Restoring Epigenetic Balance with the HDAC Inhibitor Mocetinostat
The Role of Epigenetics in Cancer

Epigenetics is the study of heritable cellular traits that are not caused by changes in the DNA sequence or by chromosomal alterations. Many cancers are associated with identifiable “epigenetic” changes, resulting in altered regulation of gene transcription, (the conversion of DNA “blueprints” to RNA) and translation (the process by which RNA is “read” by cellular machinery to create proteins).¹

TWO KEY PROCESSES INVOLVED IN REGULATION OF THE EPIGENETIC PROGRAM ARE:

1) **DNA Methylation**, the addition (methylation) or removal (demethylation) of methyl groups to/from DNA, and

2) **Covalent Histone Modifications**, such as lysine acetylation or methylation, that affect the regulation of gene expression through interaction of histone regulatory proteins with DNA or through recruitment of protein-DNA complexes that regulate gene expression.

The epigenetic regulation of gene expression is mediated by the packaging of DNA in histone protein-DNA complexes called nucleosomes, as well as the positioning of nucleosomes across the genome in different configurations of chromatin.⁸ Epigenetic regulators can be divided into distinct groups based on broad functions: epigenetic writers introduce epigenetic marks on DNA or histones; these marks are removed by epigenetic erasers and recognized by epigenetic readers.
The Role of Histone Deacetylases (HDACs) in Cancer

A key epigenetic regulatory mechanism is mediated by the lysine acetylation of histone proteins regulated by key protein families, called histone acetyl transferases (HATs) and HDACs.

HATs are generally associated with transcriptional activation and introduce acetyl groups at key lysine residues of histones (i.e., writers) and include the CREBBP/EP300, GCN5/PCAF, HAT1, and the MYST families. It should also be noted that HDACs and HATs regulate the lysine acetylation and function of non-histone proteins such as p53, MYC, p16 and others, indicating that HDAC- and HAT-dependent modification of lysine acetylation likely regulates cellular function via regulation of both gene expression and protein function. The coordinated regulation of histone and non-histone protein acetylation and deacetylation is normally tightly regulated to maintain normal tissue development and homeostasis.

HDACs remove acetyl groups from histone tails (i.e., erasers) resulting in transcriptional repression. HDACs are a large family of proteins made up of four groups, including class I (HDAC 1, 2, 3, 8), class II (HDAC 4, 5, 6, 7, 9, 10), class III (Sirtuins), and class IV (HDAC 11).

In human cancers, histone lysine acetylation can become dysregulated through the upregulation of HDACs, and/or inactivation of HATs, resulting in a net decrease in histone acetylation and silencing of important genes that are subject to regulation. For example, the dysregulation of histone acetylation can lead to aberrant gene expression, causing activation of oncogenes and inactivation of tumor suppressor genes, leading to uncontrolled cell growth and tumor progression.

HDAC inhibitors (HDACIs) have the potential to restore normal gene expression and display potent antitumor effects in vitro. As single agents, HDACIs, such as vorinostat and romidepsin, have demonstrated clinical efficacy in cutaneous T-cell lymphoma and are approved for that indication. HDACIs have also shown single agent clinical activity in diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, and Hodgkin's lymphoma and occasional responses have been observed in solid tumors. In addition, they have shown clinical activity in combination with DNA methylation inhibitors in myeloid leukemias and in combination with estrogen receptor inhibitors in patients with breast cancer. Other uses and combinations are being studied in a variety of tumors.

Loss of function mutations involving CREBBP or EP300 have been observed in between 20-25% of urothelial bladder cancers and both diffuse or follicular B-cell lymphoma, as well as significant percentages of gastric, colorectal, head and neck, and lung cancers.

Mutations That Drive Epigenetic Dysfunction

The regulation of protein acetylation is disrupted in multiple human cancers, including leukemias, lymphomas, and selected solid tumors through the overexpression of certain HDACs or gene deletion, mutation, or rearrangements involving the CREBBP and EP300 HAT family. Given this knowledge, research has focused on the precise identification, role and therapeutic targeting of mutations in these epigenetic regulators. For example, loss of function mutations involving CREBBP or EP300 have been observed in between 20-25% of urothelial bladder cancers and both diffuse or follicular B-cell lymphoma, as well as significant percentages of gastric, colorectal, head and neck, and lung cancers. Recurrent mutations in these cancers result in the loss of acetyltransferase activity which results in hypoacetylation of key lysine residues and suggests that these HATs have tumor suppressive activity. The genetic alteration of the CREBBP/EP300 HATs in a unique spectrum of cancers provides a rationale for targeting HDACs as a potential therapeutic strategy.

Past Studies of HDAC Inhibitors Were Not Based on a Tumor's Genetic Signature

HDAC inhibitors have been described to have clinical activity in cancer by causing tumor cell apoptosis, growth arrest, senescence, differentiation and angiogenesis inhibition. However, historical results with HDAC inhibitors have been somewhat disappointing, potentially because earlier generations of HDAC inhibitors were tested in unselected patient populations which may have underestimated their effect. In addition, the early HDAC inhibitors non-selectively targeted all types of HDACs, which often leads to significant side effects, like myelosuppression, that can limit dosing and may limit clinical benefit.
Mocetinostat, a HDAC Inhibitor for Tumors with CREBBP and EP300 Inactivating Mutations

Mocetinostat, a spectrum selective HDAC inhibitor, has activity in preclinical tumor models with CREBBP and EP300 inactivating mutations. Due to their role in regulating histone acetylation, Mirati hypothesized that loss of function mutations of CREBBP and/or EP300 would sensitize tumors to mocetinostat therapy and identify a subset of patients with increased probability of therapeutic benefit. Though similar in structure and functionality, CREBBP and EP300 work independently, creating a rationale for targeting both mutations. While some reports of tumors with mutations in CREBBP and EP300 indicate a high percentage of tumors have these mutations, not all mutations are functionally important. Mocetinostat studies are focused only on inactivating mutations in CREBBP and EP300 that reduce acetylation and may play a role in the development of bladder cancer and non-Hodgkin’s lymphoma, including DLBCL. These mutations selectively affect the acetylation of lysine residues that are affected by HDACs 1 and 2.

Mocetinostat selectively targets HDACs 1, 2, 3 and 11 and is the first HDAC inhibitor being developed using genetic sequencing tools to identify bladder cancer and DLBCL patients whose tumors carry inactivating mutations of the CREBBP or EP300 genes. We believe that by blocking these HDACs, mocetinostat may compensate for inactivating mutations of CREBBP and EP300 by restoring the delicate HAT-HDAC balance needed to keep tumors from growing.

**Using Mocetinostat to Restore Epigenetic Balance**

**HDACs:**
Histone deacetylases remove acetyl groups from histone "tails", causing the DNA to wind tightly around the chromatin center, preventing gene expression of important tumor suppressor genes.

**HATs:**
Histone acetyl transferases (HATs) add acetyl groups to histone "tails", allowing gene expression.

Mocetinostat can selectively target HDACs 1, 2, 3 and 11 and is the first HDAC inhibitor being developed using genetic sequencing tools to identify bladder cancer and DLBCL patients whose tumors carry inactivating mutations of the CREBBP or EP300 genes. We believe that by blocking these HDACs, mocetinostat may compensate for inactivating mutations of CREBBP and EP300 by restoring the delicate HAT-HDAC balance needed to keep tumors from growing.
Mocetinostat in Bladder Cancer and DLBCL: Diseases with High Unmet Need

A single agent Phase 2 study of mocetinostat in patients with refractory DLBCL, unselected for any specific genetic mutations, demonstrated a reduction in tumor size in 77% of patients, with confirmed responses in 17% of patients, including 6 partial responses (PRs) and 1 complete response (CR). However, that study did not pre-select patients for genetic mutations. Mirati believes that pre-selecting patients with CREBBP and EP300 inactivating mutations represents a promising patient enrichment approach that has the potential to improve response rates. Mirati has initiated two single-agent proof-of-concept clinical studies with mocetinostat, one in bladder cancer and the other in DLBCL, both in patients selected for CREBBP and EP300 mutations.

Patients with bladder cancer or certain Non-Hodgkins Lymphomas — DLBCL — that carry inactivating mutations of the CREBBP and EP300 genes represent significant populations with high unmet patient need. There are no approved therapies available in the U.S. after failure of initial treatment with chemotherapy for patients with bladder cancer, and about half of DLBCL patients relapse or are refractory to first line treatment.

- Inactivating mutations of CREBBP or EP300 occur in ~20% of patients with bladder cancer, and ~25% of patients with DLBCL. Mirati is pursuing 2nd-line indications for both, representing 4,480 bladder cancer patients and 5,300 DLBCL patients in the U.S. alone.

- If the patient selection strategy proves successful, patients with other tumor types with these same mutations could be explored to expand to additional indications.

Use of HDAC Inhibitors with Other Epigenetic or Immuno-Oncology Agents

The combination of mocetinostat with other epigenetic agents has strong scientific rationale as the epigenetic mechanisms of methylation and acetylation interact synergistically to control gene expression and resulting tumor formation. Mirati is exploring combinations of mocetinostat with other epigenetic agents as a potential next step in the mocetinostat development program.

Mirati is also assessing combinations of mocetinostat with immune therapies. There is growing evidence that HDACs may be able to increase the efficacy of immuno-oncology therapies. Initial preclinical data suggests that spectrum-selective HDAC inhibitors "prime" the immune system to attack a cancer by inducing immunogenic cell death and upregulating programmed death ligand-1 (PD-L1) and major histocompatibility (MHC) Class 1 and 2 molecules. In addition, spectrum selective HDAC inhibitors were demonstrated to increase the number of CD8+ tumor infiltrating lymphocytes while depleting immunosuppressive cell types, such as T-regulatory cells and myeloid-derived suppressor cells. These data indicate that mocetinostat could potentially be used to enhance the efficacy of immune check-point inhibitors, such as PD1 or PD-L1 antagonists.
Endnotes

Forward Looking Statements

Certain statements contained in this Backgrounder, other than statements of fact that are independently verifiable at the date hereof, contain "forward-looking" statements, within the meaning of the Private Securities Litigation Reform Act of 1995, that involve significant risks and uncertainties. For more detailed disclosures and discussions regarding such forward looking statements, please refer to Mirati’s filings with the U.S. Securities and Exchange Commission ("SEC"), including without limitation Mirati’s filings on Forms 10-K, 10-Q, and 8-K. Forward looking statements are based on the current expectations of management and upon what management believes to be reasonable assumptions based on information currently available to it. Such statements can usually be identified by the use of words such as “may,” “would,” “believe,” “intend,” “plan,” “anticipate,” “estimate,” “expect,” and other similar terminology, or by statements that certain actions, events or results “may” or “would” be taken, occur or be achieved. Such statements include, but are not limited to, statements regarding Mirati’s development plans and timelines, potential regulatory actions, expected use of cash resources, the timing and results of clinical trials, and the potential benefits of and markets for Mirati’s product candidates. Forward looking statements involve significant risks and uncertainties and are neither a prediction nor a guarantee that future events or circumstances will occur. Such risks include, but are not limited to, potential delays in development timelines or negative clinical trial results, reliance on third parties for development efforts, changes in the competitive landscape, changes in the standard of care, as well as other risks described in Mirati’s filings with the SEC. We are including this cautionary note to make applicable, and to take advantage of, the safe harbor provisions of the Private Securities Litigation Reform Act of 1995 for forward-looking statements. The information in this Backgrounder is given as of the date below and Mirati expressly disclaims any obligation to update or revise any forward-looking statements, whether as a result of new information, future events or otherwise, unless required by law.
About Mirati Therapeutics

Mirati Therapeutics develops molecularly targeted cancer treatments that are intended to inhibit tumor growth. Mirati’s approach combines the three most important factors in oncology drug development, 1) researching and developing drug candidates that target genetic and epigenetic drivers of cancer, 2) designing creative and agile clinical development strategies that select for patients whose tumors are dependent on specific driver alterations, and 3) leveraging a highly accomplished targeted oncology leadership team. The Mirati team uses a blueprint – proven by their prior work – for developing potential breakthrough cancer therapies, with accelerated development paths, in order to improve outcomes for patients. Mirati is advancing three drug candidates through clinical development for multiple oncology indications.