

Molecular characteristics of CTA056, a novel Itk inhibitor which selectively targets malignant T cells and modulates oncomirs

Wenchang Guo, Ruiwu Liu, Yoko Ono, Aihong Ma, Anthony Martinez, Eduardo Sanchez, Yan Wang, Wenzhe Huang, Anisha Mazloom, Jixian Li, Jinying Ning, Emanuel Maverakis, Kit S. Lam, and Hsing-Jien Kung

Supplemental Figure 1

A

Induction of tumor suppressive MiRs and decrease of oncogenic MiRs in Jurkat cells following treatment with CTA056

Tumor suppressive MicroRNAs	Fold change	Relation to cancer	References
hsa-miR-135a*	285.3	Downregulation of JAK2	Navarro et al., 2009
hsa-miR-1224-5p	112.9	Downregulation of TNF α	Niu et al., 2011
hsa-miR-1226*	66.9	Downregulation of mucin1	Jin et al., 2010
hsa-miR-487b	59.4	Downregulated in high risk neuroblastoma	Gattolliat et al., 2011
hsa-miR-520b	57.1	Inhibition of MEKK2 and IL-8	Hu et al., 2011 Zhang et al., 2012
hsa-miR-365*	28.8	Downregulation of BCL2	Nie et al., 2012
hsa-miR-195*	27.0	Downregulation of BCL2	Chen et al., 2011
hsa-miR-601	26.7	Inhibition of NFkB	Ohdaira et al., 2009
hsa-miR-370	26.0	Downregulation of TGF β -RII	Lo et al., 2012
hsa-miR-198	25.9	Inhibition of HGF/c-MET	Tan et al., 2011
hsa-miR-622	14.9	Inhibition of k-RAS	Han et al., 2012
hsa-miR-623	14.1	Upregulated in drug sensitive gastric cancer cells	Belian et al., 2010
hsa-let-7d*	11.5	Downregulation of KRAS	Yu et al., 2011 Jiao et al., 2012
hsa-miR-630	10.7	Upregulated by NGX6	Wang et al., 2010
hsa-miR-150*	7.8	Downregulation of NOTCH3	Ghisi et al., 2011
hsa-miR-1246	5.9	Upregulated by p53	Zhang et al., 2011
hsa-miR-494	4.3	Downregulation of KIT	Kim et al., 2011
hsa-miR-1915	2.5	Inhibition of BCL2	Xu et al., 2011
hsa-miR-874	2.5	Downregulation of PPP1CA	Nohata et al., 2011
hsa-miR-2861	2.2	Downregulation of HDAC5	Li et al., 2009
Oncogenic MicroRNAs	Fold change	Relation to cancer	References
hsa-miR-142-3p	-2.2	Downregulation of cAMP/ PKA and GR α	Lv et al., 2011
hsa-miR-93	-2.2	Downregulation of p53	Yeung et al., 2008
hsa-miR-191*	-2.3	Downregulation of TIMP3	He et al., 2011
hsa-miR-30e*	-2.5	Downregulation of I κ B α	Jiang et al., 2012
hsa-miR-744	-3.3	Upregulation of Cyclin B1	Huang et al., 2012
hsa-miR-15b*	-37.8	Induced by E2F	Myklebust et al., 2011
hsa-miR-450a	-68.5	Upregulated in EMT	Castilla et al., 2011
hsa-miR-378*	-69.7	Mediates cMyc activity, inhibits TOB2	Feng et al., 2011
hsa-miR-421	-76.2	Downregulation of ATM	Hu et al., 2010

B

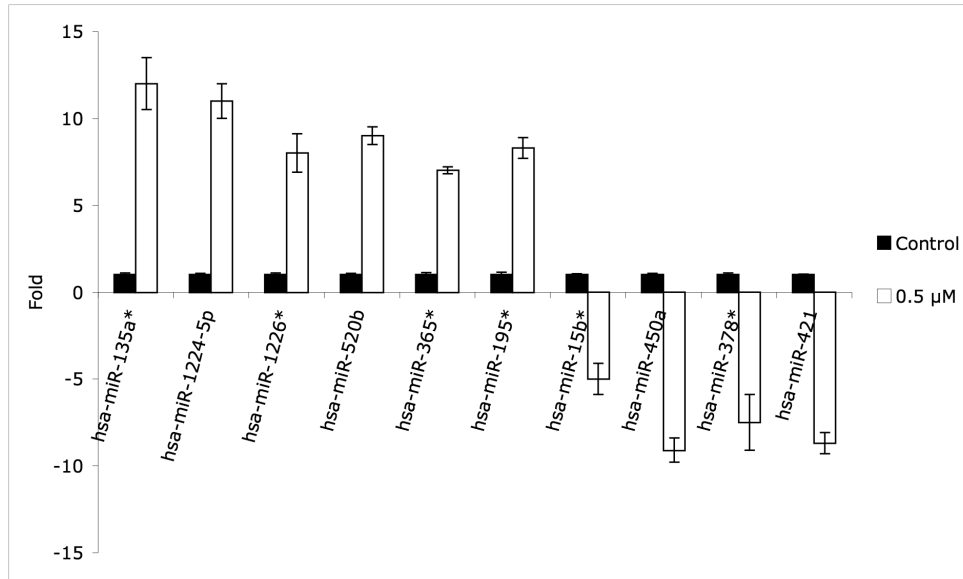


Figure legend: Jurkat cells were treated with 0.5 μ M CTA056 for 8h. RNA was isolated and miR profile was examined using miR array. The fold change between treatment and control was indicated. The function of the miRs was obtained through Pubmed study (A). The miRs with the most significant changes from miR array were further confirmed using real-time PCR (B). For up regulation, fold = treatment/control; for down regulation, fold = control/treatment. Columns, mean; bars, standard deviation; n=3.

References

Belian E, Kurucz R, Treue D, Lage H (2010) Effect of YB-1 on the regulation of micro RNA expression in drug-sensitive and drug-resistant gastric carcinoma cells. *Anticancer Res.* 30(2):629-33.

Chen YQ, Wang XX, Yao XM, Zhang DL, Yang XF, Tian SF, Wang NS (2011) MicroRNA-195 promotes apoptosis in mouse podocytes via enhanced caspase activity driven by BCL2 insufficiency. *Am J Nephrol.* 34(6):549-59.

Feng M, Li Z, Aau M, Wong CH, Yang X, Yu Q (2011) Myc/miR-378/TOB2/cyclin D1 functional module regulates oncogenic transformation. *Oncogene.* 30(19):2242-51.

Castilla MÁ, Moreno-Bueno G, Romero-Pérez L, Van De Vijver K, Biscuola M, López-García MÁ, Prat J, Matías-Guiu X, Cano A, Oliva E, Palacios J (2011) Micro-RNA signature of the epithelial-mesenchymal transition in endometrial carcinosarcoma. *J Pathol.* 223(1):72-80.

Gattolliat CH, Thomas L, Ciafrè SA, Meurice G, Le Teuff G, Job B, Richon C, Combaret V, Dessen P, Valteau-Couanet D, May E, Busson P, Douc-Rasy S, Bénard J (2011) Expression of miR-487b and miR-410 encoded by 14q32.31 locus is a prognostic marker in neuroblastoma. *Br J Cancer.* 105(9):1352-61.

Ghisi M, Corradin A, Basso K, Frasson C, Serafin V, Mukherjee S, Mussolin L, Ruggero K, Bonanno L, Guffanti A, De Bellis G, Gerosa G, Stellin G, D'Agostino DM, Basso G, Bronte V, Indraccolo S, Amadori A, Zanovello P (2011) Modulation of microRNA expression in human T-cell development: targeting of NOTCH3 by miR-150. *Blood.* 117(26):7053-62.

Han Z, Yang Q, Liu B, Wu J, Li Y, Yang C, Jiang Y (2012) MicroRNA-622 functions as a tumor suppressor by targeting K-Ras and enhancing the anticarcinogenic effect of resveratrol. *Carcinogenesis.* 33(1):131-9.

He Y, Cui Y, Wang W, Gu J, Guo S, Ma K, Luo X (2011) Hypomethylation of the hsa-miR-191 locus causes high expression of hsa-mir-191 and promotes the epithelial-to-mesenchymal transition in hepatocellular carcinoma. *Neoplasia.* 13(9):841-53.

Hu H, Du L, Nagabayashi G, Seeger RC, Gatti RA (2010) ATM is down-regulated by N-Myc-regulated microRNA-421. *Proc Natl Acad Sci U S A.* 107(4):1506-11.

Hu N, Zhang J, Cui W, Kong G, Zhang S, Yue L, Bai X, Zhang Z, Zhang W, Zhang X, Ye L (2011) miR-520b regulates migration of breast cancer cells by targeting hepatitis B X-interacting protein and interleukin-8. *J Biol Chem.* 286(15):13714-22.

MOL #79889

Huang V, Place RF, Portnoy V, Wang J, Qi Z, Jia Z, Yu A, Shuman M, Yu J, Li LC (2012) Upregulation of Cyclin B1 by miRNA and its implications in cancer. *Nucleic Acids Res.* 40(4):1695-707.

Jiang L, Lin C, Song L, Wu J, Chen B, Ying Z, Fang L, Yan X, He M, Li J, Li M (2012) MicroRNA-30e* promotes human glioma cell invasiveness in an orthotopic xenotransplantation model by disrupting the NF- κ B/I κ B α negative feedback loop. *J Clin Invest.* 122(1):33-47.

Jiao LR, Frampton AE, Jacob J, Pellegrino L, Krell J, Giamas G, Tsim N, Vlavianos P, Cohen P, Ahmad R, Keller A, Habib NA, Stebbing J, Castellano L (2012) MicroRNAs Targeting Oncogenes Are Down-Regulated in Pancreatic Malignant Transformation from Benign Tumors. *PLoS One.* 7(2):e32068.

Jin C, Rajabi H, Kufe D (2010) miR-1226 targets expression of the mucin 1 oncoprotein and induces cell death. *Int J Oncol.* 37(1):61-9.

Kim WK, Park M, Kim YK, Tae YK, Yang HK, Lee JM, Kim H (2011) MicroRNA-494 downregulates KIT and inhibits gastrointestinal stromal tumor cell proliferation. *Clin Cancer Res.* 17(24):7584-94.

Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, Xu K, Sheng ZF, Zhou HD, Wu XP, Luo XH (2009) A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J Clin Invest.* 119(12):3666-77.

Lo SS, Hung PS, Chen JH, Tu HF, Fang WL, Chen CY, Chen WT, Gong NR, Wu CW (2012) Overexpression of miR-370 and downregulation of its novel target TGF β -RII contribute to the progression of gastric carcinoma. *Oncogene.* 31(2):226-37.

Lv M, Zhang X, Jia H, Li D, Zhang B, Zhang H, Hong M, Jiang T, Jiang Q, Lu J, Huang X, Huang B (2011) An oncogenic role of miR-142-3p in human T-cell acute lymphoblastic leukemia (T-ALL) by targeting glucocorticoid receptor- α and cAMP/PKA pathways. *Leukemia.*

Myklebust MP, Bruland O, Fluge Ø, Skarstein A, Balteskard L, Dahl O (2011) MicroRNA-15b is induced with E2F-controlled genes in HPV-related cancer. *Br J Cancer.* 105(11):1719-25.

Navarro A, Diaz T, Martinez A, Gaya A, Pons A, Gel B, Codony C, Ferrer G, Martinez C, Montserrat E, Monzo M (2009) Regulation of JAK2 by miR-135a: prognostic impact in classic Hodgkin lymphoma. *Blood.* 114(14):2945-51.

MOL #79889

Nie J, Liu L, Zheng W, Chen L, Wu X, Xu Y, Du X, Han W (2012) microRNA-365, down-regulated in colon cancer, inhibits cell cycle progression and promotes apoptosis of colon cancer cells by probably targeting Cyclin D1 and Bcl-2. *Carcinogenesis*. 33(1):220-5.

Niu Y, Mo D, Qin L, Wang C, Li A, Zhao X, Wang X, Xiao S, Wang Q, Xie Y, He Z, Cong P, Chen Y (2011) Lipopolysaccharide-induced miR-1224 negatively regulates tumour necrosis factor- α gene expression by modulating Sp1. *Immunology*. 133(1):8-20.

Nohata N, Hanazawa T, Kikkawa N, Sakurai D, Fujimura L, Chiyomaru T, Kawakami K, Yoshino H, Enokida H, Nakagawa M, Katayama A, Harabuchi Y, Okamoto Y, Seki N (2011) Tumour suppressive microRNA-874 regulates novel cancer networks in maxillary sinus squamous cell carcinoma. *Br J Cancer*. 105(6):833-41.

Ohdaira H, Nakagawa H, Yoshida K (2009) Profiling of molecular pathways regulated by microRNA 601. *Comput Biol Chem*. 33(6):429-33.

Tan S, Li R, Ding K, Lobie PE, Zhu T (2011) miR-198 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the HGF/c-MET pathway. *FEBS Lett*. 585(14):2229-34.

Wang XY, Wu MH, Liu F, Li Y, Li N, Li GY, Shen SR. (2010) Differential miRNA expression and their target genes between NGX6-positive and negative colon cancer cells. *Mol Cell Biochem*. 345(1-2):283-90.

Xu K, Liang X, Cui D, Wu Y, Shi W, Liu J (2011) miR-1915 inhibits Bcl-2 to modulate multidrug resistance by increasing drug-sensitivity in human colorectal carcinoma cells. *Mol Carcinog*.

Yeung ML, Yasunaga J, Bennasser Y, Dusetti N, Harris D, Ahmad N, Matsuoka M, Jeang KT (2008) Roles for microRNAs, miR-93 and miR-130b, and tumor protein 53-induced nuclear protein 1 tumor suppressor in cell growth dysregulation by human T-cell lymphotropic virus 1. *Cancer Res*. 68(21):8976-85.

Yu ML, Wang JF, Wang GK, You XH, Zhao XX, Jing Q, Qin YW (2011) Vascular smooth muscle cell proliferation is influenced by let-7d microRNA and its interaction with KRAS. *Circ J*. 75(3):703-9.

Zhang W, Kong G, Zhang J, Wang T, Ye L, Zhang X (2012) MicroRNA-520b Inhibits Growth of Hepatoma Cells by Targeting MEKK2 and Cyclin D1. *PLoS One*. 7(2):e31450.

Zhang Y, Liao JM, Zeng SX, Lu H (2011) p53 downregulates Down syndrome-associated DYRK1A through miR-1246. *EMBO*. 12(8):811-7.