



Category: Experimental And Molecular Therapeutics 21

Session Title: Conjugates And Other Targeted Approaches

#3654 ON 01910.Na, a novel clinical grade PLK-1 inhibitor, selectively induces apoptosis in human B-cell chronic lymphocytic leukemia (B-CLL). Colby M. Chapman, Patricia Perez-Galan, Adrian Wiestner. NHLBI, NIH, Bethesda, MD.

Polo-like kinase 1 (PLK1) is a Ser/Thr kinase that plays a critical role in cell cycle progression and recently has been shown to be an attractive target for cancer therapy. Overexpression of PLK1 is detected in several cancers and often correlates with more aggressive disease. ON 01910.Na, a multi-kinase inhibitor with preferential activity against PLK1, has anti-tumor activity in solid tumor models and is currently undergoing clinical testing. B-CLL is a lymphoid neoplasm with deregulated apoptosis that is incurable with chemotherapy. Only a few agents display activity in refractory CLL. We found high PLK1 mRNA expression in CLL B-cells compared to normal B-cells suggesting that the PLK pathway could also be a therapeutic target in CLL. This study aimed to determine activity of ON 01910.Na in CLL and investigate the mechanism of action. After 48 hours of *in vitro* exposure we measured the effect of ON 01910.Na on leukemic cells and primary lymphocytes by flow cytometry using AnnexinV-PE. We co-stained with CD3-APC, and CD19-FITC to assess the effect on B and T cells separately. ON 01910.Na induced apoptosis of the leukemic cells in all CLL samples tested (n=15). At concentrations that induced apoptosis of the leukemic cells, ON 01910.Na did not affect the T-cells in the same PBMC sample. Also B and T cells from normal donors were unaffected. Twelve CLL samples demonstrated high sensitivity towards ON 01910.Na with mean B-cell IC₅₀ 0.78 μ M; three samples were slightly less sensitive with mean B-cell IC₅₀ 3.8 μ M. In contrast, the IC₅₀ of T-cells was >8 μ M in all samples. ON 01910.Na was also effective against leukemic cells carrying p53 deletions (n=2). Interestingly, leukemic cells of the more progressive IgVH unmutated CLL subtype (n=6) were more sensitive to ON 01910.Na (mean B-cell IC₅₀ of 0.85 μ M) than cells of IgVH mutated subtype (n= 7, mean B-cell IC₅₀ 2.0 μ M). To investigate the mechanism of ON 01910.Na against B-CLL cells, we determined whether PLK1 was inhibited in the leukemic cells. CLL cells exposed to 1 μ M of ON 01910.Na for 14 hours were lysed, and PLK1 was immunoprecipitated. Using a PLK1 activity assay (Cyclex), we found that ON 01910.Na treatment reduced PLK1 activity >50% in the leukemic cells. ON 01910.Na activated the mitochondrial apoptosis pathway as shown by flow cytometry using conformation specific antibodies to detect activation of Bax and Bak, leading to mitochondrial depolarization (measured by mitotracker) and caspase-3 activation. Overexpression of the antiapoptotic protein Mcl-1 is a central mechanism of CLL cell survival. ON 01910.Na decreased Mcl-1 protein expression in leukemic cells and this preceded the onset of apoptosis, suggesting that a reduction of Mcl-1 expression could play an important role in the antileukemic effect of ON 01910.Na. These results support the development of ON 01910.Na in CLL, and a clinical trial in this indication is being developed.

Citation Format

Chapman C M, Perez-Galan P, Wiestner A. ON 01910.Na, a novel clinical grade PLK-1 inhibitor, selectively induces apoptosis in human B-cell chronic lymphocytic leukemia (B-CLL) [abstract]. In: Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; 2009 Apr 18-22; Denver, CO. Philadelphia (PA): AACR; 2009. Abstract nr 3654.

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Category: Experimental And Molecular Therapeutics 24

Session Title: Kinase Inhibitors 3

#3717 Identification of a dual JAK-2/BCR-ABL kinase inhibitor for treatment of myeloproliferative disorders. Shashidhar S. Jatiani¹, Stephen C. Cosenza², Venkat R. Pallela², Ji Hee Ha², Stacey J. Baker², M V Ramana Reddy², E. Premkumar Reddy¹. ¹Fels Institute for Cancer Reseach, Temple University School of Medicine, Philadelphia, PA; ²Temple University School of Medicine, Philadelphia, PA.

Myeloproliferative disorders (MPD) are clonal malignancies characterized by overproduction of one or more hematopoietic lineages with relatively normal differentiation. The molecular pathogenesis of several MPDs has been well characterized, and is frequently attributable to mutations that result in constitutive activation of a protein tyrosine kinase. The classic MPDs are subdivided into chronic myeloid leukemia (CML i.e. BCR-ABL⁺) and the BCR-ABL⁻ classic MPDs, i.e. polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Imatinib, which is an inhibitor of the BCR-ABL tyrosine kinase, has been a remarkable success for the treatment of CML. However, a significant proportion of patients chronically treated with imatinib develop resistance due to acquisition of mutations in the kinase domain of BCR-ABL. A novel gain-of-function mutation in the JAK2 tyrosine kinase (*JAK2V617F*) has been observed in about 95% of patients with PV and 50% of those with either ET or PMF. This mutation has also been found in patients with non-classic MPDs such as refractory anemia with ringed sideroblasts associated with thrombocytosis (RARS-T), chronic neutrophilic leukemia (CNL), atypical CML and chronic myelomonocytic leukemia (CMML) at incidences of 50%, 20%, 20% and 3%, respectively. *JAK2V617F* has been found to confer erythropoietin-independent growth of the mutant cells *in vitro* due to deregulation of signaling pathways downstream of JAK2. These findings have opened new avenues for the diagnosis and classification of patients with these disorders, and identify a new molecular target for drug discovery. In our quest to develop targeted therapies for MPD, we screened a compound library of molecules and identified an α -stryl benzyl sulfone, ON044580 that exhibits potent JAK2 inhibitory activity. ON 044580 is a substrate-competitive inhibitor of JAK2 and is unaffected by physiological levels of ATP. ON044580 interferes with IL-3 mediated proliferation pathways that signal via JAK2 to inhibit the growth and survival of Ba/F3-*JAK2V617F* cells. Growth of two patient cell lines, HEL and SET-2, which express the activating *JAK2V617F* mutation is also inhibited by ON044580. ON 044580 is also a potent inhibitor of the tyrosine kinase activity of BCR-ABL purified from mammalian cells. Further, ON 044580 inhibits the phosphorylation of BCR-ABL and Stat5 in cultured CML cells. Finally, not only does ON044580 induce apoptosis in cells expressing imatinib-sensitive and imatinib-resistant forms of BCR-ABL, it does so in primary cells from CML patients. Taken together, our results suggest that ON 044580 is a dual JAK2/BCR-ABL kinase inhibitor with therapeutic potential to treat *JAK2V617F* positive MPDs and CML.

Citation Format

Jatiani S S, Cosenza S C, Pallela V R, Ha J, Baker S J, Reddy M R, Reddy E P. Identification of a dual JAK-2/BCR-ABL kinase inhibitor for treatment of myeloproliferative disorders [abstract]. In: Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; 2009 Apr 18-22; Denver, CO. Philadelphia (PA): AACR; 2009. Abstract nr 3717.

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Category: Experimental And Molecular Therapeutics 27

Session Title: Mechanisms Of Drugs Targeting Cell Cycle Controls

#3866 Modulation of Chk1, Chk2, p-Chk2 dimer and p-ATM in cancer cells treated with ON 01910.Na, a clinical stage mitotic inhibitor. [Irina Oussenko](#)¹, James F. Holland¹, E. Premkumar Reddy², Takao Ohnuma¹. ¹Mount Sinai School of Medicine, New York, NY; ²Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA.

Introduction: The benzyl styryl sulfone analog ON 01910.Na (here abbreviated as 1910) is a novel anticancer agent that inhibits mitotic progression and induces apoptosis in a number of cancer cells in culture. The compound is currently in Phase 1 and Phase 2 trials. Available data show that the drug produces 3 major abnormalities in tumor cells: (a) aberrant cell division including irregular chromosomal segregation and cytokinesis; (b) G2/M arrest and apoptosis in many tumor cells; and (c) decreased expression of Cdc25C.

Materials and Methods: We assessed all DNA damage checkpoints throughout the cell cycle (Niida-H et al, *Mutagenesis* 2006;21:3-9) and effects on signaling molecules upstream of Cdc25C following treatment with 1910. Camptothecin and doxorubicin were used as positive controls. DU145 prostate cancer cells were incubated with 1910 or camptothecin or doxorubicin for 4, 16 and 24 hr, cell lysate was resolved on 8% SDS-PAGE (7% for phospho (p)-ATM) and subjected to Western blot analysis for Chk1, Chk2, ATM and their phosphorylated forms.

Results and Conclusions: As reported in literature, incubation with doxorubicin resulted in activation of Chk1 through phosphorylation, whereas no p-Chk1 was detected in 1910 treated cells. Similarly, incubation with camptothecin resulted in over-expression of Chk2 and p-Chk2, whereas these changes were not evident after incubation with 1910. Instead, 1910 exposure resulted in concentration-dependent development of slower migrating extra bands in the Chk1, Chk2, p-Chk2 dimer and p-ATM westerns. The extra bands were reduced in intensity when caffeine was added to the incubation mixture. These aberrant forms of regulatory proteins may represent 1910-bound to phosphorylated and non-phosphorylated proteins or other post-translational modifications and are being investigated. These changes may result in dephosphorylation of Cdc25C and activation of Cdc2, resulting in blockage of cell cycle progression in the G2/M phase. Cells cannot be arrested in mitosis indefinitely, because they will either die or exit mitosis. 1910 was originally considered as a direct Plk1 inhibitor, but our data suggest indirect effects on DNA damage checkpoint pathways and may be a part of its multi-targeted mode of action.

Citation Format

Oussenko I, Holland J, Reddy E, Ohnuma T. Modulation of Chk1, Chk2, p-Chk2 dimer and p-ATM in cancer cells treated with ON 01910.Na, a clinical stage mitotic inhibitor [abstract]. In: Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; 2009 Apr 18-22; Denver, CO. Philadelphia (PA): AACR; 2009. Abstract nr 3866.

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Category: Experimental And Molecular Therapeutics 24

Session Title: Kinase Inhibitors 3

#3718 Novel Bcr-abl kinase inhibitors active against T315I imatinib resistant mutation: (Z)-3-benzylideneindolinones: structure activity relationship and biological evaluation. M V Ramana Reddy¹, Stephen C. Cosenza¹, Vinay K. Billa¹, Venkat R. Pallela¹, Muralidhar R. Mallireddigari², E Premkumar Reddy¹. ¹Fels Institute for Cancer Research, Philadelphia, PA; ²Onconova Therapeutics Inc, Newtown, PA.

BCR-ABL, a transforming tyrosine kinase active in >90% of CML cases, and it has been possible to inhibit of BCR-ABL kinase in leukemic cells without adversely affecting the normal cell population. However, a significant proportion of patients treated chronically with imatinib (Gleevec) develop drug resistance because of the acquisition of mutations in kinase domain of BCR-ABL. While new compounds like dasatinib and nilotinib are active against most of these mutant enzymes, one mutation, the T315I, has been refractory to most new inhibitors.

Here, we describe new small molecule inhibitors of both wild type and T315I mutant BCR-ABL kinases. These compounds belong to indolinone family, which has not previously been used in bcr-abl inhibition, and inhibit BCR-ABL and T315I mutation variant in a non-ATP competitive fashion. These compounds are water soluble with suitable pharmacokinetic profile and are. These compounds exhibit potent activity against K562 cells and Baf3 cells carrying mutant bcr-abl genes. One of these compounds, ON 88210 showed inhibition of BCR-ABL (WT and T315I forms) auto-phosphorylation and STAT-5 phosphorylation in cultured cells. The mechanism of action of ON 88210 seems to be different from imatinib (Gleevec) as this compound does not affect the phosphorylation of CRK-L. Further development and differentiation of these compounds from imatinib will be described.

Citation Format

Reddy M, Cosenza S C, Billa V K, Pallela V R, Mallireddigari M R, Reddy E. Novel Bcr-abl kinase inhibitors active against T315I imatinib resistant mutation: (Z)-3-benzylideneindolinones: structure activity relationship and biological evaluation [abstract]. In: Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; 2009 Apr 18-22; Denver, CO. Philadelphia (PA): AACR; 2009. Abstract nr 3718.

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Category: Experimental And Molecular Therapeutics 35

Session Title: Novel Agents 3

#4702 Pathway-based comparison approach for the identification of responders to the mitotic modulator ON 01910.Na in head and neck cancer (HNC). Aik Choon Tan, Ryan Anderson, Barbara Frederick, David Raben, Antonio Jimeno. University of Colorado Denver, Aurora, CO.

Purpose: This work is aimed at determining the efficacy of the novel clinical stage mitotic inhibitor ON 01910.Na in HNC, and to elucidate gene expression-based predictors of efficacy, in comparison with approved therapeutic agents. We used a rational pathway-driven comparison of global gene expression (Affymetrix U133) to correlate changes in expression with efficacy. **Methods:** We used 29 HNC cell lines and tested ON 01910.Na and a panel of 10 approved agents (including cetuximab, erlotinib, docetaxel and bortezomib). We then correlated the antitumor efficacy with gene expression profiles of these cell lines, using unbiased gene set expression analysis (GSEA; www.broad.mit.edu/gsea/) of 198 pathways. GSEA permits elucidating modest but coordinated gene expression differences at the pathway level. **Results:** We obtained a mosaic of susceptibilities in the panel. At clinically relevant concentrations (1 μ M) docetaxel, bortezomib and ON 01910.Na significantly inhibited growth in 27, 27, and 25 cell lines respectively, whereas only 9 were inhibited with erlotinib and only two by cetuximab. Expression analysis for ON 01910.Na indicated that at 0.1 μ M, the “cell cycle pathway” was the tenth most expressed gene set in comparing the sensitive vs resistant cell lines, whereas the “p53 pathway” was the most highly expressed in resistant vs sensitive strains. At 1 μ M, the “cell cycle” pathway was the most differentially expressed pathway in comparing sensitive vs resistant cell lines. Of the 98 genes in the “cell cycle” pathway, those acting as “driver” genes included CHEK1, CCNE2, CDC6, CDC7, SMAD4, and CCND2. The analysis of bortezomib showed that the “proteasome” pathway was the fifth most expressed of 198 pathways in sensitive vs resistant. Similar analysis with docetaxel indicated that pathways over-expressing cytoskeleton components were particularly expressed in sensitive strains. **Conclusions:** ON 01910.Na is a highly effective mitotic inhibitor active in a broad panel of HNC cell lines, with potency equivalent or superior to approved agents. Expression analysis revealed that sensitive strains had over-expression of the cell cycle pathway (providing further evidence of mechanisms relevant in the action of ON 01910.Na in HNC). The only four resistant cell lines shared over-expression of the p53 pathway as the leading expression alteration. The “cell cycle” pathway changes were drug dose dependent. Overall this suggests that cells with a de-regulated cell cycle are more sensitive to ON 01910, and that a functional p53 pathway dictates resistance. These results combined with the observations with established therapeutic agents suggest that pathway comparisons provide a rational method for identifying targets for new drugs and biomarkers for personalized therapy. Complete pathway expression profiles for all drugs tested and in vivo data will be presented.

Citation Format

Tan A, Anderson R, Frederick B, Raben D, Jimeno A. Pathway-based comparison approach for the identification of responders to the mitotic modulator ON 01910.Na in head and neck cancer (HNC) [abstract]. In: Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; 2009 Apr 18-22; Denver, CO. Philadelphia (PA): AACR; 2009. Abstract nr 4702.

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