SUPPLEMENTARY APPENDIX

Targeting Wnt signaling in acute myeloid leukemia stem cells

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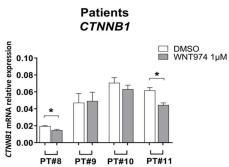
Supplementary Table S1. Cytogenetics and molecular features of the primary AML samples.

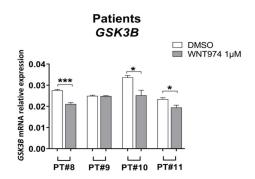
Patient # Cytogenetics Mutations 1 46,XX[20] NPM1 2 46,XY[20] RUNX-1, TET2 3 46,XX[20] FLT3-ITD 46,XY,t(9;22)(q34;q11.2)[18]/nonclonal w/clonal[2]* 4 NPM1 5 46,XY[19]/nonclonal[1] FLT3-ITD, NPM1, TET2 6 46,XX[20] FLT3-ITD, IDH1 46,XX[20] FLT3-ITD, NPM1 7 FLT3-ITD, IDH2, NPM1, DNMT3A 8 46,XY[19]/nonclonal[1] 9 46,XX,t(1;10)(q21;q22)[2]/46,XX[18] 10 46, X, -Y, t(1;19)(p13;q13.3), +8, t(8;21)(q22;q22)[19]/46, XY[1]46,XY,t(8;21)(q22;q22)[20] 11 12 46,XY,del(9)(q21.2q34),del(11)(p13p15)[12]

^{*} Chronic Myeloid Leukemia in Myeloid Blast Crises

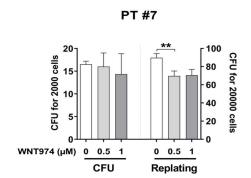
Supplementary Figures

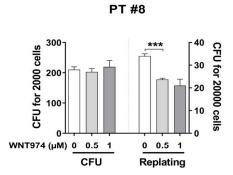




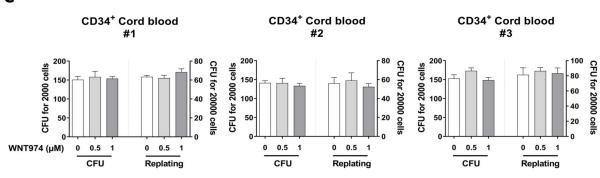


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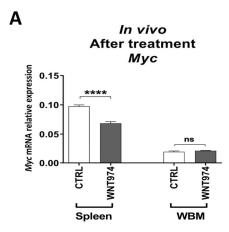


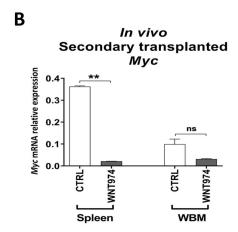
C

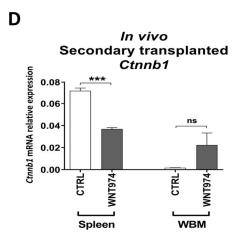


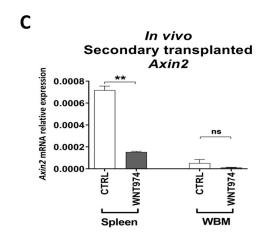
Supplementary Figure S1.

(A) CTNNB1 (left panel) and GSK3B (right panel) mRNA relative expression on GAPDH of BM-CD34+ selected primary AML treated with WNT974 1uM for 24 hours (*: p<0.05, **: p<0.01, ***: p<0.001, by t-test). (B) Colony forming unit (CFU) assay of BM CD34⁺ primary AML cells treated with WNT974 drug at different concentration or vehicle (DMSO 0.1% volume). After 2 week, colonies were scored and replated. A second scoring was done after 2 weeks of replating. In the graph we reported mean and standard deviation from a technical triplicate (**: p<0.01, ***: p<0.001 by t-test). (C) Colony forming unit (CFU) assay of CD34⁺ cells from cord blood, treated with WNT974 drug at different concentration or vehicle (DMSO 0.1% volume). After 2 week, colonies were scored and replated. A second scoring was done after 2 weeks of replating. In the graph we reported mean and standard deviation from a technical triplicate.

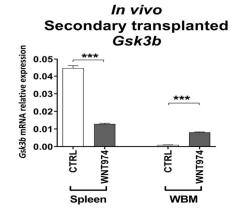








Ε



Supplementary Figure S2.

(A) *Myc* mRNA relative expression on *Actinb* of spleen and whole bone marrow (WBM) from BoyJ transplanted mice with *MII*^{PTD/WT}/ *FIt3*^{ITD/WT} murine cells and treated with 5mg/Kg/twice at day with WNT974 by oral gavage for one week before retransplantation. (****: p<0.0001 by t-test). (B) *Myc* mRNA relative expression on *Actinb* of spleen and WBM from BoyJ secondary transplanted mice with spleen from *MII*^{PTD/WT}/ *FIt3*^{ITD/WT} murine cells and treated with 5mg/Kg/twice at day with WNT974 by oral gavage for one week before secondary transplantation. Spleen and WBM were harvested when natural death occurred (**: p<0.01 by t-test). (C) *Axin2* mRNA relative expression on *Actinb* of spleen and WBM from BoyJ secondary transplanted mice with spleen from *MII*^{PTD/WT}/ *FIt3*^{ITD/WT} murine cells and treated with 5mg/Kg/twice at day with WNT974 by oral gavage for one week before secondary transplantation. Spleen and WBM were harvested when natural death occurred (**: p<0.01 by t-test). (D) *Ctnnb1* mRNA relative expression on *Actinb* of spleen and WBM from BoyJ secondary transplanted mice with spleen from *MII*^{PTD/WT}/ *FIt3*^{ITD/WT} murine cells and treated with 5mg/Kg/twice at day with WNT974 by oral gavage for one week before secondary transplantation. Spleen and WBM were harvested when natural death occurred (***: p<0.001 by t-test). (E) *Gsk3b* mRNA relative expression on *Actinb* of spleen and WBM from BoyJ secondary transplanted mice with spleen from *MII*^{PTD/WT}/ *FIt3*^{ITD/WT} murine cells and treated with 5mg/Kg/twice at day with WNT974 by oral gavage for one week before secondary transplantation. Spleen and WBM were harvested when natural death occurred (***: p<0.001 by t-test). (E) *Gsk3b* mRNA relative expression on *Actinb* of spleen and WBM from BoyJ secondary transplanted mice with spleen from *MII*^{PTD/WT}/ *FIt3*^{ITD/WT} murine cells and treated with 5mg/Kg/twice at day with WNT974 by oral gavage for one week before secondary transplantation. Spleen and WBM were harvested when natural