved in chromosomal rearrangements.

Targeted Therapy

GSK2849330 was administered on an NSCLC expansion cohort of a phase I trial (NCT01966445) conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects after approval by institutional board review. Informed written consent was obtained from subjects. Patients were eligible if they had ERBB3/NRG1-expressing tumors and advanced disease. GSK2849330 was administered at the recommended phase II dose (30 mg/kg i.v. weekly for 5 doses, followed by 30 mg/kg every other week). Imaging was performed at baseline and every 8 weeks. Response
was assessed by RECIST v1.1. An exploratory assessment of volumetric tumor response was performed. Adverse events were captured using CTCAE v4.0. Commercial afatinib was administered at 40 mg daily.

**Cell Lines and Culture**

MDA-MB-175-VII, NCI-292, and MCF7 were obtained from the ATCC in 2015. HCC-95 cells were obtained from the Korean Cell Line Bank in 2016. Cells were grown in a humidified incubator (37°C, 5% CO2), authenticated by short tandem repeat profiling, and tested for Mycoplasma (ATCC Universal Mycoplasma Detection Kit upon receipt and every 6 months, last tested in September 2017). MCF7 was cultured in Eagle Minimum Essential Medium (Gibco) supplemented with 0.01 mg/mL insulin and 10% FBS. All other cell lines were cultured in RPMI supplemented with 10% FBS.

**In Vitro and In Vivo Experiments**

NRG1 fusion testing (MDA-MB-175-VII, HCC-95) was performed by RT-PCR and targeted RNA-seq (MSK Solid Fusion Assay). RTK phosphorylation was assessed using a phospho-RTK array (profiles the activation state of 49 RTKs; ref. 15). To generate growth curves for cells treated with GSK2849330 or control IgG, MDA-MB-175-VII (500 cells/well), HCC-95 (200 cells/well), NCI-H292 (200 cells/well), and MCF7 (300 cells/well) were plated in 96-well plates (Nunc 136102) in triplicate. Cells were treated with 10 μg/mL GSK2849330 or control IgG. Cell proliferation was measured (CellTiter-Glo Luminescent Cell Viability Assay, Promega). For shRNA infection, 250,000 cells were plated (6-well plates) and infected (24 hours later) with viral supernatant. Infected cells were selected with 5 μg/mL puromycin. For caspase-3/7 activation, 25,000 puromycin-selected cells were plated (white clear bottom 96-well plates) and caspase-3/7 enzymatic activity measured (APO-ONE homogenous caspase-3/7 Activity Assay Kit, Promega). 0V-10-0050 was treated (vehicle, IgG control at 25 mg/kg, or GSK2849330 at 25 mg/kg biweekly) on day 35 postimplantation at an average tumor size of approximately 163 mm³. Tumor size was measured twice in two dimensions and used for calculations of both T-C and T/C values.

**TCGA Sample Analysis**

RNA-seq data from a total of 8,984 cancer samples (dbGaP Study Accession: phs000178.v9.p8) from 33 different cancer types in TCGA were analyzed using the MediSapiens FusionSCOUT fusion gene detection pipeline. Candidate fusion genes were filtered on the basis of gene–gene distance, sequence homology, either partner annotated as a pseudogene, and the presence of the fusion in RNA-seq data from ~600 normal tissue samples. Fusion junctions were identified by constructing all possible exon–exon combinations between each pair of genes, followed by alignment of unmapped reads against synthetic junctions. Reading frame status was predicted on the basis of the end and start phases of junction exons. NRG1 expression by cancer type was drawn using the RSEM scaled estimate expression values (Broad Institute GDAC Firehose pipeline).

Additional details regarding cell lines, molecular profiling, shRNA infection, caspase-3/7 activity, western blotting, PDX generation and treatment, fusion gene analysis for TCGA samples, and expression/copy-number analysis of NSCLC lines are provided in the Supplementary Methods.

**Disclosure of Potential Conflicts of Interest**

A. Ruusulehto has ownership interest (including patents) in MediSapiens Ltd. G.J. Riely reports receiving commercial research support from Ariad/Takeda, Novartis, Pfizer, and Roche/Genentech. M.G. Kris is a consultant/advisory board member for AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

**Conception and design:** A. Drilon, R. Somwar, B.P. Mangatt, M.G. Kris, M.E. Arcila, C. Matheny, G. Ganji

**Development of methodology:** A. Drilon, J. Sabari, W.W. Lockwood, M.E. Arcila

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** A. Drilon, R. Somwar, P. Desmeules, R.S. Smith, L. Delasos, M. Vojnic, A.J. Plodkowski, J. Sabari, J. Montecalvo, W.W. Lockwood, V. Martinez, G.J. Riely, C.M. Rudin, M.G. Kris, M.E. Arcila, C. Matheny, R. Benayed, N. Rekhtman, G. Ganji

**Analysis and interpretation of data (e.g., statistical analysis, bio-statistics, computational analysis):** A. Drilon, R. Somwar, H. Edgern, G.J. Riely, C.M. Rudin, M.G. Kris


**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** A. Drilon, P. Desmeules, M. Vojnic, H. Tai, M.G. Kris, G. Ganji

**Study supervision:** A. Drilon, M.G. Kris, M. Ladanyi, G. Ganji

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