The Rationale and Therapeutic Promise of Combining HDAC Inhibitors with Immune Checkpoint Inhibitors
The Hypothesis for Combining HDAC Inhibitors with Immune Checkpoint Inhibitors

Major breakthroughs in the treatment of patients with multiple types of cancer have been achieved by drugs targeting the immune checkpoint pathway. Treatment with agents targeting the programmed death receptor-1 (PD-1) and ligand (PD-L1) have resulted in robust tumor responses and survival benefit in patients with a number of tumor types, including melanoma, renal, lung, head and neck, and bladder cancers.\(^{23,24}\) Despite the promise of PD-1/PD-L1 inhibitors as single-agent therapies, the majority of patients either do not respond or eventually progress due to innate or acquired resistance. Possible mechanisms of resistance to immune checkpoint inhibitors include (i) downregulation of PD-L1; (ii) a decrease in the number of tumor-infiltrating lymphocytes; (iii) the inability of T-effector lymphocytes to recognize tumor cells; and (iv) the presence of negative (immune suppressive) immune regulatory cells in the tumor microenvironment including regulatory T cells [Tregs] and myeloid-derived suppressor cells [MDSCs].\(^{16}\) Research is now focused on the co-administration of PD-L1 inhibitors with other therapeutic agents to treat or prevent resistance potentially resulting in enhanced activity of PD-1/PD-L1 pathway inhibitors. Based on scientific rationale, one key class of molecules for immune checkpoint inhibitor-based combination therapy are spectrum-selective histone de-acetylase (HDAC) inhibitors.

There is growing evidence that the combined anti-tumor and immunomodulatory effects of Class I HDAC inhibitors may increase the efficacy of immune checkpoint inhibitors.\(^{17,22,31}\)

CLASS I HDAC INHIBITORS

**PD-1 serves as an “on/off” switch on cytotoxic T cells, while its ligand, PD-L1, is overexpressed in a variety of tumors as well as tumor immune-infiltrating cells. Binding of PD-1 to PD-L1 deactivates CD4+ and CD8+ T cells and inhibits cytokine production, allowing tumors to escape and survive.\(^{5}\)** Antibodies that block the PD-1 receptor have been shown to restore T-cell function and result in profound clinical responses in some patients with various solid tumors, including non–small cell lung cancer (NSCLC), bladder, and head and neck carcinomas.\(^{24}\) Similarly, antibody-mediated blockade of PD-L1 has induced durable tumor regression (objective response rate of 6%-32%) and prolonged stabilization of disease (rates of 12%-41% at 24 weeks) in patients with several advanced cancers, including NSCLC, melanoma and renal-cell cancer.\(^{5,23,28}\)
The rationale for combining these two therapeutic classes is based on the ability of Class I HDAC inhibitors to:

- "Prime" the adaptive immune system to program and activate naïve T cells to target tumor antigens\(^2,19\)
- "Prime" the innate immune system to stimulate natural killer (NK) cell activity against tumor cells\(^14,26\)
- Increase the number of cancer-killing CD8+ and CD4+ tumor-infiltrating lymphocytes\(^2,14\)
- Deplete immnosuppressive cells, such as Tregs and MDSCs\(^17,25\)
- Make tumors more recognizable to the immune system by upregulating PD-L1 and the major histocompatibility (MHC) Class I and II molecules that present tumor antigens\(^13,14,17\)

In addition, combination therapy with checkpoint blockade immunotherapy has the added advantage of inducing a memory response unattainable with single-agent cytotoxic and targeted therapies.\(^13\)

The Complementary Mechanisms of Action of HDAC Inhibitors and Checkpoint Inhibitors

In addition to the immunostimulatory effects noted above, several studies show that certain HDAC inhibitors are relatively nontoxic to normal cells or tissues, but are selectively cytotoxic against a wide range of cancer cells. These characteristics make them an attractive partner for combination therapy with treatments such as PD-L1 inhibitors that reverse the defective immune checkpoint regulation associated with cancer cells.\(^1\) Supporting this concept, recent data demonstrate that Mirati’s Class I/IV HDAC inhibitor, mocetinostat, generated robust and durable upregulation of PD-L1 and PD-L2 in melanoma cell lines, as well as patient tumor samples,\(^31\) and also addressed other potential immune checkpoint inhibitor resistance mechanisms by increasing tumor cell immunogenicity and enhancing the T cell–mediated adaptive immune response.\(^17\)

The immunomodulatory properties of mocetinostat and other Class I HDAC inhibitors are mediated through several distinct routes that complement checkpoint inhibition, as summarized in Table 1.
Mocetinostat

efficiency of tumor antigenicity by:
  •  upregulating antigens necessary for co-stimulation in multiple
tumor types\textsuperscript{19,20,26}
  •  elevating proteins associated with the processing and
presentation of tumor peptides.\textsuperscript{9,15} This increases the
expression of certain glycoproteins on tumor cells (but not
normal cells) making them sensitive to destruction by the
innate immune system\textsuperscript{4,32}
  •  Increasing expression of other phagocytosis ("eat-me") signals\textsuperscript{9}

Induction of immunogenic cell death:
  •  Vorinostat (pan-inhibitor of Class I and Class II HDACs)
triggered apoptosis and stimulated the release of important
mediators of immunogenic cell death\textsuperscript{17,27}
  •  Christiansen et al. showed that MC38 colon carcinoma cells
treated with a pan-HDAC inhibitor, were efficiently taken up
by dendritic cells\textsuperscript{10}

Enhance T-effector cell function:
  •  HDAC inhibitors enhance cytotoxic CD8\textsuperscript{+} T-cell activity and
have no (or minimal) effects on CD4\textsuperscript{+} T cells\textsuperscript{5,17}

Decreased Treg cell function:
  •  Class I HDAC inhibitors reduced Treg numbers and Treg
activity in multiple tumor models\textsuperscript{6}
  •  In addition to effects on Tregs, treatment with Class I HDAC
inhibitors transformed tumor-induced myeloid-derived
suppressor cells into cancer-eliminating cells in vitro\textsuperscript{13,25}
  •  Class I HDAC inhibitors can restore antigen expression on
tumor-associated macrophages, reversing immune suppression
and delaying tumor growth\textsuperscript{7,12}

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easier for T-effector cells to recognize and eliminate tumors\textsuperscript{9} |
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innate immune system\textsuperscript{4,32} | •  Enhance dendritic cells’ recognition of the tumor as "non-self,"
which primes T cells and other cancer-killing immune cells
against the tumor\textsuperscript{11} |
| •  Increasing expression of other phagocytosis ("eat-me") signals\textsuperscript{9} | |
| **Induction of immunogenic cell death:** | •  Drive the elimination of tumor cells and reduce tumor-induced
immune suppression. Eliminating cancer cells on the periphery
of the tumor makes the core tumor mass more accessible to
cytotoxic immune cells\textsuperscript{17} |
| •  Vorinostat (pan-inhibitor of Class I and Class II HDACs)
triggered apoptosis and stimulated the release of important
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inducing apoptotic tumor cell death, but also by producing and
presenting tumor-specific antigens\textsuperscript{17} |
| •  Christiansen et al. showed that MC38 colon carcinoma cells
treated with a pan-HDAC inhibitor, were efficiently taken up
by dendritic cells\textsuperscript{10} | •  Increase ligands expressed by tumor cells that activate the
innate immune system’s natural killer cells\textsuperscript{17} |
| **Enhance T-effector cell function:** | •  Activate tumor-destroying CD8\textsuperscript{+} cytotoxic T cells and
upregulate CD4\textsuperscript{+} and CD8\textsuperscript{+} memory T cells that recognize
tumor antigens and lock them into memory for future
identification and defense\textsuperscript{29} |
| •  HDAC inhibitors enhance cytotoxic CD8\textsuperscript{+} T-cell activity and
have no (or minimal) effects on CD4\textsuperscript{+} T cells\textsuperscript{5,17} | •  Reverse the immune-suppressive tumor microenvironment
by reducing tumor-driven recruitment of Tregs and myeloid-
derived suppressor cells\textsuperscript{17} |

| NOTE: CLASS II HDACS (HDACs 4–7/HDACS 8–10) AND PAN-HDAC INHIBITORS CAN HAVE IMMUNO-SUPPRESSIVE EFFECTS THAT COULD COUNTERACT THE IMMUNOSTIMULATORY ACTIONS OF CLASS I HDAC INHIBITION.\textsuperscript{17} |

Table 1: Immune Functions Associated With Class I HDAC Inhibitors

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Mocetinostat, a Spectrum-Selective Class I/IV HDAC Inhibitor, May Be Better Suited Than Pan-HDAC Inhibitors for Combination Treatment with PD-1/PD-L1 Inhibitors

HDAC inhibitors are categorized by the types of HDACs they inhibit (see INSET, Page 3: The Role of HDAC Inhibitors in Cancer).

Mocetinostat is a spectrum-selective Class I/IV HDAC inhibitor specifically targeting HDACs 1, 2, 3, and 11. Class I HDACs are of particular interest from an immunostimulatory and immune-priming perspective because of their effects on a broad range of immune system functions. For example, treatment with Class I HDAC inhibitors was shown to enhance NK cell and CD8+ T cell functions and reduce Treg numbers and function in multiple tumor models, result in an enhanced tumor immune response. In contrast, Class II HDAC inhibitors directly enhance the immunosuppressive function of murine Treg cells. In addition, Class II HDAC inhibitors (HDACs 4–7/HDACs 8–10) and pan-HDAC inhibitors can have immunosuppressive effects that could counteract the immunostimulatory actions of Class I HDAC inhibition. Class I and II inhibitors have different effects on MDSCs that may be implicated in resistance to immune checkpoint inhibitors. This is highlighted by a study showing that inhibition of Class I HDACs caused highly immunosuppressive, tumor-induced MDSCs in the bone marrow to differentiate into macrophages and dendritic cells capable of stimulating an immune system attack on the tumor cells. This effect is distinct from the effects of pan-HDAC inhibition, which causes myeloid cells to differentiate into immunosuppressive MDSCs that limit immune system responsiveness. The immunosuppressive activity of pan-HDAC inhibitors is highlighted by the fact that they have been used to limit cytokine production and immune responses following allogenic transplant in autoimmune diseases, including rheumatoid arthritis. Therefore, data suggest that the use of mocetinostat, a Class I/IV HDAC inhibitor, would be more appropriate in combination with a checkpoint inhibitor than a Class II or pan-HDAC inhibitor, such as vorinostat (which could result in immunosuppression and a decrease in the activity of the checkpoint inhibitor).

Further, while mocetinostat generated robust and durable upregulation of PD-L1 and PD-L2 in melanoma cell lines and patient tumor samples, these effects were not observed with HDAC 6 or Class IIa–specific inhibitors. This observation is consistent with a related study by Woods et al. showing that a treatment with a Class I HDAC inhibitor and PD-1 blocking antibodies in murine models of metastatic melanoma resulted in delayed tumor growth and increased survival compared to either single agent. Ongoing studies confirm the induction of PD-L1 expression following mocetinostat treatment of non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), and urothelial cancer cell lines on mouse models (Mirati, data on file). In addition, mocetinostat induced the expression of molecules that mediate the presentation of tumor antigens including HLA-DR, HLA-A, and HLA-B or markers facilitating an innate NK cell–directed immune response, including MICA and MICB. Studies in syngeneic mouse models indicated that mocetinostat increased CD4/CD8+ T-effector lymphocytes and decreased tumor T regs in vivo (Mirati, data on file). These preclinical studies indicate that mocetinostat exhibits immunomodulatory properties consistent with Class I HDAC inhibitors.
Conclusion: Immunogenicity and Priming of the Tumor Microenvironment

There is increasing evidence that indicates Class I HDAC inhibitors may enhance the efficacy of immune checkpoint inhibitors such as PD-L1 antagonists, especially spectrum-selective Class I HDAC inhibitors, like mocetinostat, that selectively target specific HDAC isoforms.

This evidence suggests that combining a spectrum-selective HDAC inhibitor with an immuno-oncology therapy may provide an important therapeutic option in treating a wide range of cancers, and provides a strong scientific rationale for further research in this area.

Mocetinostat, a Class I/IV HDAC inhibitor, "primes" the immune system to attack a cancer cell by inducing immunogenic cell death and upregulating MHC Class I and II molecules, making the cancer cell more recognizable. Mocetinostat also upregulates PD-L1, the target of PD-L1 antibodies. In addition, spectrum-selective HDAC inhibitors, such as mocetinostat, increase the number of CD8+ tumor-infiltrating lymphocytes while depleting immunosuppressive cell types, such as T regs and MDSCs.

**FIGURE 1. MOCETINOSTAT: KEY ANTI-CANCER ACTIVITIES COMPLEMENTING PD-1/PD-L1 INHIBITION**

Tumor cell markers and immune cell types associated with increased tumor cell immunogenicity.
Endnotes

17. Kresse M. HDAC inhibitors and immunotherapy; a double edged sword? Oncoarget. 5.16 (2014):6558-6572.

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, single agent and immuno-oncology combination therapies intended to treat cancer. 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.