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## Panobinostat (LBH589): a novel pan-deacetylase inhibitor with activity in T cell lymphoma



A B S T R A C T

Panobinostat is a novel cinnamic hydroxamic acid pan-deacetylase inhibitor able to induce hyperacetylation of lysine residues on both histone and non-histone targets in cancer cells. Panobinostat is currently in clinical development, as both an intravenous and oral formulation, across multiple tumor types, and as proof of principle has resulted in prolonged histone hyperacetylation in tumor cells along with impressive anti-tumor activity in phase I and II trials, in particular T cell lymphomas, Hodgkin lymphoma and myeloid malignancies. Initial concerns regarding cardiac toxicities appear to have been answered by alterations in dose scheduling. Future studies may help unravel molecularly- or cytogenetically-defined patient sub-groups who are most likely to benefit from panobinostat, either as a single agent, or, ultimately more likely, in combination with other therapies.

### Introduction

Histone deacetylase (HDAC) enzymes and histone acetylases (HAT) control the relative degree of histone acetylation and deacetylation of the chromatin in DNA. In this way, HDACs/HATs regulate the level to which a gene is transcribed by controlling the accessibility of DNA for transcription.<sup>1</sup> HDACs, which result in a net decrease in acetylation and thus result in transcriptional silencing, have been divided into four general classes. Class I, located within the cell nucleus; Class II and the Class IV HDAC, HDAC 11 which shuttle between the cell cytoplasm and nucleus. Class III HDACs consist of the NAD<sup>+</sup>-dependent sirtuin family 1 to 7.<sup>2</sup> By affecting HDACs and altering gene expression, the new anti-cancer drugs the deacetylase inhibitors (DACi)

upregulate pro-apoptotic genes and the downregulate anti-apoptotic genes,<sup>3</sup> activate cell-cycle checkpoints, induce cellular differentiation, suppress angiogenesis and enhance host immune surveillance.<sup>3-5</sup> Acetylation-dependent changes also occur in the activities of important *non-histone* cytoplasmic proteins such as p53, Ku70,  $\alpha$ -tubulin and Hsp90.<sup>5</sup> Multiple cellular pathways are therefore affected simultaneously, resulting in cancer cell death. Whether pan-DACi, which inhibit both Class I and II HDACs, are superior to selective DACi inhibiting class I alone, is unknown. Panobinostat is a cinnamic hydroxamic acid pan-deacetylase inhibitor with a marked DAC inhibitor activity at low nanomolar concentrations both *in vitro* and *in vivo*, which has undergone extensive preclinical and clinical scrutiny.

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## Preclinical data

### *In vitro*

Panobinostat is a pan-DACi, with low nanomolar concentration high inhibitory activity against all Class I, II and IV HDACs. Panobinostat appears to be at least 10-fold more effective as a pure DACi when compared with vorinostat, the only currently FDA approved HDACi. Marked anti-tumor activity across a broad range of cancer cell lines of hematologic malignancies has been demonstrated, including cutaneous T cell lymphoma (CTCL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), multiple myeloma (MM), Hodgkin lymphoma (HL), as well as solid tumors cell lines such as breast, colon, prostate and pancreas. Moreover, panobinostat was up to 100-fold more effective than vorinostat in the HCT116, BT474 and HH cell lines in its ability to cause inhibition of cancer cell proliferation and cell viability. It should be noted, that although the cytotoxic effects of panobinostat were evident over multiple cancer cell lines, there does appear to be differences in sensitivity between haematological and solid tumor cell lines, with lower concentrations required for cytotoxicity seen in AML, CML, HL and CTCL cells. Despite the marked anti-tumor activity seen with multiple cancer cell lines at nanomolar concentrations, panobinostat only resulted in apoptosis of normal human cell lines at far greater concentrations, indicating a selective toxicity for cancer cells.<sup>6</sup> A similar phenomenon was also seen in human myeloma cell lines and myeloma cells from patients with MM resistant to standard chemotherapeutic agents, where panobinostat was able to arrest growth in tumor cells without affecting normal peripheral blood mononuclear cells (PBMCs) or granulocytes.<sup>7,8</sup>

### *In vivo*

Early murine experiments have confirmed

that panobinostat has potent anti-tumor activity against both hematologic and solid tumors *in vivo*. A HH CTCL mouse xenograft model showed complete tumor regression on intravenous panobinostat at 10 mg/kg when compared to vehicle,<sup>9</sup> and a dose-related reduction in tumor burden with preservation of bone integrity in a MM xenograft mouse model. Further experiments confirmed a significant synergistic cytotoxicity between panobinostat and the proteasome inhibitor bortezomib without additional toxicity to normal bone marrow stromal cells *in vitro*.<sup>10</sup> Potent tumor regression was also reported with panobinostat in colon, pancreatic and small cell lung cancer (SCLC) mouse xenograft models.<sup>9,11</sup>

The E $\mu$ -myc B cell lymphoma model has been used to demonstrate that activation of the intrinsic apoptotic pathway is required for the apoptotic and therapeutic activity of panobinostat. Conversely, a functional extrinsic apoptotic pathway or functional apoptosome is not required for panobinostat mediated tumor cell death or therapeutic efficacy (Johnstone R.W *et al.* submitted for publication). Moreover, knockout murine experiments allow essential components of the apoptosome such as apaf-1 or caspase-9, to be deleted, resulting in delayed panobinostat-induced lymphoma killing *in vitro* and *in vivo*, associated with suppression of a number of biochemical indicators of apoptosis. Despite this, prolonged exposure to panobinostat did result in tumor regression, with morphological and biochemical features of autophagy apparent, suggesting that in the absence of a functional intrinsic apoptosis pathway, panobinostat may initiate an alternative cell death pathway. These pre-clinical studies demonstrate that loss of viability, primarily through induction of apoptosis via the intrinsic apoptotic pathway but also through additional cell death pathways, produces therapeutic efficacy in response to panobinostat.

**Table 1.** Phase I/II studies of Panobinostat in haematological malignancies.

	<i>Disease</i>	<i>Phase</i>	<i>No.</i>	<i>Responses</i>
Giles <i>et al.</i> <sup>12</sup>	Advanced leukemia/MDS	I	15	HI 1
Sharma <i>et al.</i> <sup>14</sup>	NHL/Solid tumors	I	44	PR 3 (CTCL, PTCL, HRPC)
Prince <i>et al.</i> <sup>15</sup>	NHL/Solid tumors	I	19	CR 2 (CTCL), PR 2 (CTCL)
Ellis <i>et al.</i> <sup>16</sup>	CTCL	I	10	CR 2, PR 4
Duvic <i>et al.</i> <sup>17</sup>	CTCL	II	95	CR 4, Skin CR 2, PR
Ottmann <i>et al.</i> <sup>21</sup>	AML/MDS/MF/MM/HL/NHL	I/II	146	CR 3, CRi 1, PR 17, SD 14
Wolf <i>et al.</i> <sup>22</sup>	MM	II	38	PR 1, SD 4
Siegel <i>et al.</i> <sup>23</sup>	MM (in combination with Bort)	I	18	CR 1, VGPR 3, PR 5

NHL: Non Hodgkin lymphoma; HL: Hodgkin lymphoma; CTCL: Cutaneous T-cell lymphoma; MDS: Myelodysplasia; AML: Acute myeloid leukaemia; MF: Myelofibrosis; MM: Multiple myeloma; Bort: Bortezomib; CR: Complete response; PR: Partial response, SD: Stable disease; HI: haematological improvement; VGPR: Very good partial response.

### Clinical studies involving panobinostat

In a two-arm, dose-escalation phase IA/II study in patients with AML, acute lymphocytic leukemia (ALL) or myelodysplastic syndrome (MDS), panobinostat was administered intravenously daily for seven days on a 21-day cycle. This dosing schedule was based on the presumption that leukemic cells may need prolonged exposure to the drug for disease control. Fifteen patients were treated, and despite a reduction in blasts in peripheral blood in seven patients, dose limiting cardiac toxicity was noted early, with Grade 3 QTcF prolongation in four patients, resulting in premature discontinuation of the study.<sup>12</sup> In view of this, subsequent studies have utilized an intermittent dosing schedule with minimal cardiac effects observed to date.<sup>13</sup>

A further phase I study, examined a dose-escalating, intravenous schedule of 10-20 mg/m<sup>2</sup> given weekly for three weeks out of four, was evaluated in 44 patients with advanced solid tumors or NHL.<sup>14</sup> This schedule was chosen primarily on the basis that a sustained pharmacodynamic effect would be present, whilst avoiding progressive QTcF prolongation seen with consecutive daily

doses of panobinostat. For this schedule the maximum tolerated dose (MTD) was 20 mg/m<sup>2</sup> with three dose-limiting toxicities (DLTs) reported: fatigue, hyperglycemia (both Grade 3) and thrombocytopenia (Grade 4). Despite intensive ECG monitoring, effects on the QTcF interval appeared mild. One patient known to have had complete left bundle branch block on enrolment had a QTcF interval  $\geq 500$  milliseconds, and two further patients having transient asymptomatic increases in QTcF (>60 milliseconds). Three patients had a partial response (PR), including one patient with CTCL, one with peripheral T cell lymphoma (PTCL), and one heavily pre-treated patient with nodal hormone refractory prostatic cancer. Dose-dependent increases in histone H3 acetylation were reported in PBMCs seven days after both the first and second doses of panobinostat at all dose levels, suggesting sustained hyperacetylation of tumor cells had been achieved as intended. However, a high rate of thrombocytopenia resulted in several delays and dose reductions. This has prompted investigation of a schedule administering panobinostat on weekly for two weeks in a three week cycle, with the intention of reducing the incidence and depth of thrombocytopenia with only a moderate reduction in

dose intensity.

A phase I dose-escalation trial of oral panobinostat given on three days of each week over a 28-day cycle on weekly for advanced solid tumors or NHL enrolled 10 CTCL patients, with two achieving CR, four PR, two SD, and two patients PD. Two patients actually achieved CR several weeks after treatment cessation. The MTD was 20 mg, with three DLTs seen in total; diarrhea, transient thrombocytopenia and fatigue. The incidence of Grade  $\geq 2$  QTf prolongation throughout was only 6.3%.<sup>15</sup> Pharmacodynamic analysis of histones from normal PBMCs reveal increased acetylation at doses of 20 mg and above, and for patients receiving doses of 20 mg and 30mg, hyperacetylation was seen beyond 72 hours in 50% of the patients following their last dose. Given the accessibility of tumor cells, correlative skin biopsies were taken from six patients at several timepoints within the first 24 hours of drug administration. Gene expression profiling of these biopsies demonstrated that of genes whose expression as shown to be altered, the majority were down-regulated, while 23 genes were commonly up- or down-regulated in all patients tested. These genes had wide ranging functions important in cancer cell growth, including cell cycling, proliferation, angiogenesis and immune regulation.<sup>16</sup>

Based on the results of the above study, a phase II study of oral panobinostat MWF is enrolling patients with refractory CTCL. The modified Severity Weighted Assessment Tool (mSWAT) was used to monitor skin response and CT to monitor systemic disease. Currently ninety five patients have been treated, and the 62 patients who had previously received bexarotene, there have been 11 confirmed skin responses, including two complete skin responses. In the bexarotene naïve group four out of 33 patients have confirmed skin and CT CR's. Most common adverse events include

diarrhoea, thrombocytopenia and fatigue. Notably, despite intensive ECG monitoring, only two patients have had a QTcF  $>480$  ms, and four had a QTcF increase of  $>60$  ms from their baseline.<sup>17</sup> Comparison of the results of panobinostat with other HDACi in advanced clinical development for this disease, such as vorinostat and romidepsin, is problematic given the lack of consensus on the definition of response and disease progression in CTCL. The different endpoints in studies used for cutaneous manifestations of CTCL, such as the mSWAT and the modified Physicians Global Assessment [PGA] tool) means meaningful comparison is near impossible given that the interpretation of key efficacy data, including progression-free survival varies widely.<sup>18-20</sup>

An ongoing two-arm phase IA/II study, has enrolled 77 patients thus far, receiving either weekly MWF or alternate weekly MWF treatment, with doses ranging from 20 mg - 80 mg. The arms are further sub-divided, allowing enrolling of patients with acute leukaemias and myeloid neoplasms, as well as enrolling patients with myeloma, HL and NHL. At doses  $\geq 20$  mg, increased histone acetylation in both PBMCs and bone marrow core biopsies was seen. No responses were observed in patients treated at doses  $<40$  mg, nor at any dose level in those treated alternate weekly. However, anti-leukemic activity was evident in seven of 12 evaluable patients at dose levels  $\geq 40$  mg, with two CR, one CRi and two patients with prolonged SD. Moreover, of the other three responders, one patient achieved CR several weeks after discontinuation of treatment, another had peripheral blood count recovery following the end of treatment and one patient had SD for 1.5 treatment cycles and 10% blasts in bone marrow 10 months after the end of treatment. Clinical activity in AML appears to be both dose- and schedule-dependent, although whether responses are purely related to the duration and intensity of acetylation is

not yet known.

Impressive interim results were also seen in this study in HL, where response was assessed by both <sup>18</sup>FDG-PET (PET) and CT. A total of 12 out of 18 patients demonstrated a metabolic PR, and eight an anatomical PR. Furthermore, seven of nine patients had resolution of their constitutional symptoms, while two patients have been on therapy for more than 13 months. On the basis of these results an international multi-centre trial for relapsed or refractory HL has been initiated, and is already accruing at a starting dose of 40 mg three times per week.<sup>21</sup>

Results from phase II study of oral panobinostat in patients with MM have also been reported. Of 38 patients enrolled, only one PR was seen, with a further four patients having SD. This response rate was somewhat lower than expected given the pre-clinical data available, and may relate to the comparatively low dose of 20 mg three weekly.<sup>22</sup> The combination of oral panobinostat (10 or 20 mg three weekly continuously) with the bortezomib in patients with relapsed myeloma is under investigation. Thus far, nine clinical responses among 18 evaluable patients have been seen, including one CR, three very good PR and five PR). Moreover, three of the PR occurred in patients who had previously failed to respond to bortezomib.<sup>23</sup> The multitude of recent novel biological agents approved in myeloma ensures that future studies of panobinostat in combination with bortezomib, thalidomide, lenalidomide as well as traditional chemotherapeutic agents such as melphalan and steroids are guaranteed.

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#### Adverse effects

Both formulations of panobinostat appear to be generally well tolerated with a good safety profile. Similar to reports of other DACi, the most common toxicities appear to be dose

related rather than related to the pharmacokinetic profile of the drug and include constitutional symptoms, particularly fatigue, gastrointestinal symptoms and myelosuppression.<sup>17,21</sup> Fatigue and transient thrombocytopenia has been dose-limiting in several studies but generally is rapidly reversible on drug cessation and subsequent dose reductions. In general, neutropenia and anemia are less common among panobinostat-treated patients than thrombocytopenia. Gastrointestinal AEs, including nausea, diarrhea and vomiting are also common but are generally of Grade 1/2 severity and manageable in most patients. Initial concerns surrounding clinically significant QTc interval prolongation, an apparent class effect of the DACi, appear to have been answered with the use of alternative drug schedules.<sup>13</sup>

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#### Conclusion and future prospects

Early results of phase I and II studies of panobinostat suggest efficacy against a range of hematologic malignancies, with particular promise shown in CTCL, AML and HL. Despite recent progress in these diseases, large numbers of patients remain in need of novel and effective treatments for relapsed and refractory disease. Early combination studies have already begun, and given the acceptable toxicity profile and synergy with multiple anti-cancer agents, it seems likely phase II followed by phase III combination studies will follow in patients with poor prognosis disease in the up-front setting. Other areas of potential interest for panobinostat include maintenance therapy, its use as a potential radio-sensitizing agent, combination with other immunomodulatory drugs, and a greater knowledge of the influence of the bone marrow and lymph node microenvironment on drug response.

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