Sarcoma Cell Line Screen of Oncology Drugs and Investigational Agents Identifies Patterns Associated with Gene and microRNA Expression

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Abstract

The diversity in sarcoma phenotype and genotype make treatment of this family of diseases exceptionally challenging. Sixty-three human adult and pediatric sarcoma lines were screened with 100 FDA-approved oncology agents and 345 investigational agents. The investigational agents’ library enabled comparison of several compounds targeting the same molecular entity allowing comparison of target specificity and heterogeneity of cell line response. Gene expression was derived from exon array data and microRNA expression was derived from direct digital detection assays. The compounds were screened against each cell line at nine concentrations in triplicate with an exposure time of 96 hours using Alamar blue as the endpoint. Results are presented for inhibitors of the following targets: aurora kinase, IGF-1R, MEK, BET bromodomain, and PARP1. Chemical structures, IC_{50} heat maps, concentration response curves, gene expression, and miR expression heat maps are presented for selected examples. In addition, two cases of exceptional responders are presented. The drug and compound response, gene expression, and microRNA expression data are publicly available at http://sarcoma.cancer.gov. These data provide a unique resource to the cancer research community.

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Introduction

Sarcomas are cancers of mesodermal origin that arise from connective tissue (soft-tissue sarcoma, STS) or bone (osteosarcoma, chondrosarcoma; ref. 1). Sarcomas are rare tumors, about 1% of all human cancers. Many of these tumors affect children and young adults accounting for 15% of all pediatric cancers. There are approximately 13,000 cases of sarcoma diagnosed per year in the United States and an estimated death rate around 4,500 patients. STS is a diverse group of tumors comprising over 50 subtypes, the majority of which are liposarcoma, derived from adipose tissue and leiomyosarcoma, derived from smooth muscle. Certain sarcoma types are primarily pediatric, for example, osteosarcoma, Ewing sarcoma/primitive neuroectodermal tumors (PNET, sometimes classified with the bone sarcomas) and rhabdomyosarcoma, whereas others are most common in adults over 55 years of age, for example, leiomyosarcoma, synovial sarcoma, and liposarcoma (2, 3).

Sarcomas are classified by the abnormalities that drive their pathogenesis. However, most sarcoma subtypes are still treated with traditional therapeutic modalities. Surgery with or without adjuvant or neoadjuvant radiation is the most common treatment for localized disease. Over half of sarcoma patients develop metastatic disease, which is treated with chemotherapy. Doxorubicin and ifosfamide are the two most active agents in advanced STS with an average response rate of 20% (4). Several core molecular determinants of sarcomagenesis have been identified and have the potential to transform the care of sarcoma patients (5). Chromosomal translocations occur in about one third of sarcomas (6). The majority of sarcomas have nonspecific genetic changes with a complex karyotype (7).

The challenge in sarcoma research for diseases such as chondrosarcoma is finding therapeutically tractable targets. Approximately 30% of mesenchymal tumors carry a specific translocation with an otherwise relatively simple karyotype. The fusion proteins act either as transcription factors, upregulating genes responsible for tumor growth, as for Ewing sarcoma, or translocate a highly active promoter in front of an oncogene-driving tumor formation, as for aneurysmal bone cyst (8). Molecular studies have identified oncogenic pathways in sarcomas that can be targeted by drugs that include histone deacetylases in translocation associated sarcomas of young adults, Akt/mTOR inhibitors in pleomorphic sarcomas, and macrophage colony-stimulating factor in giant cell tumor of bone (6). Although in many cancers, the age of the patient influences treatment, this is less often the case with sarcoma (9).

The rare incidence of each sarcoma subtype makes clinical trials challenging. Trials often enroll patients with any sarcoma subtype, despite diverse epidemiologies, pathogeneses, etiologies and clinical manifestations, resulting in highly heterogeneous patient cohorts (4, 10). The promise of molecular personalized medicine
is being realized in sarcoma with the success of imatinib mesylate and sunitinib in gastrointestinal stromal tumors (GIST; refs. 11, 12). In addition, imatinib has shown activity in metastatic dermatofibrosarcoma protuberosans (DFSP) and fibrosarcomatous DESP (13). Cetirizine, a targeted ALK inhibitor, has shown activity in pediatric inflammatory myofibroblastic tumor and shows promise in clear cell sarcoma (14). The mTOR inhibitor everolimus has been approved as a single agent for the treatment of TSC-associated perivascular epithelioid cell tumor (PEComa; ref. 15). Cediranib, a potent inhibitor of all three VEGFRs, has demonstrated an overall response rate of 35% and a disease control rate of 84% at 24 weeks in alveolar soft part sarcoma (16). Another antiangiogenic kinase inhibitor, pazopanib, has been approved for treatment of metastatic STS (17, 18).

This study was undertaken to explore the response of a wide spectrum of sarcoma cell lines to approved anticancer drugs and to a library of investigational agents in conjunction with exon arrays and microRNA array results to allow correlation of molecular characteristics with compound response. These data are publicly available at: http://sarcoma.cancer.gov.

Materials and Methods

Cell lines
Division of Cancer Treatment and Diagnostics of the National Cancer Institute (DCTD/NCI) collected a panel of 63 human adult and pediatric sarcoma cell lines. Cells were purchased from the ATCC, or obtained from Dr. Samuel Singer (Memorial Sloan Kettering Cancer Center, NY, NY), the Children’s Oncology Group (COG; Dr. Patrick Reynolds, Texas Tech University Health Sciences Center, Lubbock, TX) and Dr. Peter Houghton (Nationwide Children’s Hospital, OHSU). The atypical synovial sarcoma cell line, SW982 expresses SSX gene, but not SYT-SSX or HLA-A24 (Supplementary Fig. S1). The ASPS-1 aveolar soft part sarcoma line was developed at NCI (19). The sarcoma lines were stored frozen at 10⁶ cells per ml in liquid nitrogen. The sarcoma lines were authenticated using the Applied Biosystems Identifier Kit for short tandem repeat analysis (15 loci). The lines were thawed from the banked stock and samples were taken for Identifier analysis within passages 2 to 5. New cells from the same frozen stock were thawed after a maximum of 20 passages, which did not exceed 5 continual months in culture. The human A549 NSCLC line purchased from the ATCC was run on each plate as a screen control. The lines were maintained in the medium specified for each line supplemented with FBS and other additives (Supplementary Table S1).

Compounds
Approximately 100 FDA-approved anticancer drugs (available from NCI at: http://dtp.nci.nih.gov/branches/dsdb/ontology_drugset_explanation.html) and a library of 345 investigational oncology agents, composed primarily of targeted small molecules currently in clinical and/or preclinical studies acquired by synthesis or purchase were screened against the 63 sarcoma cell line panel (Supplementary Table S2).

Screen
Twelve lines (11 sarcoma and A549 human NSCLC cell line control) were screened per run. Each of the 12 lines was grown and harvested using standard tissue culture procedures. On day 1, the cells were collected and suspended at the desired density in 300 mL of media. The cells were plated using a Tecan Freedom Evo robotic device. A cell inoculum (42 μL) was added to designated wells in 384-well plates (15 test plates, 1 Control plate). After cell inoculation, the plates were moved to a humidified 37°C incubator with 5% CO₂. The next day, the Tecan Evo was used to perform compound addition. Each compound was tested at nine concentrations ranging from 10 nmol/L to 1.5 nmol/L (final DMSO concentration 0.25%). After compound addition, the plates were returned to the humidified 37°C incubator for 96 hours incubation. The controls were: topotecan (10 nmol/L); doxorubicin (10 nmol/L); tamoxifen (200 nmol/L); and DMSO (0.25%). The incubation was terminated by adding Alamar blue solution (15 μL) to each well using the Tecan Evo, the plates were incubated 4 hours in a humidified 37°C incubator and fluorescent signal in the wells were read on a Tecan plate reader.

Exon and microRNA arrays
Total RNA, including the miRNA fraction, was extracted from samples using the Qiagen miRNasey Mini Kit (Qiagen) according to the manufacturer’s instructions. Agilent RNA Integrity Number (RIN) >8.5 indicated good quality RNA for all samples. Sense strand CDNA from 100 ng total RNA was fragmented and labeled using the Affymetrix WT Terminal Labeling Kit. Samples were hybridized with Human Exon 1.0 ST Arrays (Affymetrix) at 45°C, 60 rpm for 16 hours. Arrays were washed and stained using Affymetrix Fluidics Station 450 and scanned on Affymetrix GenChip scanner 3000 7G. Expression data from miRNA were normalized using Robust Multiset Array Average (RMA) and summarized at the gene level using AROMA (20). Exon array data are available at GSE68591.

For microRNA profiling, total RNA (100 ng) was ligated to unique oligonucleotide tags to increase the length of the short miRNA for detection without amplification using the NanoString Kit. Samples were hybridized for 16 hours to NanoString human miRNA probedset, which has probe pairs specific for each miRNA with different fluorescent barcode labels. Each consists of a Reporter Probe, with the fluorescent signal on its 5’ end, and a Capture Probe with biotin on the 3’ end. Purification of bound probes was performed with a two-step magnetic bead-based wash on the nCounter Prep Station followed by immobilization in the cartridge for data collection. The miRNA data were scale normalized and log₂ plus 1 transformed. The miRNA expression data were available at GSE69470.

Data analysis
Concentration response data were fit with a 4 parameter curve fit and IC₅₀s determined. The data are publicly available at: http://sarcoma.cancer.gov. Hierarchical clustering of gene expression was performed for drugs with greatest IC₅₀ variability across the cell lines. Average linkage clustering using 1—Pearson correlation distance was performed. Pair-wise Pearson correlations between the negative log₁₀ IC₅₀ and log₂, gene or miRNA expression are presented. The Affymetrix mRNA and the NanoString microRNA datasets supporting the results of this article are available for direct download from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/). The accession number for the overall project is GSE69524 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69524), Affymetrix: GSE68591 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68591), and Nanostring: GSE69470 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69470).
Results

The sarcoma lines performed well in the screen with an average $Z'$ value of 0.85 and an average signal to background of 15.8. Topotecan, doxorubicin, and tamoxifen were included in each run to assess screen stability (Supplementary Fig. S2). When the data were analyzed by unsupervised clustering of compounds and cell lines, clear patterns emerged (Supplementary Fig. S3).

To analyze the relationships between the compounds tested, any agents with a log$_{10}$ IC$_{50}$ range $<0.5$ across the 63 cell lines were removed, as these agents will have low statistical power to detect pair-wise associations. After the filtering, 345 compounds (445 tested) remained. A constellation relational map for the approved and investigational agents tested in the sarcoma lines was developed. The drugs and compounds were connected with a line if the pair-wise correlation of the log$_{10}$ IC$_{50}$ was greater than 0.75 with thickness of the line indicating greater correlation (Fig. 1). The relational map identified clusters of compounds that had highly correlated patterns of cell line sensitivity. Many of the compound clusters could be classified on the basis of the same molecular target or pathway and others based on similar cellular effects such as DNA damage. Some of the clusters such as the MEK cluster included compounds developed to target the same protein while other clusters included compounds developed to target varied proteins.

The investigational agents tested included 8 aurora kinase inhibitors (Fig. 2A). The heat map (generated using the website) for the aurora kinase inhibitors across 63 sarcoma lines showed the great heterogeneity in cell line response (as measured by 50% inhibitory concentration, IC$_{50}$) to the aurora kinase inhibitors with the Ewing sarcoma and the synovial sarcoma lines being more sensitive as a group to these compounds than the other sarcoma line panels (Fig. 2B). The differential response of the sarcoma lines to the aurora kinase inhibitors spanned the entire concentration range tested from 10 $\mu$mol/L to 1 nmol/L. For the heat maps and IC$_{50}$ estimates, cell lines for which an IC$_{50}$ was not reached are included as the lowest or highest concentration tested. The mean IC$_{50}$s for these compounds was from 0.23 $\mu$mol/L to $<0.013$ $\mu$mol/L. The most clinically advanced aurora kinase

Stringency 0.75

Figure 1.
Constellation relational map showing response similarity connections among the approved and investigational anticancer agents tested in the sarcoma lines at a stringency of 0.75. The line thickness is directly proportional to the pair-wise correlation.
inhibitor, alisertib (NSC759677), is currently in phase III clinical trial. The mean IC50 for alisertib in the sarcoma lines was 0.135 μmol/L and the mean IC50 in the Ewing lines was 0.028 μmol/L. The clinical Cmax concentration of alisertib is 2 to 6 μmol/L; thus, the screen results suggest that Ewing sarcoma and synovial sarcoma may be susceptible to alisertib at clinically achievable concentrations. Barasertib (NSC 757444) is in phase II clinical trial and has an active metabolite clinical steady state concentration of 0.45 μmol/L. The mean IC50 for barasertib in the sarcoma lines was 0.23 μmol/L and the mean IC50 in the Ewing sarcoma lines was 0.019 μmol/L. However, even among the Ewing lines there appears to be a range of sensitivities upon exposure to this compound with the less sensitive lines comprising 4 of 19 of the panel (Fig. 2C).

The IGF-1R kinase inhibitors presented a more complex pattern (Fig. 3A and B). There was a consistent sub-group of rhabdomyosarcoma lines (Rh-30, SICRH30, Rh-28, RH-28 PX11/1PAM, and Rh-41) that were more sensitive to the IGF-1R kinase inhibitors (Fig. 3C). All of these lines are alveolar rhabdomyosarcoma with relatively high expression of ALK, anaplastic lymphoma tyrosine kinase. The embryonal rhabdomyosarcoma lines (HS 729, RD, and RH36) are less sensitive to IGF1R inhibitors and are lower expressers of ALK kinase. Sensitivity to the IGF-1R inhibitor linsitinib (NSC756652) compared with gene-expression data for ALK (r = 0.54), IGF-2 (r = 0.03), IGF-1 (r = 0.51), and IGF-1R (r = 0.11) and microRNAs miR-9 (r = 0.59), miR-100 (r = −0.58), miR-222 (r = −0.57), miR-125 (r = −0.52), miR-22 (r = −0.52), and miR-21 (r = −0.61). This indicates that high levels of miR-9 and low levels of the other miRs correlated better with sensitivity to linsitinib than high gene expression for the putative protein targets (Fig. 3D). The expression of miR-9-5p was higher in Ewing sarcoma, synovial sarcoma, and subsets of rhabdomyosarcoma and osteosarcoma lines than in normal cells and other sarcoma lines (Supplementary Fig. S4). All of the miRs that were negatively correlated with response to linsitinib were expressed at higher levels in normal cells, chondrosarcoma, liposarcoma, leiomyosarcoma, and malignant peripheral neural sheath tumor lines than in other sarcoma lines.

The sarcoma lines were, generally, insensitive to the MEK inhibitors; however, a subset of lines was sensitive to certain MEK inhibitors (Fig. 4A and B). Among the MEK inhibitors, trametinib (NSC758246) in combination with dabrafenib (BRAF inhibitor) is approved for treatment of mutant β-Raf melanoma, selumetinib (NSC764042) is in Phase III trial (NCT01933932, NCT01843062,
and NCT01974752) and refametinib (NSC765866) is in phase II trial (NCT02168777, NCT01915589, and NCT01915602). Four sarcoma lines, HT-1080 fibrosarcoma, which expresses mutant NRASp.Q61K, ES-4 Ewing sarcoma, RD rhabdomyosarcoma, and Rh36 rhabdomyosarcoma, which all express mutant NRAS(p.Q61K), are sensitive to trametinib; however, the concentration response curves lack sigmoid shape. The mean IC50 across the four lines of 0.016 μmol/L is less than the clinical Cmax of 0.036 μmol/L for the drug (Fig. 4C). In those same lines, selumetinib had a mean IC50 of 5 μmol/L, which is slightly above the clinical Cmax of 3.2 μmol/L for the compound. The mean IC50 for the eight MEK inhibitors across the sarcoma lines was 3.55 μmol/L with a range of 0.032 to 10 μmol/L (Fig. 4B and C).

The BET bromodomain inhibitors JQ1 (NSC764043) and I-BET-151 (NSC767599) are shown in Fig. 5A. By gene expression, MYC (cMyc) was, generally, highest in Ewing sarcoma and synovial sarcoma, MYCN (nMyc) expression was highest in the rhabdomyosarcoma lines, and RUNX2, another potential bromodomain binding transcription factor, was most highly expressed by the osteosarcoma lines and the bone/muscle lines. The liposarcoma line LS141 has high expression of both MYC and RUNX2 (Fig. 5C). The mean IC50 for the BET bromodomain inhibitors JQ1 and I-BET-151 across the sarcoma lines was low micromolar; however, the primary targets of the compounds may be different in the sarcoma subgroups. The mean IC50 for JQ1 across the sarcoma lines was 0.83 μmol/L with a range of 0.065 to 10 μmol/L (Fig. 4B and C). Synovial sarcoma was the most sensitive subgroup with a mean IC50 of 0.25 μmol/L and a range of 0.065 to 0.59 μmol/L and the line SYO-1 was the most sensitive sarcoma line with an IC50 of 0.065 μmol/L. For osteosarcoma the mean IC50 was 0.8 μmol/L and the range was 0.093 to 7.41 μmol/L with the OHS line being most sensitive (IC50 0.93 μmol/L) and for rhabdomyosarcoma the mean IC50 was 2.29 μmol/L with a range of 0.16 μmol/L to...
10 μmol/L with Rh41 being the most sensitive (IC<sub>50</sub> 0.158 μmol/L; Fig. 5D). The Pearson correlation coefficient for expression of cMYC and sensitivity to JQ1 was 0.35, indicating that high expression of MYC mRNA was positively associated with sensitivity to JQ1.

The chemical structure for the potent PARP inhibitor talazoparib (BMN673 and NSC767125) is shown in Fig. 6A. The expression of genes related to response to DNA damage, BRCA1, BRCA2, PARP1, PARP2, and RAD51, in the sarcoma lines are shown in Fig. 6B. Five sarcoma lines, including the chondrosarcoma SW1353, the two malignant peripheral neural sheath tumor lines MPNST and ST8814, the rhabdomyosarcoma Rh36, and the atypical synovial sarcoma SW982 have relatively low expression of all five genes. Five PARP1 inhibitors were tested in the sarcoma lines (Fig. 6B). The mean IC<sub>50</sub> for talazoparib across the sarcoma lines was 0.29 μmol/L with a range of 0.0015 to 10 μmol/L (Fig. 6C). The mean IC<sub>50</sub> for the Ewing sarcoma lines to talazoparib was 0.051 μmol/L with a range of <0.0015 to 0.83 μmol/L. The malignant peripheral neural sheath tumors, which by gene expression had very low levels of PARP1 had a mean IC<sub>50</sub> to talazoparib of 0.24 μmol/L, very near the mean of all of the lines, with a range of 0.067 to 0.85 μmol/L. The mean IC<sub>50</sub> of talazoparib for the synovial sarcoma lines was 0.049 μmol/L with a range of <0.0018 to 0.41 μmol/L. The SYO1 synovial sarcoma line was greater than 1 log more sensitive to talazoparib than were the other two synovial sarcoma lines. The Fig. 6D heat map shows a trend that higher expression of PARP1 (r = 0.54), and to a lesser extent, BRCA1 (r = 0.14), and BRCA2 (r = 0.16) correlated with greater sensitivity to talazoparib while expression of PLK2 was negatively correlated with sensitivity to talazoparib (r = −0.76). The exceptional lines, MPNST, ST8814, SW982, Rh36, and SW1353, with very low gene expression can be seen on the heat map. High levels of the microRNAs, miR-150 (r = 0.55) and miR-9 (r = 0.64) correlate positively with sensitivity to talazoparib whereas low levels of microRNAs miR-574 (r = −0.58), miR-22 (r = −0.71), and miR-30 (r = −0.62) correlate negatively with sensitivity to talazoparib. Four of the miRs that correlate with response to talazoparib are the same as those that correlate with response to linsitinib. Of the remaining, miR-150 that correlated positively with sensitivity to talazoparib has low expression in normal cells and most sarcoma lines and was higher in Ewing sarcoma (Supplementary Fig. S6). MiR-574 was expressed at higher levels in normal cells than in most sarcoma lines and miR-30 has a mixed pattern.

Exceptional responders can provide important leads for molecular targets. There were examples of exceptionally responsive sarcoma lines. The A-204 rhabdoid tumor line was exceptionally responsive to the IAP inhibitor birinapant...
(NSC767128) with an IC_{50} of 0.058 μmol/L compared with >10 μmol/L for all other sarcoma lines except HSSY-II, which had an IC_{50} of 2 μmol/L (Supplementary Figs. S7 and S8). MiRNA expression was highly positively correlated with the A-204 birinapant response (r = 0.82). The A-673 Ewing sarcoma line was exceptionally responsive to three kinase inhibitors, sara
catinib (NSC758872), ZM-336372 (NSC756654), and WZ-4002 (NSC755927). With each of these compounds the sen
tivity of the A-673 line reached an IC_{50} of <0.0015 μmol/L, the lower limit of the screen. The expression of FRG2, which codes for a protein normally expressed only in myoblasts and whose overexpression has been associated with facioscapulo
dermal muscular dystrophy, was highly correlated with the response of the A-673 cells to the three kinase inhibitors (r = 0.68, 0.78, and 0.77, respectively; Supplementary Fig. S9; ref. 21).

Discussion

The great diversity in sarcoma phenotypes and genotypes make this disease family exceptionally challenging. Phenotypically diverse human adult and pediatric sarcoma lines were screened with a defined set of drugs and compounds (22). Sarcoma genomics have been explored using cell lines and clinical speci
ments (12, 23). This study presents gene-expression data derived from exon array data as well as microRNA data, which are available in GEO.

One study goal was to identify small-molecule drugs for further examination in sarcoma. The constellation relational map provides visualization of the sarcoma screen for compounds with a dynamic range of at least one log in IC_{50} across the panel (Fig. 1). The clusters are compounds with similar patterns of response. Aurora kinase inhibitors form a distinct cluster adjacent to bifunc
tional alkylating agents, topoisomerase I and II inhibitors. Taxanes and microtubule fragmenters form a cluster adjacent to topoisomerase I and II inhibitors. The results are consistent with DNA damage being an important factor in the cytotoxicity of these agents. Other classes of agents such as proteasome inhibitors, dihydrofolate reductase inhibitors, and HSP90 inhibitors form clusters that are not connected, supporting the notion that these compounds have unique mechanisms. Some clusters include compounds with more than one putative target, for example, mTOR and Akt inhibitors cluster together. The results underscore the ability of a cell line panel response pattern to elucidate putative molecular targets and cell similarities. The article high-
lights examples (not the only examples) of cases in which there...
were several compounds directed toward a target and that produced a sarcoma-type pattern.

Aurora kinases are nuclear serine/threonine kinases essential for cell division (24). The potency of aurora kinase inhibitors led to their classification as cytotoxic agents (25). The response of the sarcoma lines to aurora kinase inhibitors was heterogeneous, with clear selectivity for Ewing sarcoma and synovial sarcoma lines (Fig. 2). Aurora kinase inhibitors are in clinical trials in hematologic malignancies and solid tumors (26) and are in phase II clinical trials in a broad spectrum of adult STS. Preclinically, pediatric sarcoma are sensitive to aurora kinase inhibitors; however, clinical trials have not yet been initiated (27). In pediatric sarcoma xenograft studies, alisertib (NSC759677) had activity in rhabdomyosarcoma xenografts, but little activity against Ewing sarcoma xenografts (28); however, higher doses than those administered in humans were used (29).

The insulin-like growth factors (IGF) and the insulin-like growth-1 receptor (IGF-1R) are targeted by multiple small molecules and multiple antibody therapeutics. A predictive biomarker for IGF/IGF-1R-targeted agents is being sought (30). The current screen data highlighted some of the challenges (Fig. 3). Although subgroups of several sarcoma types respond to the IGF-1R inhibitors, others do not. There was a positive correlation between response to the IGF-1R inhibitor linsitinib and ALK gene expression ($r = 0.54$) and weak correlation with IGF-1R ($r = 0.11$). Similar results have been seen in clinical studies where IGF-1R expression was not predictive of clinical response (31). MiR-9-5p expression was positively correlated ($r = 0.59$) with sensitivity to linsitinib whereas the expression of miRs 100-5p, 222-3p, 125b-5p, 22-3p, and 21-5p were negatively correlated. MiR-9 and miR-21 are associated with insulin resistance and diabetes-associated pancreatic cancer, and thus with the insulin–IGF signaling pathway (32). Although miR-9 is an oncogenic miR, linked with high c-Myc expression (33), it is also associated with BRCA1 downregulation and decreased DNA damage repair, thus, improving response to chemotherapy (34).
MiR-21, another oncogenic miR (35), downregulated expression of the tumor-suppressor IGFBP3 (36). Data indicate that miR-100 acts as a tumor suppressor in chondrosarcoma and overexpression in chondrosarcoma and can increase sensitivity to chemotherapy (37). MiR-100 appears to target the FBSP51 and IGFR1-mTOR signaling pathways (38). These data indicate that microRNAs may be important in determining response to IGF-1R inhibitors.

The RAS–RAF–MEK–ERK and the PI3K–AKT–mTOR pathways are frequently altered in cancer (39). The single-agent MEK inhibitor activity across the sarcoma lines was modest; however, specific lines from varied sarcoma types were sensitive to MEK inhibition (Fig. 4A–C). Among the sensitive lines, the HT-1080 fibrosarcoma and the rhabdomyosarcoma lines RD and Rh36 express mutant RAS (40). However, the MEK inhibitor, selumetinib (NSC764042) was not effective against the Rh36 xenograft (30). Hu09 osteosarcoma was sensitive to inhibitors of RAS/RAF/MEK/ERK whereas the KRAS lines were not. Finally, the Rh41, Rh28 PX11/LPAM, and SICRH30 (RMS13) rhabdomyosarcoma lines were sensitive to inhibitors of the PI3K–AKT–mTOR pathway whereas the Rh36 and Rh28 lines were not (41). Both Akt inhibitors and mTOR inhibitors had little activity against rhabdomyosarcoma and Ewing sarcoma xenografts (42–45). The MEK inhibitor PD-0325901 is in clinical trial for adolescent and adults with neurofibromatosis type-1 (NF1), a genetic disease with a predisposition for patients to rhabdomyosarcoma. The MEK inhibitors, trametinib, trametinib and MEK162 are undergoing broad spectrum clinical trials (ClinicalTrials.gov Identifiers NCT02096471, NCT01991379, NCT01725100) and others.

The bromodomain and extraterminal (BET) protein, Brd4, described as a general transcriptional regulator (e.g., Myc), recruits transcriptional regulatory complexes to acetylated chromatin (46). The therapeutic effects of bromodomain inhibitors have been attributed to a specific set of downstream target genes whose expression are sensitive to BET protein targeting. JQ1 and I-BET151 have high affinity for bromodomains of the BET family (47). Among sarcoma, rhabdomyosarcoma express MYCN; in addition, Myc is positively regulated by EWS-FL1 in Ewing sarcoma likely through an indirect mechanism (48). MYC transcript can be downregulated by siRNA against EWS-FL1 (49). MYC was expressed broadly by the sarcoma lines with exceptions being ASPS-1 and osteosarcoma, and lower expression by fibrosarcoma and rhabdomyosarcoma (Fig. 5). MYCN was expressed by a subset of rhabdomyosarcoma lines. Bromodomain inhibitors are in phase I clinical trial in hematologic malignancies, NUT (nuclear protein in testis) midline carcinoma, lymphoma, and advanced solid tumors (ClinicalTrials.gov Identifiers NCT01713582, NCT01587703, NCT01949883, and NCT01987362).

PARP1, a highly expressed DNA-binding protein, is involved in chromatin modification, transcription, and DNA repair (50). Ewing sarcoma express high PARP1 and are sensitive to PARP1 inhibitors (51, 52). The EWS-FL1 and EWS-ERG fusion proteins expressed in Ewing sarcoma induce DNA damage, which is increased in the presence of PARP1 inhibition (53). However, a small clinical study of olaparib in refractory Ewing sarcoma resulted in no significant responses or durable disease control (54). Talazoparib as a single agent was not active against Ewing sarcoma xenografts (55). Rhabdomyosarcoma also tend to high in PARP1 (Fig. 6; ref. 56). Expression of polo-like kinase 2 (PLK2) and miRs-100, -574, -22, -21, and -30 were negatively correlated with talazoparib sensitivity (Fig. 6).

MiR-22 is repressed in Ewing sarcoma expressing EWS-FL1, and was low in leiomyosarcoma and miR-22 expression was negatively correlated with sensitivity to talazoparib (57). In addition, c-Myc expression can produce a downregulation of miR 22 (58). MiR-30, a tumor suppressor, is frequently downregulated in malignant disease (59). MiR-150 is upregulated in serum exosomes in colon cancer patients and downregulated in pancreatic cancer patients (60). In the sarcoma panel, high miR-150 was associated with sensitivity to talazoparib (Fig. 6). Talazoparib is in active clinical trials, including phase II trials (ClinicalTrials.gov Identifiers NCT02116777, NCT02049593, NCT01286987, NCT01989546, NCT02127151, NCT01945775, and NCT02034916).

Inhibitors of apoptosis protein (IAP) are involved in regulating the caspase activation of NF-kB signaling (61). Birinapant is a small molecule that mimics the binding of the endogenous IAP antagonist Smac to IAP proteins (62). The A-204 rhabdoid tumor line was an exceptional responder to the IAP inhibitor birinapant (Supplementary Figs. S7 and S8). High miR-204 correlated with the sensitivity of the A-204 line to birinapant. High miR-204 is associated with slower tumor growth in malignant peripheral nerve sheath tumors and cholangiocarcinoma (63). On the other hand, miR-204 upregulation increased cell motility and migration in mesenchymal neural crest cells during development (64).

The A-673 Ewing sarcoma was an exceptional responder to three kinase inhibitors: saracatinib, an inhibitor of the non-receptor protein tyrosine kinase c-Src (65), WZ-4002, an EGFR T790M-mutant selective kinase inhibitor (66), and ZM-336372, a Raf inhibitor that blocks the Raf–MEK–ERK signaling pathway (Supplementary Fig. S9; ref. 67). A-673 cells have high phosphorylated Src protein and are sensitive to Src inhibition (68). High expression of FRG2 mRNA correlated with A-673 cell exceptional kinase inhibitor sensitivity. FRG2 is implicated in the genetic disease facioscapulohumeral muscular dystrophy and under normal condition in myogenesis (21, 69).

Many sarcoma lines screened in this study have been in culture for >20 years. Over time, the cultures may have genetically drifted from the original tumor, so that interpretation of the data based solely on histology of the tumor of origin may not be sufficient. Developing new xenografts and cell lines is especially important in sarcoma where many rare diseases are underrepresented or not represented by cell lines. In the sarcoma cell line panel, the bone sarcomas are well represented (both Ewing sarcoma and osteosarcoma); however, other sarcomas are absent or underrepresented, such as uterine LMS, myxoid liposarcomas, dedifferentiated liposarcoma, undifferentiated pleomorphic sarcomas, and other rare subtypes.
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