Kevetrin™ targets both MDM2-p53 and Rb-E2F pathways in tumor suppression

Ashok Kumar, Sylvia A. Holden, Karima Chafai-Fadelo, Siya Ram, Krishna Menon
Cellceutix Pharmaceuticals (a publicly traded company, stock ticker: CTIX), Beverly, MA, USA

ABSTRACT

Our studies showed that Kevetrin™, a compound currently under development, has potent antitumor activity in several wild type and mutant p53 human tumor xenografts, e.g., A549, MIA PaCa-2, PC-3, MDA-MB-231, H226, H460, HT1080, HCT116, SH-SY5Y, and LoVo. To investigate the mechanism of action of its antitumor activity in different xenograft models, we assessed Kevetrin’s effects on apoptosis and cell cycle progression. HDAC inhibitors regulate mediator cell death through several pathways: HDAC1 and HDAC2 are deregulated in multiple cancers and are the main deacetylases involved in human cancer types. Downregulation of HDAC2 has been shown to inhibit tumor growth. Kevetrin downregulated HDAC2 and HDAC6 in many mutant p53 and wild-type cancer cell lines. Kevetrin strongly induced apoptosis in multiple tumor cell lines characterized by activation of PARP. Kevetrin upregulated pro-apoptotic proteins, including Bax, which was observed in A549 and 8226 cells that were treated with Kevetrin and MDA-MB-231 and MIA PaCa-2 cell lines. Kevetrin induced downregulation of anti-apoptotic protein p53-1 and HDAC6-Hsp90 axis. Kevetrin enhanced activation via dephosphorylation of cell cycle-dependent kinase inhibitor p21 in many wild type cells, mutant and null cell lines. In wild type p53 human lung carcinoma (A549), Kevetrin showed a concentration dependent increase in activation of p21 by Western blot analysis. Kevetrin induced apoptosis in a transcriptional independent way by altering the E2F1 gene expression of MDA-MB-231. Immunoprecipitation and Western blot analysis were used to determine the interaction of Kevetrin with E2F1. E2F1 is known to be involved in DNA damage responses, chromatin remodeling and cell cycle progression. We have also demonstrated that Kevetrin in non-genotoxic DNA damaging drugs result in rapid phosphorylation of HDAC at Ser 166 by Western blot analysis, however, Kevetrin did not induce phosphorylation of HDAC3. Kevetrin was well tolerated in GLP safety pharmacology and toxicity studies, and has shown unique mechanism of action with potential antitumor activity while being non-genotoxic; therefore, we have expanded an IND Application for a Phase I clinical trial.

CONCLUSION

Due to the complexity of pro-apoptotic and antiangiogenic pathways with multiple players involved in molecular signaling network, targeting only one anti-apoptotic factor may not result in antitumor activity. Kevetrin acts via multiple targets to produce efficacy in a wide range of xenograft tumor models. Kevetrin has potent antitumor activity in several wild type and mutant p53 human tumor xenografts, e.g., A549, MIA PaCa-2, PC-3, H226, H460, HT1080, HCT116, SH-SY5Y, and LoVo. Kevetrin modulated the expression of HDAC2 and HDAC6. HDAC inhibitor downregulation has been shown to modulate cell death through several pathways, including cell cycle arrest, induction of apoptosis, antiangiogenesis and effects mediated protein expression. Kevetrin acts through alteration of a chromatin modification and gene expression, modulation of the expression and function of the HDAC-chromatin axis and modulation of transcription factor expression. Kevetrin enhanced phosphorylation by depleting mutant p53. In many mutant p53 tumors, gain of function and hyperstability of p53 drives tumor formation, invasion and metastasis. Kevetrin downregulated mutant p53 (MDM2 mediated) expression in response to Kevetrin. Kevetrin strongly induced apoptosis in multiple tumor cell lines characterized by activation of PARP. Kevetrin upregulated pro-apoptotic proteins, including Bax, which was observed in A549 and 8226 cells that were treated with Kevetrin and MDA-MB-231 and MIA PaCa-2 cell lines. Kevetrin induced downregulation of anti-apoptotic protein p53-1 and HDAC6-Hsp90 axis. Kevetrin enhanced activation via dephosphorylation of cell cycle-dependent kinase inhibitor p21 in many wild type cells, mutant and null cell lines. In wild type p53 human lung carcinoma (A549), Kevetrin showed a concentration dependent increase in activation of p21 by Western blot analysis. Kevetrin induced apoptosis in a transcriptional independent way by altering the E2F1 gene expression of MDA-MB-231. Immunoprecipitation and Western blot analysis were used to determine the interaction of Kevetrin with E2F1. E2F1 is known to be involved in DNA damage responses, chromatin remodeling and cell cycle progression.

Kevetrin’s multimechanistic activity is in many wild and mutant p53 human tumor xenografts, e.g., A549, MIA PaCa-2, PC-3, H226, H460, HT1080, HCT116, SH-SY5Y, and LoVo. Kevetrin modulated the expression of HDAC2 and HDAC6. HDAC inhibitor downregulation has been shown to modulate cell death through several pathways, including cell cycle arrest, induction of apoptosis, antiangiogenesis and effects mediated protein expression. Kevetrin acts through alteration of a chromatin modification and gene expression, modulation of the expression and function of the HDAC-chromatin axis and modulation of transcription factor expression. Kevetrin induced phosphorylation by depleting mutant p53. In many mutant p53 tumors, gain of function and hyperstability of p53 drives tumor formation, invasion and metastasis. Kevetrin downregulated mutant p53 (MDM2 mediated) expression in response to Kevetrin. Kevetrin strongly induced apoptosis in multiple tumor cell lines characterized by activation of PARP. Kevetrin upregulated pro-apoptotic proteins, including Bax, which was observed in A549 and 8226 cells that were treated with Kevetrin and MDA-MB-231 and MIA PaCa-2 cell lines. Kevetrin induced downregulation of anti-apoptotic protein p53-1 and HDAC6-Hsp90 axis. Kevetrin enhanced activation via dephosphorylation of cell cycle-dependent kinase inhibitor p21 in many wild type cells, mutant and null cell lines. In wild type p53 human lung carcinoma (A549), Kevetrin showed a concentration dependent increase in activation of p21 by Western blot analysis. Kevetrin induced apoptosis in a transcriptional independent way by altering the E2F1 gene expression of MDA-MB-231. Immunoprecipitation and Western blot analysis were used to determine the interaction of Kevetrin with E2F1. E2F1 is known to be involved in DNA damage responses, chromatin remodeling and cell cycle progression. We have also demonstrated that Kevetrin in non-genotoxic DNA damaging drugs result in rapid phosphorylation of HDAC at Ser 166 by Western blot analysis, however, Kevetrin did not induce phosphorylation of HDAC3. Kevetrin was well tolerated in GLP safety pharmacology and toxicity studies, and has shown unique mechanism of action with potential antitumor activity while being non-genotoxic; therefore, we have expanded an IND Application for a Phase I clinical trial.

Kevetrin has potent antitumor activity in several wild type and mutant p53 human tumor xenografts, e.g., A549, MIA PaCa-2, PC-3, H226, H460, HT1080, HCT116, SH-SY5Y, and LoVo. Kevetrin modulated the expression of HDAC2 and HDAC6. HDAC inhibitor downregulation has been shown to modulate cell death through several pathways, including cell cycle arrest, induction of apoptosis, antiangiogenesis and effects mediated protein expression. Kevetrin acts through alteration of a chromatin modification and gene expression, modulation of the expression and function of the HDAC-chromatin axis and modulation of transcription factor expression. Kevetrin induced phosphorylation by depleting mutant p53. In many mutant p53 tumors, gain of function and hyperstability of p53 drives tumor formation, invasion and metastasis. Kevetrin downregulated mutant p53 (MDM2 mediated) expression in response to Kevetrin. Kevetrin strongly induced apoptosis in multiple tumor cell lines characterized by activation of PARP. Kevetrin upregulated pro-apoptotic proteins, including Bax, which was observed in A549 and 8226 cells that were treated with Kevetrin and MDA-MB-231 and MIA PaCa-2 cell lines. Kevetrin induced downregulation of anti-apoptotic protein p53-1 and HDAC6-Hsp90 axis. Kevetrin enhanced activation via dephosphorylation of cell cycle-dependent kinase inhibitor p21 in many wild type cells, mutant and null cell lines. In wild type p53 human lung carcinoma (A549), Kevetrin showed a concentration dependent increase in activation of p21 by Western blot analysis. Kevetrin induced apoptosis in a transcriptional independent way by altering the E2F1 gene expression of MDA-MB-231. Immunoprecipitation and Western blot analysis were used to determine the interaction of Kevetrin with E2F1. E2F1 is known to be involved in DNA damage responses, chromatin remodeling and cell cycle progression. We have also demonstrated that Kevetrin in non-genotoxic DNA damaging drugs result in rapid phosphorylation of HDAC at Ser 166 by Western blot analysis, however, Kevetrin did not induce phosphorylation of HDAC3. Kevetrin was well tolerated in GLP safety pharmacology and toxicity studies, and has shown unique mechanism of action with potential antitumor activity while being non-genotoxic; therefore, we have expanded an IND Application for a Phase I clinical trial.

Kevetrin’s multimechanistic activity is in many wild and mutant p53 human tumor xenografts, e.g., A549, MIA PaCa-2, PC-3, H226, H460, HT1080, HCT116, SH-SY5Y, and LoVo. Kevetrin modulated the expression of HDAC2 and HDAC6. HDAC inhibitor downregulation has been shown to modulate cell death through several pathways, including cell cycle arrest, induction of apoptosis, antiangiogenesis and effects mediated protein expression. Kevetrin acts through alteration of a chromatin modification and gene expression, modulation of the expression and function of the HDAC-chromatin axis and modulation of transcription factor expression. Kevetrin induced phosphorylation by depleting mutant p53. In many mutant p53 tumors, gain of function and hyperstability of p53 drives tumor formation, invasion and metastasis. Kevetrin downregulated mutant p53 (MDM2 mediated) expression in response to Kevetrin. Kevetrin strongly induced apoptosis in multiple tumor cell lines characterized by activation of PARP. Kevetrin upregulated pro-apoptotic proteins, including Bax, which was observed in A549 and 8226 cells that were treated with Kevetrin and MDA-MB-231 and MIA PaCa-2 cell lines. Kevetrin induced downregulation of anti-apoptotic protein p53-1 and HDAC6-Hsp90 axis. Kevetrin enhanced activation via dephosphorylation of cell cycle-dependent kinase inhibitor p21 in many wild type cells, mutant and null cell lines. In wild type p53 human lung carcinoma (A549), Kevetrin showed a concentration dependent increase in activation of p21 by Western blot analysis. Kevetrin induced apoptosis in a transcriptional independent way by altering the E2F1 gene expression of MDA-MB-231. Immunoprecipitation and Western blot analysis were used to determine the interaction of Kevetrin with E2F1. E2F1 is known to be involved in DNA damage responses, chromatin remodeling and cell cycle progression. We have also demonstrated that Kevetrin in non-genotoxic DNA damaging drugs result in rapid phosphorylation of HDAC at Ser 166 by Western blot analysis, however, Kevetrin did not induce phosphorylation of HDAC3. Kevetrin was well tolerated in GLP safety pharmacology and toxicity studies, and has shown unique mechanism of action with potential antitumor activity while being non-genotoxic; therefore, we have expanded an IND Application for a Phase I clinical trial.