Abstract 4711

Broad Anti-Tumor Activity of a Novel BET Bromodomain Inhibitor

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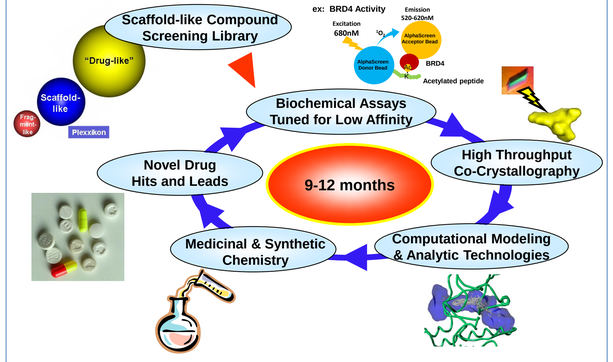
Abstract

Inhibitors against the bromodomain and extra terminal domain (BET) family of proteins have been pursued as promising oncology agents based on growing understanding of epigenetic control of disease processes. Through scaffold-based and crystallography-guided drug design, we discovered PLX51107, a potent and selective small molecule inhibitor of the BET family bromodomains. PLX51107 is structurally unrelated to the benzodiazepines such as JQ1, I-BET762, and OTX015 and other published BET inhibitors. PLX51107 exhibits low nanomolar potency in blocking interactions mediated by the four BET family proteins BRD2, BRD3, BRD4, and BRDT. Pharmacologic inhibition of BET proteins by PLX51107 suppresses the transcription of genes essential for tumor growth and survival and leads to selective killing of cancer cell lines across a broad range of hematologic malignancies (e.g. leukemia, lymphoma and multiple myeloma). A subset of solid tumors (e.g. melanoma) is also sensitive to growth inhibition by the BET inhibitor PLX51107. Novel biomarkers in these diseases have been identified. PLX51107 is well tolerated and has sufficient potency and oral bioavailability to demonstrate *in vivo* efficacy in animal models of a variety of tumor types, representing both hematologic and solid tumors of diverse genetic backgrounds. In combination studies, PLX51107 showed potential to improve efficacy (response rates and duration of response) of other anticancer treatments without increasing toxicity. These results support further development of PLX51107 as an epigenetic-based therapy for a variety of cancer indications.

Plexxikon's Discovery Platform

Figure 1. Scaffold-Based Drug Discovery



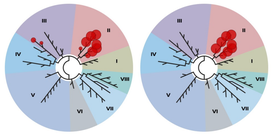
Results

Figure 2. Discovery of PLX51107 as a Pan-BET Inhibitor and Comparison with OTX-015

A.

in vitro Assay Data (IC_{50} nM)		
Assay	PLX51107	OTX-015
BRD2 (BD1,BD2) binding	52	73
BRD4 (BD1,BD2) binding	23	48
MV-4-11 Proliferation	63	52
MYC reporter assay	134	165

B.



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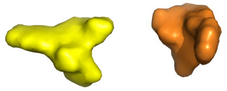

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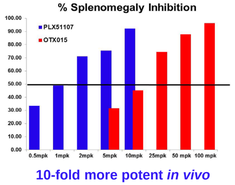


10-fold more potent *in vivo*

A. Potency of PLX51107 and OTX-015 in biochemical and cell-based assays.
B. Selectivity profile of PLX51107 and OTX-015 showing percentage inhibition using BROMO scan technology (DiscoveRx, Fremont CA). Image generated using TREEspot™ Software Tool and reprinted with permission from KINOMEscan®, a division of DiscoveRx Corporation, © DISCOVERX CORPORATION 2010.
C. Surface diagram showing PLX51107 and OTX-015 accessing the acetyl binding pocket of BRD4-BD1.
D. *In vivo* activity of PLX51107 and OTX-015 in a BaF3 splenomegaly model.

Figure 3. PLX51107 Inhibits Melanoma Cell Proliferation *in vitro*

	Cell Line	PLX51107 IC_{50} (μM)
BRAF V600E	MALME-3M	0.405
	SK-MEL-5	0.716
	A2058	1.91
	SK-MEL-28	2.2
	SK-MEL-3	3.77
	UACC-257	4.17
	A375	4.46
NRAS Q61 mutant	IPC-298	0.884
	SK-MEL-30	0.892
	SK-MEL-2	6.13

Figure 4. PLX51107 + MEK Inhibitor Combinations Exert Synergistic Apoptosis in NRAS Mutant Melanoma Cells

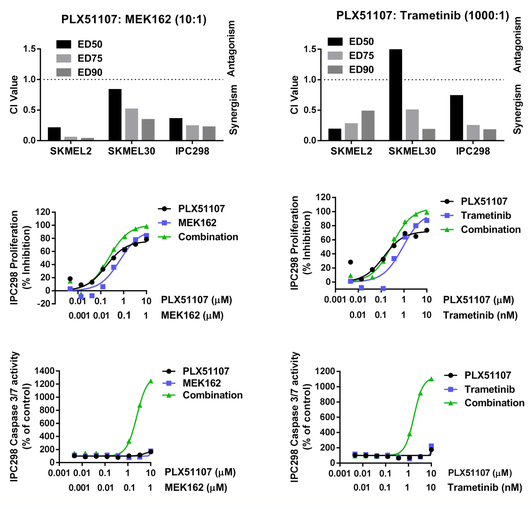


Figure 5. PLX51107 + MEK Inhibitor Combinations Suppress IPC298 Growth *in vivo*

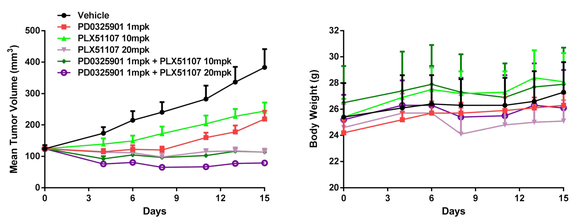
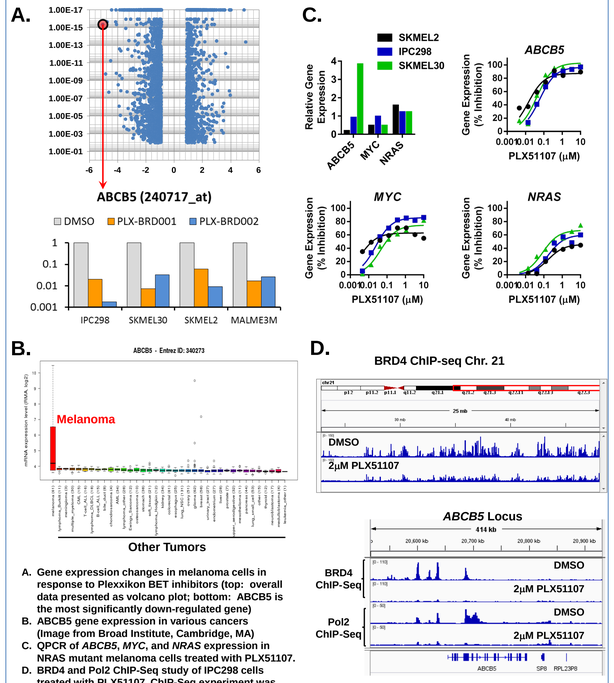


Figure 6. Microarray and ChIP-Seq Studies Identify BET Inhibitor-Regulated Genes in Melanoma Cells



A. Gene expression changes in melanoma cells in response to Plexxikon BET inhibitors (top: overall data presented as volcano plot; bottom: ABCB5 is the most significantly down-regulated gene)
B. ABCB5 gene expression in various cancers (Image from Broad Institute, Cambridge, MA)
C. QPCR of *ABCB5*, *MYC*, and *NRAS* expression in NRAS mutant melanoma cells treated with PLX51107.
D. BRD4 and Pol2 ChIP-Seq study of IPC298 cells treated with PLX51107. ChIP-Seq experiment was performed by Active Motif (Carlsbad, CA)

Conclusion

1. PLX51107 is a novel BET bromodomain inhibitor that is currently undergoing clinical study (NCT02683395).
2. PLX51107, as a single agent and in combination with MEK inhibitor, inhibits melanoma cell growth *in vitro* and *in vivo*.
3. Microarray and ChIP-Seq studies identify direct target genes of PLX51107 including the known oncogenes *MYC* and *NRAS*, and the melanoma-initiating cell marker *ABCB5* in melanoma cells.