## CG'806, a Novel Pan-FLT3/BTK Multi-kinase Inhibitor, Induces Cell Cycle Arrest, THE UNIVERSITY OF TEXAS ΑΡΤΏSΕ MDAnderson Apoptosis, or Autophagy in AML Cells Depending on FLT3 Mutational Status BLOSCIENCES Cancer Center Guopan Yu<sup>1</sup>, Weiguo Zhang<sup>1</sup>, Charlie Ly<sup>1</sup>, Hongying <sup>1</sup>Section of Molecular Hematology and Therapy, Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, Texas; Making Cancer History® Zhang<sup>2</sup>, William G Rice<sup>2</sup>, and Michael Andreeff<sup>, 1,3</sup> Abstract: 4629 <sup>2</sup>Aptose Biosciences, San Diego, California; <sup>3</sup> Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA CG'806 Induces G1 Arrest by Repressing FLT3, c-Myc, and Modulates Abstract MSC/hypoxia Triggers Autophagy in FLT3- ITD-mutated AML Cells, which Is Background: CG'806 Targets FLT3, BTK and AurK Cell Cycle Checkpoint Proteins in FLT3-ITD Mutant AML Suppressed by Chloroquine and Eradicates the Protection in CG'806 Treatment MOLM14 0 1<sub>16</sub> 3<sub>16</sub> 0 10 20 (nM) (C) (a) MOLM14 (b) .... (a) Gain-of-function mutations of the fms-like tyrosine kinase 3 (FLT3) play a pivotal role in hematopoietic malignancies (Gilliland et al., 2002). 1002 1008 1002 Therefore, FLT3 kinase inhibitors are an important component in the treatment of acute myeloid leukemia (AML). However, relapse/resistance 1.034 to these inhibitors is frequent because of secondary acquired mutations in c-Mvc GAPDH FLT3 gene. Recently, we reported on a novel small molecular multi-CDK6 THE REP. LEWIS CO., LANSING MICH. \*MSC = mesenchymal stem cells kinase inhibitor, CG'806, which showed promising effects in AML GAPDH (d (c) harboring FLT3-internal-tandem-deletion (ITD) mutations, tyrosine kinase w/o MSC with MSC G1 arrest (24 h) domain (TKD) mutations, or both by inhibiting FLT3/Bruton's tyrosine CG'806: 0 1 5 0 1 5 (nM) kinase (BTK)/aurora kinase (AuroK) activation. We observed an (d) LC3-I (e) 100 LC3impressive inhibition of leukemic cell proliferation (i.e., IC50s of subc-Myc-KD (%) <sub>80</sub> control control GAPD c-Myc-KD nanomolar or low nanomolar concentrations, Zhang, et al., AACR %G1:580 %S1373 %G2:374 60 Hematological Malignancies, 2017). (e) <sub>100</sub> Nucleus G1: 49.8% 1: 59.6% 40 100 without MSCs with MSCs w/ MSCs w/o chloroquince To further characterize the mechanisms underlying this antiw/MSCs w/ chloroquinc Blood. 2015; 125: 3236; Oncotarget. 2017; 8:28359; Nature Reviews Cancer. 2014; 14, 219 280 8 leukemia effect, we investigated the impact of CG'806 on cell cycle 20 80 % progression. Measuring BrdU incorporation by flow cytometry. CG'806 i<u>€</u> 60 60 Results triggered profound G1 cell cycle arrest in FLT3-ITD-mutated cells. control 1 8<sub>40</sub> Interestingly, CG'806 triggered G2/M phase arrest in FLT3 wild type (WT) 0.14-0 (24 h) (G'806 (nM) 4٨ cells. Immunoblotting demonstrated that the G1 arrest was mediated by CG'806 Induces G1 Arrest in FLT3 Mutant AML <u>ਵ</u> 20 20 downregulation of signaling associated with p-FLT3-ITD, the p-AKT/p-CG'806 Induces G2/M Arrest by Repressing Phospho-Aurora and G2/M Arrest in FLT3-WT AML Activation in FLT3 WT AML Cells mTOR/cyclin D1/p-Rb axis, and that of cyclin B1/A2, cdk1/cdc2, and 0 1 5 0 1 5 0 2.5 cdk4/6 in FLT3-ITD-mutated cells. This was not observed in FLT3 WT 5 (a) (a) MOLM14 THP-1 FLT3 ITD FLT3 ITD FLT3 W CG'806 (nM) 48hr FITS WT CG'806 (nM) AC220 (nM) cells. In addition, c-Myc, a primary regulator of the G1/S transition, was downregulated in FLT3-mutated but not in WT cells treated with CG'806. Combined CG'806 with Conventional Chemotherapeutics Exerts Enhanced Pro-apoptotic Effects in FLT3 WT and mutant AML Cells In support of this finding, knockdown of c-Myc with siRNA increased the G1-arrested population in FLT3-mutated cells, suggesting a critical role of (a) (h) $CI = 0.77 \pm 0.35$ $CI = 0.87 \pm 0.20$ THP-1 (48h) 80 c-Myc in the CG'806-induced G1 arrest. However, only suppression of p-70 CG'806 BTK and p-AuroK, but not p-FLT3, and no modulation of G1 arrest-70 CG'806 <u>F 60</u> Ara-C 8.60 related proteins were observed in FLT3 WT cells. D-FIT3 . <u>8</u> 50 G2/M arrest CG'806+Ara-0 CG'806+IDA .<u></u>2 50 Interestingly, we observed autophagy induction in FLT3 WT cells GAPDI <sup>.</sup>รี 40 iti 40 / 30 compared to the induction of apoptosis in FLT3 mutated cells (as (FLT3 ITD) 24h (FLT3 WT) 5 30 evidenced by modulated LC3-II and cleaved-caspase 3 levels, 1044 THP-1 KG1 BA/F2-FLT3 VIOLM14 THP-1 THP-1 e dra m ·<u></u> 20 CG'806 Inhibits Autophagy in FLT3-mutant AML Cells respectively) after exposure to CG'806 for 24 hours. Inhibiting autophagy Quizartinib Giltertinib (24 h) Ĕ 10 . ₽ 10 CG'8 with 3-methyladenine (3-MA) partially reversed CG'806-induced G2/M 0 0 (h) arrest in FLT3 WT cells, suggesting that autophagy induction may also be MOLM14 THP-1 **MOL M14** MV/4-11 THP-1 OCI-AML3 Ida 0 0.5 1 2.5 (µM) 2.5 0 0.5 1 5 (μM) Ara-C involved in G2/M arrest in addition to suppression of AuroK and BTK in 0 10 20 0 5 10 (nM) CG'806 0 1 3 0 1 5 0 0.005 0.01 0.025(µM) 0 0.005 0.01 0.025 0.05 (μM) CG'806 CG'806 FLT3 WT cells LC3 { | Next, we investigated if nucleoside analogues and intercalating ATG7 Conclusions agents enhance activity of CG'806, and observed that combination of Beclin-1 CG'806 with conventional chemotherapeutics cytarabine or idarubicin CG'806 exerts profound suppression of cell proliferation by arresting profoundly enhanced pro-apoptotic effects. c-Myc cell cycle progression at G1 phase in FLT3-mutant AML cells, which is Conclusions: CG'806 exerts profound suppression of cell CDK4 associated with inhibition of mutant FLT3 and downstream p-AKT/pproliferation by arresting cell cycle progression at G1 phase in FLT3mTOR/cyclin D1/p-Rb signaling axis. CDK6 mutant AML cells, which is associated with inhibition of mutant FLT3 and CG'806 exerts a G2/M arrest in FLT3 WT cells, which is associated with p-mTOR downstream p-AKT/p-mTOR/cvclin D1/p-Rb axis, CG'806 exerts a G2/M inhibition of AuroK and downstream cyclin B/CDK1 signaling pathway. p-S6K MSC/hypoxia induce autophagy of FLT3-ITD mutated cells, which can arrest in FLT3 WT cells associated with inhibition of aurora and BTK be abrogated by chloroquine and therefore enhances CG'806-induced kinases and induction of autophagy. CG'806 sensitized AML to standard p-Rb pro-apoptotic effect. chemotherapeutic agents. Taken together, these data support the GAPDH CG'806 sensitizes AML to standard chemotherapeutic agent-mediated development of CG'806 for AML patients with FLT3-ITD and FLT3-ITD cvtotoxicity. (FLT3 ITD (FLT3 WT) and additional TKD mutations, as well as FLT3-WT patients. (24 h \* H. Zhang and W. Rice are employees of Aptose Biosciences; M. Andreeff serves on Aptose Biosciences SAB.