Epigenetic targets have emerged as an exciting area for drug discovery. The discovery that histone deacetylase (HDAC) inhibitors had marked anticancer activity in T-cell lymphoma gave impetus to the field. In a phase I study published in *Clinical Cancer Research* in March 2002, romidepsin (depsipeptide), a potent HDAC inhibitor, was found to be tolerable, with a side effect profile that was later understood to be characteristic of this class of agents. Evidence of activity in this key phase I trial provided momentum for the further study of epigenetic agents. *Clin Cancer Res;* 21(10); 2195–7. ©2015 AACR. See related article by Sandor et al., *Clin Cancer Res* 2002;8(3) Mar 2002;718–28

As *Clinical Cancer Research* celebrates 20 years of bringing translational cancer research to its readers and contributing to the progress in understanding the biology and treatment of cancer, we have the opportunity to highlight an article that was part of this progress. In 2002, we reported the phase I study of romidepsin, then depsipeptide or FR901228, administered on a day 1 and 5 schedule of a 21-day cycle (1). In the paragraphs that follow, we discuss observations made in that phase I study that provided a first view of aspects of romidepsin and histone deacetylase (HDAC) inhibitor clinical development that emerged over time. HDAC inhibitors, added to DNA methyltransferase inhibitors, provided impetus to a whole field of epigenetic therapies. They did so first in being well-tolerated and effective agents for T-cell lymphomas.

Romidepsin was selected for development by the NCI Developmental Therapeutics Program based on its potency and unique profile in the NCI-60 cell line screen. It had been submitted to the NCI drug screen by the Fujiwasa Pharmaceutical Company, which had isolated it from the fermentation broth of a soil bacterium, *Chromobacterium violaceum*, after it scored in an anticancer screen using *Hras*-transformed NIH3T3 cells (2). The unique profile in the NCI-60 cell line panel was generated by its marked avidity for P-glycoprotein–mediated efflux and its similarity to taxanes, despite the lack of measurable interaction with tubulin (3). Subsequently, while this agent was still in phase I testing, it emerged that romidepsin was an HDAC inhibitor (4). Although its ability to potently inhibit HDAC-mediated deacetylation is now well-documented, it is remarkable that after 20 years of study, and FDA approval of several HDAC inhibitors, we still do not understand exactly how HDAC inhibitors exert their antitumor effects in patients.

Numerous laboratory studies have sought to answer the simple question, how do they work? In the laboratory, HDAC inhibitors can induce cell-cycle arrest, differentiation, or apoptotic cell death in cancer cells. Which effect is observed depends upon cell context and cell sensitivity (5). In 2002, when the phase I trial was published, we were mostly cognizant of the cell cycle and differentiating effects of the HDAC inhibitors. Indeed, our phase I report included experiments showing that serum obtained from patients during the romidepsin infusion could induce a G1 and G2 cell-cycle arrest in an *ex vivo* assay (1). This meant that patients treated with romidepsin were receiving concentrations that were effective in laboratory models, and that mechanistic studies of the agent performed in the laboratory could be clinically relevant. Since then, we have observed that cell types that undergo a cell-cycle arrest in G1 are actually on the less sensitive side of the spectrum, and that some cell types, such as T-cell lymphoma cells, undergo rapid cell death via apoptosis (5). The importance of the intrinsic apoptosis pathway in cancer cell death from romidepsin has now been clearly demonstrated, and efforts to identify combinations of agents that will enhance apoptosis are under way in several laboratories (6–9).

An unresolved mechanistic question is whether the altered gene regulation that results after HDAC inhibition is the actual mechanism of cell death. According to the canonical explanation of how HDAC inhibitors work, unrestrained histone acetyltransferase activity in the presence of HDAC inhibition leads to increased global histone acetylation. This increased histone acetylation opens chromatin, allowing access to transcription factors that may also be acetylated, and leads to induction of genes that induce differentiation, cell-cycle arrest, or apoptosis. Although histone acetylation occurs at multiple different lysine residues on all histone proteins, the alteration in gene expression appears more specific—that is, only about 5% of genes are induced and about the same number are repressed. How global acetylation can result in specific gene expression has not yet been explained. Further, in clinical samples obtained before and after romidepsin treatment, we observed that the main determinant of which genes are induced is the individual patient examined—gene expression pre- and post-romidepsin clusters strongly by patient, rather than by treatment effect (10).
In the 2002 phase I study, we examined histone acetylation as a potential pharmacodynamic marker to confirm that the drug found its target and effected increased histone lysine acetylation (1). As with the in vivo cell-cycle experiments, this study confirmed increased histone acetylation in circulating mononuclear cells obtained from patients, and at levels comparable with those observed in vitro. We, and others, would go on to include histone acetylation as a pharmacodynamic marker in phase II studies (11). Most reports suggest no correlation of histone acetylation with response, but data from our phase II trial in T-cell lymphoma suggested that persistence of histone acetylation to 24 hours was important for treatment response (12). A likely explanation is that there is a threshold required for efficacy and, beyond that, global acetylation plateaus.

Unique in the laboratory, romidepsin also proved to be unique in the clinic. In the 2002 phase I study, we saw at the highest doses various novel features: a reversible thrombocytopenia without signs of cumulative bone marrow toxicity, delayed nausea, fatigue that led some patients to discontinue therapy, and transient ECG changes that at the highest doses were suggestive of myocardial ischemia but without echocardiographic evidence of myocardial dysfunction (1). These effects were later recognized as class effects reported for other HDAC inhibitors in phase I testing (13). Romidepsin was well-tolerated at the recommended dose, and even better tolerated when in the subsequent phase II study we adopted the schedule that had been studied by John Marshall at Georgetown University (14): 14 mg/m² administered on days 1, 2, 3, and 15 every 28 days.

It was also on this phase I study that clinical activity was first observed. In the original article in Clinical Cancer Research, we reported that 1 patient with renal cell cancer had a >50% reduction in the size of supraclavicular, mediastinal, and retroperitoneal adenopathy that persisted for 6 months (1). In an expansion cohort enrolled after the phase I study was completed (in order to perform a more detailed analysis of pharmacodynamic markers), a patient with peripheral T-cell lymphoma who had experienced disease progression on combination chemotherapy had a marked reduction in skin and nodal disease on romidepsin, eventually reaching a complete remission (15). Major responses were observed in over 50% of the 10 patients with T-cell lymphoma subsequently enrolled. At that time, there were few therapies for T-cell lymphoma, whether peripheral T-cell (PTCL) or the cutaneous T-cell (CTCL) subtype. Relatively rare compared with B-cell lymphomas, PTCL had been inadequately treated with drugs. Treatment with therapies developed for B-cell lymphomas led to some responses, but these were never durable, and patients with PTCL were often treated with intensive combination chemotherapy regimens. CTCL, or mycosis fungoides and Sézary syndrome, was a more ‘indolent’ disease with skin manifestations, including erythema, pain, and itching, treated mostly with skin-directed therapies. When skin-directed therapies failed, patients were treated with systemic chemotherapy and often died of infection. Based on the activity seen in the expanded cohort in the phase I study, a phase II trial was launched in CTCL and PTCL. Vorinostat, another HDAC inhibitor in development at that time, was also investigated in CTCL, for which it received FDA approval in 2006 (16).

The NCI1312 phase II trial enrolled patients with CTCL or PTCL, and activity was confirmed, with durable responses observed (10). Lessons learned in the phase I study and reported in the Clinical Cancer Research article were followed up. The ECG changes seen in phase I, ST-T wave flattening and T wave inversion were studied in detail in the NCI phase II trial (17). Ultimately, over 3,700 ECGs were obtained and analyzed in addition to troponin levels, echocardiograms, and ejection fraction analyses (18). From these studies, we reported predictable and consistent ST-T wave changes and a small increase in the heart rate, a mean of 10 to 12 bpm in treated patients. Minimal change in the QT interval was observed. We also noted that for 55% of doses, potassium and magnesium levels met the protocol-mandated replacement requirement. Later, the prescribing information would include recommendations for monitoring potassium and magnesium and for caution in patients with underlying cardiac disease, considerations evolving from observations made in the phase I study first reported in these pages.

The NCI1312 phase II trial went on to accrue 131 patients with either CTCL or PTCL. Overall response rates were 33% in CTCL and 38% in PTCL (10). Gloucester Pharmaceuticals licensed romidepsin and launched separate registration trials in both diseases, with comparable response rates. Data from NCI1312 and the Gloucester Pharmaceuticals trials were submitted to the FDA, and romidepsin received approval for CTCL in 2009 and for PTCL in 2011. These observations, and those with vorinostat in CTCL, led to the evaluation of other HDAC inhibitors for patients with T-cell lymphoma, and the approval of belinostat for PTCL (16, 19). These findings encouraged the development of HDAC inhibitors in other tumor types—panobinostat received FDA approval on February 23, 2015, for the treatment of multiple myeloma in combination with bortezomib (20). Ongoing studies are combining HDAC inhibitors with other agents in an attempt to expand the unique activities of these epigenetic agents to different tumor types. Also, the success of HDAC inhibitors has energized the study of other epigenetic agents—with numerous new targets now being described. Basic science has aimed at understanding the epigenetic basis of cancer, and identification of mutations in multiple epigenetic genes that encode proteins regulating chromatin structure and function has led to the identification of multiple new potential targets for anticancer treatment (21). These include EZH2, with an activating mutation driving a subset of B-cell lymphomas; inactivating EZH2 mutations and deletions in leukemia; histone H3.3 mutations in glioblastoma; DNMT, IDH1/2, and TET2 mutations in acute myelogenous leukemias and peripheral T-cell lymphomas; and Brd4 mutations in midline carcinomas. New agents have been discovered that are aimed at these targets, including inhibitors of EZH2, BRD4, DOT1L, and IDH1/2, and phase I trials are in progress. We look forward to seeing the results of these studies reported in the pages of Clinical Cancer Research.

Disclosure of Potential Conflicts of Interest

S.E. Bates reports receiving a commercial research grant from Celgene via CRADA with NCI. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: S.E. Bates, R.W. Robey, R.L. Piekarz

Writing, review, and/or revision of the manuscript: S.E. Bates, R.W. Robey, R.L. Piekarz

Received March 13, 2015; accepted March 13, 2015; published online May 15, 2015.

Downloaded from clincancerres.aacrjournals.org on April 12, 2017. © 2015 American Association for Cancer Research.
References
CCR 20th Anniversary Commentary: Expanding the Epigenetic Therapeutic Portfolio

Susan E. Bates, Robert W. Robey and Richard L. Piekarz


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/21/10/2195

Cited articles
This article cites 20 articles, 10 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/10/2195.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/21/10/2195.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.