Clinical Cancer Research

Molecular Pathways: Targeting ETS Gene Fusions in Cancer

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Abstract

Rearrangements, or gene fusions, involving the ETS family of transcription factors are common driving events in both prostate cancer and Ewing sarcoma. These rearrangements result in pathogenic expression of the ETS genes and trigger activation of transcriptional programs enriched for invasion and other oncogenic features. Although ETS gene fusions represent intriguing therapeutic targets, transcription factors, such as those comprising the ETS family, have been notoriously difficult to target. Recently, preclinical studies have demonstrated an association between ETS gene fusions and components of the DNA damage response pathway, such as PARP1, the catalytic subunit of DNA protein kinase (DNAPK), and histone deactylase 1 (HDAC1), and have suggested that ETS fusions may confer sensitivity to inhibitors of these DNA repair proteins. In this review, we discuss the role of ETS fusions in cancer, the preclinical rationale for targeting ETS fusions with inhibitors of PARP1, DNAPK, and HDAC1, as well as ongoing clinical trials targeting ETS gene fusions. *Clin Cancer Res; 20(17); 4442–8. ©2014 AACR.*

Background

ETS transcription factors are aberrantly expressed in several cancers, including prostate cancer (1), the Ewing sarcoma family of tumors (2), melanoma (3), secretory breast carcinoma (4), acute lymphoblastic leukemia (5), gastrointestinal stromal tumors (6), and rare cases of acute myelogenous leukemia (7). The ETS family consists of 28 unique genes (reviewed in ref. 8), of which *ERG*, *FLI1*, and *ETV1* are the most frequently deregulated in cancer. Prostate cancer frequently harbors rearrangements of ETS genes, in which *ERG* (50% of all prostate cancers) and *ETV1* (5%) are fused to the androgen-regulated promoter and 5′ untranslated region of the *TMPRSS2* gene (1, 9). This creates an androgen-regulated *TMPRSS2–ETS* fusion transcript that encodes a nearly full-length ETS transcription factor (Fig. 1). In addition, almost all Ewing sarcomas contain an ETS rearrangement, including EWS–FLI1 (∼90%) or EWS–ERG (∼5%–10%) gene fusions, which encode a chimeric protein notable for several features, including (i) provision of an activation domain (from the EWS gene) to the ETS fusion and (ii) replacement of the N-terminus of the ETS protein by an RNA-binding domain from the EWS protein that enhances posttranscriptional splicing of ETS target genes (10; Fig. 1).

Both prostate cancer and Ewing sarcoma ETS genomic rearrangements are thought to occur early in malignant progression. For example, *TMPRSS2–ERG* fusions are observed during the transition from high-grade prostatic intraepithelial neoplasia lesions to invasive carcinoma (9, 11) and are formed at high frequency in androgen-stimulated cell lines under genotoxic stress (12–14). However, mice genetically engineered to express androgen-regulated *ERG* or *ETV1* develop prostatic intraepithelial neoplasia-like lesions, but do not progress to frank carcinoma (9, 11, 15–17). This suggests that complete ETS-mediated transformation may require additional collaborating mutations. While this spectrum is only beginning to emerge (18–20), it is clear that ERG accelerates prostate carcinogenesis following loss of a highly recurrent prostate cancer tumor suppressor protein called *PTEN* or in the context of overexpression of the androgen receptor (15–17). Interestingly, *TMPRSS2–ERG* overexpression leads to increased self-renewal over multiple plating generations in Sca-1^{hi}/EpCAM^{+} basal/progenitor cells isolated from genetically engineered mice (21), suggesting a role for ETS fusions in prostate cancer progenitor populations. In contrast with prostate cancer, the cells from which Ewing sarcomas are derived are still unknown, limiting the interpretation of genetic mouse models. Despite this impediment, *EWS–FLI1* overexpression has been shown to induce leukemic phenotypes when expressed in hematopoietic stem cells (22), to induce skeletal disruption when expressed in mesenchymal progenitors using a *PRX1* promoter (23), and to accelerate tumor formation in conjunction with *TP53* deletion (23).

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Consistent with their role in prostate cancer and Ewing sarcoma progression, ETS transcription factors drive downstream signaling pathways with a number of functional consequences. RNAi-mediated disruption of either *TMPRSS2–ERG* or *EWS–FLI1* expression inhibits cell proliferation, invasion, metastasis, and xenograft growth of prostate cancer or Ewing sarcoma cell line models that harbor the respective fusions (24–26). Accordingly, the transcriptional program driven by overexpression of ETS gene fusions is enriched for invasion and metastasis-associated gene signatures (1, 27, 28). Recently, our group found that both prostate cancer and Ewing sarcoma ETS gene fusions induce DNA double-strand breaks (25, 26). This suggests that ETS gene fusions may drive a mutator phenotype and cause increased genomic instability in some cells.

Given the pathogenic roles of ETS fusions in the progression of both prostate cancer and Ewing sarcoma, ETS fusion products represent intriguing potential therapeutic targets. However, transcription factors, such as the ETS family, have been notoriously difficult to target (29). Potential strategies for targeting ETS fusion genes include therapies directed at the gene promoter, the RNA transcript, the fusion product itself, coregulators of the fusion product, other collaborating lesions, and downstream targets of the fusion. Although each of these strategies holds promise, this review focuses on agents available to patients or currently in clinical trials, leading to an emphasis on therapies directed at the androgen-responsive promoter (in prostate cancer) or against coregulators of the fusion product.

Clinical–Translational Advances

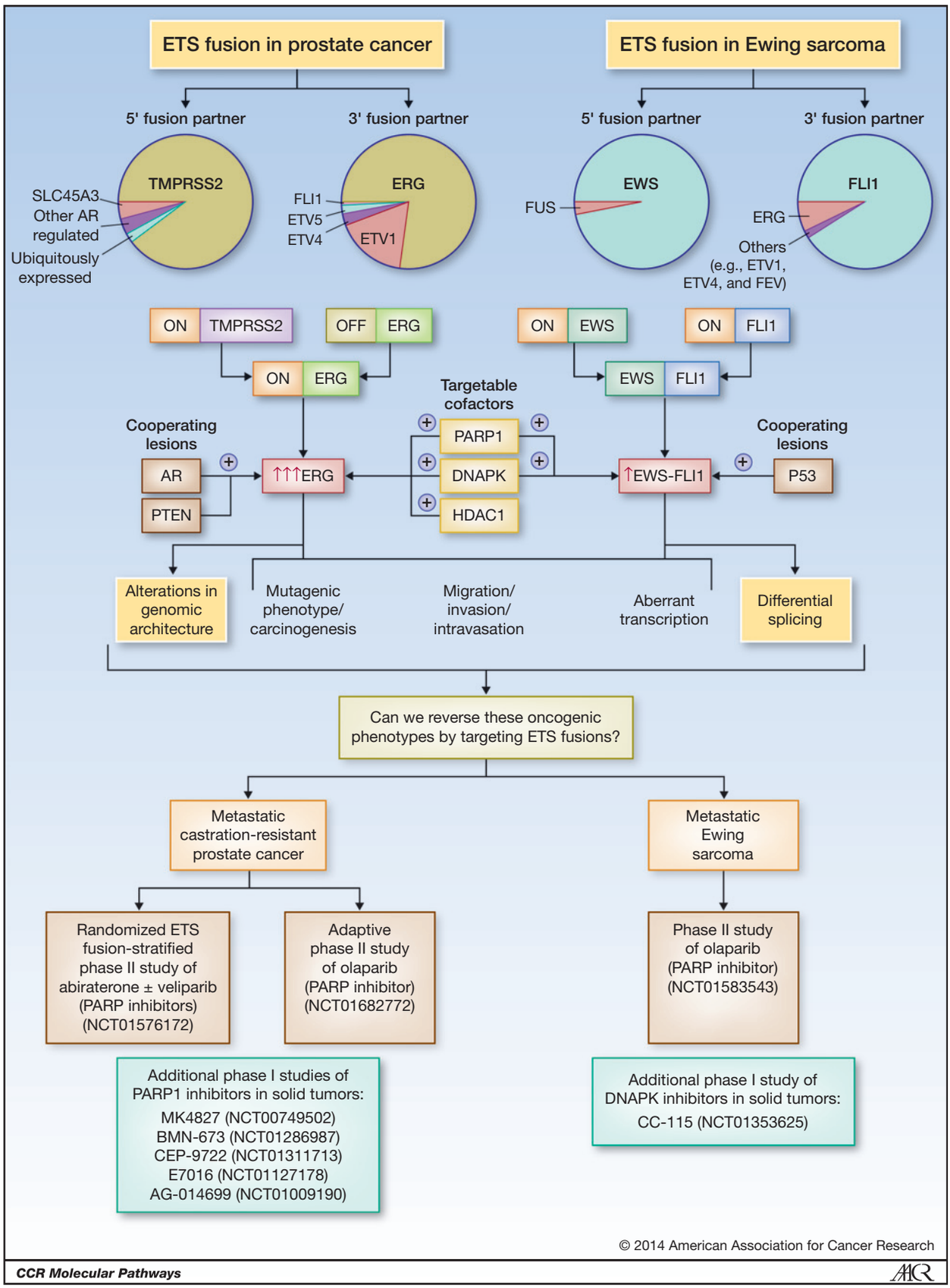
Targeting the promoter of ETS fusions

The fact that the predominant ETS fusions in prostate cancer contain an androgen-responsive promoter (1, 24, 30, 31) provides a strong rationale for treating fusion-positive prostate malignancies with approaches directed against the androgen signaling axis. However, retrospective analyses of clinical samples have not consistently supported the theory that ETS fusion–positive prostate cancers should be preferentially sensitive to androgen deprivation therapy or antiandrogen approaches. In the context of castration-sensitive disease, although data from a radical prostatectomy series suggest that ETS fusion status predicts for response to adjuvant androgen deprivation therapy (32), results from other series have suggested that there is no association between ETS fusion status and response in patients managed with either definitive or adjuvant androgen deprivation therapy or antiandrogen therapy (33, 34). This discrepancy between studies may stem from the inherent issues associated with retrospective biomarker studies, such that imbalances between comparison groups in prognostic factors are not fully taken into account. Alternatively, ETS fusions may simply not predict for response to androgen deprivation therapy in this setting, as all castration-sensitive disease may be similarly responsive to androgen deprivation therapy initially. Regardless, this relationship should be evaluated in prospective studies involving larger numbers of patients.

Following upfront androgen deprivation therapy, many patients will relapse with castration-resistant prostate cancer. The restoration of androgen signaling (35) and *TMPRSS2–ERG* expression (36) in castration-resistant disease provides a foundation for the hypothesis that ETS-positive castration-resistant prostate cancer may be preferentially responsive to next-generation antiandrogen therapy, such as abiraterone acetate. Abiraterone blocks androgen synthesis by inhibiting the enzyme cytochrome P450 17 α-hydroxysteroid dehydrogenase (37) and has improved clinical outcomes for patients with castration-resistant disease in large phase III clinical trials (38, 39). Using patient specimens from smaller phase I/II studies of metastatic patients treated with abiraterone, Attard and colleagues found that the presence of the predominant ETS fusion, the TMPRSS2:ERG rearrangement, in circulating tumor cells (CTC) correlated with prostate-specific antigen (PSA) response (40). In this study, 38% of patients with ERG fusion–positive CTCs had a >90% decline in PSA level with abiraterone, compared with 7% of patients with ERG fusion–negative CTCs (40). In contrast, Danila and colleagues (41) found that TMPRSS2:ERG status in CTCs was not associated with response to abiraterone. As with the castration-sensitive setting, these discrepancies raise additional questions, such as whether ETS fusion status in the CTCs accurately reflects fusion status in the metastatic lesions. To address these questions, a multi-institutional randomized phase II clinical trial (clinicaltrials.gov identifier: NCT01576172) was initiated by our group at the University of Michigan with the objective of assessing several key questions, including the relationships between ETS fusion status and the response to antiandrogen therapy. Specifically, this trial, which requires biopsy of metastatic prostate cancer lesions for enrollment, prospectively stratifies patients by ETS fusion status in biopsies before randomization to treatment, which includes an arm consisting of abiraterone alone. This trial represents one of the first biomarker-driven trials in prostate cancer, and in comprehensively assessing ETS status in metastases, the primary tumor, circulating blood RNA, and CTCs, the study design should provide more definitive answers about whether ETS fusion–positive castration-resistant prostate cancer can be preferentially targeted with a standard next-generation antiandrogen.

Targeting the activity of ETS fusion products

Given the uncertainty on whether antiandrogen therapies can preferentially target ETS-positive prostate cancers, it is clear that better ETS-directed therapies need to be developed. Although transcription factors themselves have conventionally been considered poor druggable targets (29), targeting cofactors necessary for functioning of the ETS gene fusion products may represent a more viable strategy. To date, the most promising cofactors, based on available clinical agents, for inhibiting ETS fusion activity include the enzymes PARP1, the catalytic subunit of DNA protein kinase (DNAPK), and histone deactylase 1 (HDAC1; Fig. 1).



PARP1 inhibition as a therapeutic approach for ETS-rearranged malignancies

Our group previously discovered an interaction between the *TMPRSS2–ERG* gene fusion product and PARP1, a protein involved in DNA damage response (26). We mapped the interaction to the conserved ETS DNA-binding domain of ERG and demonstrated that PARP1 also interacts with ETV1, EWS–ERG, and EWS–FLI1 (25, 26). Preclinical experiments demonstrated that ETS transcription factor activity was dependent on PARP1 expression and that inhibition of PARP1 could potentiate ETS-induced DNA damage leading to a long-term loss of cell viability (25, 26). Overexpression of either *TMPRSS2–ERG* or *EWS–FLI1* was sufficient to make cell line xenografts sensitive to PARP1 inhibition, indicative of a synthetic phenotype. These findings led to the hypothesis that ETS-rearranged tumors are sensitive to PARP1 inhibition. To test this hypothesis, we completed 11 different cell line or primary tumor xenografts, and of these, only the 6 xenografts with an ETS rearrangement were sensitive to PARP1 inhibition (3 *TMPRSS2–ERG*, 1 *ETV1* rearranged, 2 *EWS–FLI1*; refs. 25, 26). Subsequent independent validation of the finding that the EWS–*FLI1* fusion is associated with response to PARP1 inhibitors was performed via a high-throughput screen of 639 cell lines against 130 drugs under clinical or preclinical evaluation (42); this screen likely could not detect similar associations with prostate cancer ETS fusions as it included only one cell line harboring such a fusion.

The clinical use of PARP1 inhibitors has gained momentum secondary to previous preclinical reports demonstrating that cancers with impaired homologous recombination (HR) such as *BRCA1/2*-deficient cancers were extremely sensitive to PARP1 inhibition (43, 44). These studies proposed that PARP1 inhibitors cause replication forks to collapse, leading to increased DNA damage, which goes unrepaired in the absence of HR, and early clinical studies have suggested that the PARP1 inhibitor olaparib has activity on the context of BRCA-mutant cancers (45). Of interest, in preclinical studies, ETS fusion–positive xenografts were as sensitive to olaparib as a naturally *BRCA1*-deficient breast cancer xenograft (26), further strengthening the rationale to assess this biomarker-therapy combination clinically.

PARP1 inhibitors are now being actively evaluated in the clinic for both ETS-rearranged metastatic prostate cancer and Ewing sarcoma. Several of these trials are depicted in Fig. 1. NCT01576172, the multi-institutional phase II trial described earlier, stratifies patients with castration-resistant prostate cancer prospectively by ETS fusion status and randomizes them to abiraterone acetate alone versus abiraterone acetate combined with the PARP1 inhibitor veliparib (ABT-888). In addition to assessing the potential relationship between ETS fusion status and outcomes following abiraterone treatment, this trial also aims to prospectively determine if ETS status can predict for response to the addition of PARP1 inhibition to antiandrogen therapy. Other PARP1 inhibitors being assessed as monotherapy specifically in castration-resistant disease include olaparib (AZD-2281/KU-0059436; phase II, clinicaltrials.gov identifier NCT01682772) and niraparib (MK-4827; phase I expansion in prostate cancer, NCT00749502). The phase II olaparib study has an interesting design, as it uses a two-stage scheme; the first stage is designed to screen for potential biomarkers of response to PARP1 inhibition, and the second is an expansion cohort enriched in identified biomarkers from the first stage (J. De Bono; personal communication). Initial results from the phase I niraparib study were recently reported (46); analysis of archival tumor samples from 18 patients with metastatic castration-resistant prostate cancer did not demonstrate an association between ETS fusion status and response to therapy. Although these findings should be confirmed in larger studies with biopsies obtained immediately before treatment initiation, they do raise the issue that the response to PARP1 inhibitors is likely multifactorial in nature. The results from the prospective biomarker-stratified phase II studies described above will more conclusively determine whether the ETS–PARP1 association seen *in vitro* will hold up clinically.

Outside of prostate cancer, olaparib has been assessed as monotherapy in a phase II trial for patients with recurrent or metastatic Ewing sarcoma (NCT01583543). As only 4 of the initial 12 patients achieved stable disease (6–18 weeks) and none achieved partial or complete response, further accrual to this trial has been discontinued (47). Because molecular diagnosis was not required for this study, it is unclear whether its results stem from biologic or pharmacologic factors. In addition to this study, several phase I studies, including investigations of PARP1 inhibitors, are currently under way or near completion for patients with any solid tumor; the agents being tested include BMN-673 (NCT01286987), CEP-9722 (NCT01311713), E7016 (NCT01127178), and rucaparib (AG-014699/PF-01367338; NCT01009190).

Several issues need to be addressed when assessing PARP1 inhibitors as a strategy for targeting ETS fusion–positive malignancies. One major concern is which PARP1 inhibitor will be most efficacious in this context. Although some of

Figure 1. Overview of the role of ETS fusions in cancer and ongoing clinical trials targeting these fusions. ETS gene fusions are common driving events in both prostate cancer and Ewing sarcoma. The prevalence of different 5′ and 3′ fusion partners is depicted in a pie chart. Formation of these fusions results in an aberrant transcriptional program enriched in invasion as well as induction of DNA breaks, consistent with a mutator phenotype. ETS fusion–mediated tumorigenesis has been demonstrated to be accelerated in the presence of cooperating lesions, such as PTEN loss and androgen receptor (AR) overexpression in prostate cancer, and P53 alterations in Ewing sarcoma. Recent preclinical studies have identified targetable cofactors, such as PARP1, DNAPK, and HDAC1, and inhibition of these cofactors has conferred preferential sensitivity to ETS-positive malignancies in preclinical models. Although a number of phase I studies of PARP1, DNAPK, or HDAC1 inhibitors have been done in patient populations including ERG fusion–positive malignancies, ongoing phase II studies have focused on PARP1 inhibition as a strategy to target ETS fusion–positive disease, including a randomized study in which patients are stratified based on ETS fusion status (NCT01576172).

these studies may seem redundant in a clinical context, it is clear that not all PARP1 inhibitors behave similarly. Results from a recent study suggest that PARP inhibitors differ markedly in their ability to cause cytotoxicity by trapping PARP1 and PARP2 enzymes at damaged DNA, a difference that does not correlate with the catalytic inhibitory properties for each agent (48). This finding suggests that certain PARP1 inhibitors may be more effective than others for treating ETS-positive cancers; however, to address this concern, more investigation is needed into the mechanism by which PARP1 inhibitors are cytotoxic in the context of ETS rearrangements. A second issue is whether PARP1 inhibitors are best administered as a monotherapy or in combination with other potentiating agents. Although initial PARP1 inhibitor trials used monotherapy approaches, several PARP1 inhibitor combination studies have been completed in both the phase I and II trial settings using various chemotherapeutics for other malignancies (reviewed in ref. 49). Many of these regimens have integrated alkylating agents due to the observation that *PARP1*$^{-/-}$ mice are extremely sensitive to this class of therapeutics (49), whereas others use topoisomerase inhibitors. For example, ABT-888 has been shown to enhance the effects of topotecan in adults with refractory solid tumors or lymphomas (50). Notably, our group demonstrated that olaparib and temozolomide significantly reduced tumor volumes in a *TMPRSS2–ERG*-rearranged prostate cancer cell line xenograft and completely regressed tumors in an *EWS–FLI1*-rearranged Ewing sarcoma cell line xenograft (25, 26). These results suggest that the combination of temozolamide with a PARP inhibitor would be worthwhile to assess clinically for Ewing sarcoma; in fact, two ongoing phase I studies (NCT01858168 and NCT02044120) are exploring this regimen in patients with this disease.

DNAPK inhibition as a treatment strategy for ETS-rearranged malignancies

As an alternative to PARP1 inhibition, blocking the activity of the DNA repair protein DNAPK represents another potential strategy for targeting ETS fusion–positive cancers. Our group has previously demonstrated that the catalytic subunit of DNAPK also physically interacts with ETS fusion products, such as ERG, ETV1, EWS–ERG, and EWS–FLI1 (25, 26). *In vitro* studies demonstrated that DNAPK expression and activity were necessary for ETS transcriptional activity, and pharmacologic inhibition or genetic knockdown of DNAPK could also potentiate ETS-induced DNA damage (25, 26). Although no clinical-grade DNAPK inhibitor was available at the time of these initial studies, CC-115, a potent dual inhibitor of both DNAPK and mTOR, has since been developed and is now in evaluation in a phase I study in solid tumors (Fig. 1). mTOR is a key effector in the PI3K pathway. Given the recently demonstrated cross-talk between androgen receptor signaling and the PI3K pathway in PTEN-deficient prostate cancers (51), the associations between ERG fusions and PTEN deletion in prostate cancer (52), and the cooperativity between ERG overexpression and PTEN loss in carcinogenesis (16, 17), dual targeting of DNAPK and MTOR has intriguing potential in ETS-positive prostate cancer. In addition, because mTOR inhibition downregulates the EWS–FLI1 protein and has been demonstrated to synergize with antisense oligonucleotides against EWS–FLI1 (reviewed in ref. 53), the dual activity of CC-115 provides theoretical advantages in the treatment of Ewing sarcoma as well.

HDAC1 inhibition as a treatment approach for ETS fusion–positive cancers

Another potential strategy for targeting ETS fusions is via inhibition of HDAC1, an enzyme that modifies histones and drives epigenetic gene regulation via transcriptional corepression (reviewed in ref. 54). Previous studies have demonstrated that overexpression of ERG results in a gene expression signature notable for upregulation of HDAC1 and downregulation of its targets (55), and that HDAC1 indirectly interacts with ERG via the ERG-associated protein ESET (56, 57). In addition, inhibition of HDAC1 repressed ERG and was preferentially sensitive in ERG-positive cell lines (58). However, translation of these findings to the clinic has been less promising, as two phase II studies of HDAC inhibitors in castration-resistant prostate cancer yielded disappointing results; one of these studies, assessing the agent vorinostat, demonstrated significant toxicities that limited efficacy assessment, and the second, assessing the agent romidespin, demonstrated minimal antitumor activity (59, 60). Several other trials assessing HDAC inhibitors, as mono- or combination therapy in castration-resistant prostate cancer, are still in progress (clinicaltrials.gov identifiers; NCT01075308, NCT00878436, and NCT01174199).

Conclusions

Although ETS fusions were discovered several years ago, and are important preclinically in several aspects of prostate cancer initiation and progression, targeting of ETS fusions remains a work in progress. Although recent advances have been made in the preclinical space of targeting ETS fusions with clinically available agents, such as inhibitors of PARP1, DNAPK, and HDAC1, these findings need to be validated in clinical trials. Of these agents, the studies targeting ETS with PARP1 inhibitors are furthest along in development and should yield results within the next few years.

Disclosure of Potential Conflicts of Interest

F.Y. Feng reports receiving a commercial research grant from Celgene, speakers bureau honoraria from Ventana, and is a consultant/advisory board member for Medivation/Astellas. J.C. Brenner reports receiving royalties from the University of Michigan for its intellectual property on the use of PARP inhibitors in ETS-positive cancers, which is licensed to Hologic. A.M. Chinnaiyan is a consultant/advisory board member for Celgene, Hologic, and Oncofusion Therapeutics; reports receiving royalties from the University of Michigan for its intellectual property on the use of ETS fusions in prostate cancer and PARP inhibitors in ETS-positive cancers, which is licensed to Hologic; and has ownership interest in Oncofusion Therapeutics. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: F.Y. Feng, J.C. Brenner, A.M. Chinnaiyan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F.Y. Feng, J.C. Brenner, A.M. Chinnaiyan
Writing, review, and/or revision of the manuscript: F.Y. Feng, J.C. Brenner, M. Hussain, A.M. Chinnaiyan
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F.Y. Feng, J.C. Brenner
Study supervision: A.M. Chinnaiyan

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