SciMedCentral

Journal of Hematology & Transfusion

Editorial

Collaborating Pathways that Functionally Amplify NOTCH1 Signals in T-Cell Acute Lymphoblastic Leukemia

Nancy E. Pinnell and Mark Y. Chiang*

Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan Cancer Center, USA

Corresponding author

Mark Y. Chiang, Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan Cancer Center, Taubman Biomedical Science Research Building, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA, Tel: 734-615-7513; Email: markchia@med.umich.edu

Submitted: 10 August 2013

Accepted: 30 August 2013

Published: 02 September 2013

INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) accounts for 15% of pediatric and 25% of adult ALL. With cure rates of ~80% in children and ~40% in adults, there is an urgent need to identify and understand the signaling pathways in T-ALL in order to develop the first targeted therapies for this cancer. The most prevalent oncogene is *NOTCH1,* which is mutated in about 60% of patient samples [1]. Normally, Notch is restrained by the heterodimerization domain (HD, Figure 1). Notch becomes activated once it engages ligand. γ-secretase cleaves Notch, thus releasing the intracellular domain of Notch (ICN). ICN binds Rbpj and Maml to activate transcription. ICN has a short half-life as phosphodegron motifs in its PEST domain are recognized by the ubiquitin ligase Fbxw7, which targets it for destruction [2,3]. PEST domain deletions improve ICN stability [4]. Mutations in the HD domain trigger ligand-independent activation [5,6]. Target genes of *NOTCH1* that drive leukemia include *MYC* [7-9], *HES1* [10], *IGF-1R* [11], *TRIB2* [12], and *IL7R* [13]. Notch also activates the PI3K/Akt /mTor pathway [11,14,15].

Collaborative pathways confer resistance to *NOTCH* inhibitors

γ-secretase inhibitors (GSIs) block Notch signaling (Figure 1). GSIs are being tested in clinical trials in T-ALL and other NOTCH-driven cancers [16-18]. Initial reports show promising activity. However, resistance is an emerging problem. Approximately two-thirds of human T-ALL cell lines are resistant to GSI [1]. GSI depletes ICN in resistant cells just as it does in sensitive cells [7,15]. Thus, cancers resist GSI by activating collaborating pathways to bypass the effects of *NOTCH1* inhibition. We previously showed that *NOTCH1* mutations are moderate oncogenes in mouse models [19]. Collaborating oncogenic networks are essential to functionally enhance *NOTCH1* signaling to leukemogenic levels. These findings have shifted the field toward identifying pathways that collaborate with the NOTCH pathway.

Collaborators that act as *NOTCH1* co activators

A recent mass spectrometry screen identified coactivators that physically interact with the *NOTCH1* complex such as the SWI/SNF remodeling complex PBAF, AF4p^{12}, and the histone demethylases LSD1 and PHF8 [20]. These coactivators supported transactivation of classical *NOTCH1* target genes and leukemic growth. Also identified were master regulatory transcription factors IKAROS, HEB, BCL11B, and RUNX1. Ikaros proteins antagonize Notch-transcriptional activity. Dominant-negative

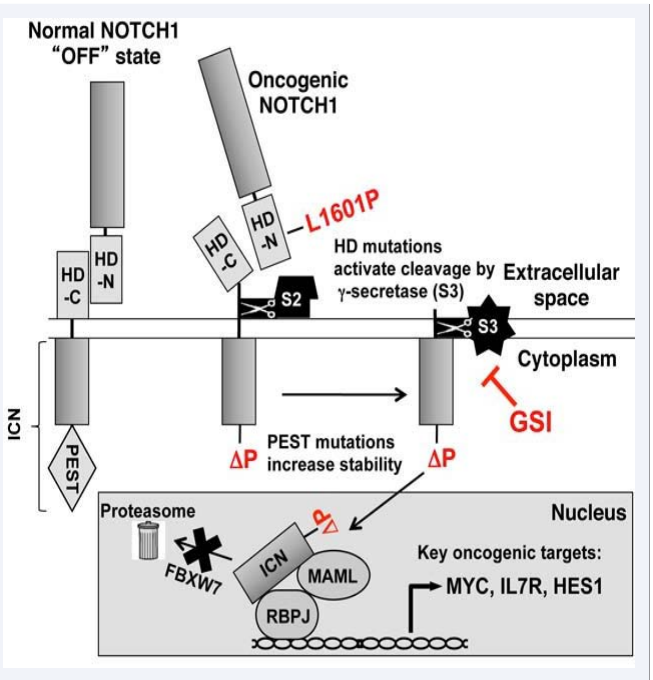

Figure 1 Model of oncogenic NOTCH1 signaling in T-ALL. NOTCH1 is normally locked in an "off " state, which blocks S2 cleavage. Mutations that discrupt the integrity of the heterodimerization domain (HD, e.g. L1601P) permit ligand-independent S2 cleavage, leading to formation of ICN, which translocates to the nucleus. ICN engages the DNA binging protein RBPJ/CSL and mastermind-like (MAML) cofactors to form a transcriptionally active complex. PEST mutations (represented by ΔP) improve protein stability by removing negative regulatory elements recognized by the ubiquitin ligase FBXW7 in the C-terminus that shorten the half-life of ICN.

IKAROS isoforms have been reported in human T-ALL [21,22] and collaborate with *NOTCH1* in mouse models [23,24]. Runx sites have been imputed adjacent to Rbpj sites [25]. Although Runx1 has been reported to act as a tumor suppressor [26-28], a recent abstract suggests that Runx factors and *NOTCH1* coordinately regulate oncogenic targets to drive proliferation in the majority of cases [29]. In addition, more than 90% of *NOTCH1*/RBPJ sites are co-bound with MYC, such as *IL7R* [30,31]. Although it has not been definitively tested whether these factors functionally collaborate with *NOTCH1* in leukemogenesis, retroviral insertional mutagenesis screens suggest that Myc and Runx1 can accelerate *NOTCH1*-induced leukemia in mice [32-34].

Collaborators that intersect with the *NOTCH1* pathway

Inactivating *FBXW7* mutations occur in approximately 20% of human T-ALL cases [2,3]. Besides Notch1, Fbxw7 degrades other cellular substrates such as Myc [35,36], Mcl1 [37,38], and mTOR [39]. In T-ALL, *FBXW7* mutations are mutually exclusive with PEST mutations, suggesting that they amplify *NOTCH1* signals by improving ICN stability. *FBXW7* mutations contribute to GSI resistance in cell lines likely by maintaining MYC protein levels despite loss of ICN. However, although MYC can rescue most human T-ALL cell lines treated with GSI, it cannot rescue all of them [7]. TAL1/SCL is a class II basic helix-loop-helix transcription factor that is over expressed in ~60% of human T-ALL cases [40]. TAL1 and *NOTCH1* collaborate in mouse models [41]. TAL1 may amplify the *NOTCH1* pathway in part by down regulating FBXW7 through miR-223 [42] and by directly inducing the *NOTCH1* target gene *TRIB2* [43]. However, TAL1 does not appear to contribute to GSI resistance. Finally, *PTEN* mutations occur in about 10% of human T-ALL cases [44]. *PTEN* mutations are thought to amplify *NOTCH1* signals through the PI3K/AKT/mTOR pathway. Activation of PI3K/AKT/mTOR was shown to rescue the proliferation of some human T-ALL cell lines treated with GSI [45].

Collaborators with unknown mechanisms of interaction with the *NOTCH1* pathway

Several pathways collaborate with *NOTCH1* through unclear mechanisms. These pathways have typically been identified through mouse models of human T-ALL [46]. An example is the HOX family transcription factor TLX1 [47,48]. To determine if the *NOTCH1*-TLX1 collaboration was targetable, we developed a TLX1-initiated T-ALL mouse model in which the expression of TLX1 was repressed by doxycycline [48]. We treated these tumors with doxycycline, GSI, or both doxycycline and GSI (Figure 2A). Mice receiving combined TLX1 and *NOTCH1* suppression had the best response (Figure 2B-C). Our work suggests that targeting collaborator proteins can improve the efficacy of anti-NOTCH therapy. Retroviral or transposon-mediated insertional mutagenesis screens in mice have been particularly effective in identifying collaborative pathways. The *NOTCH1* locus is a frequent common insertion site. These insertions frequently lead to insertions in putative collaborators such as *Rasgrp1*, *Lfng*, *Akt2*, *Erg, and Zmiz1* [34,49]. Zmiz1 is a co activator that is similar to Protein Inhibitor of Activated STAT (PIAS) family members. Our laboratory recently validated Zmiz1 as a *NOTCH1* collaborator. Ectopic *ZMIZ1* and leukemia-associated *NOTCH1*

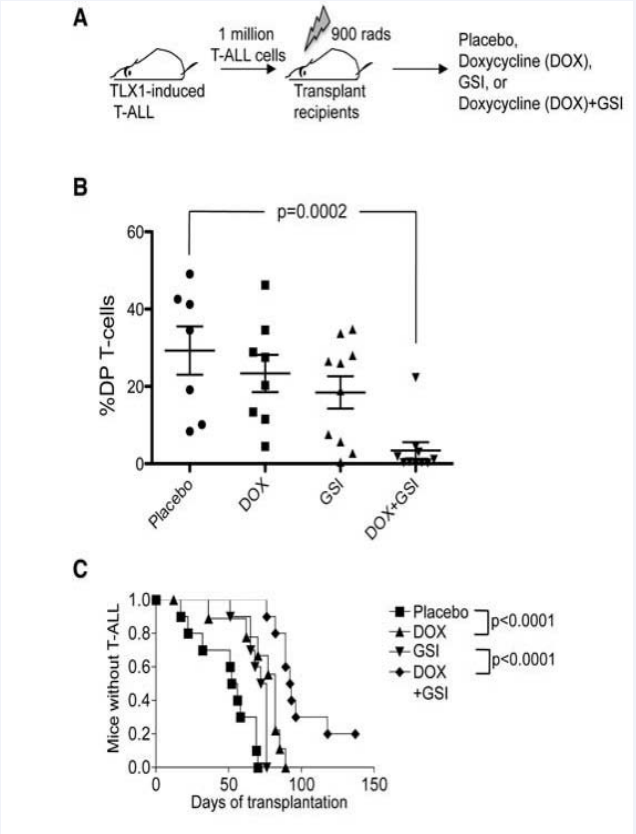

Figure 2 Long term responses with combined Notch/TLX1 inhibition in TLX1-induced T-ALL. (A) Schematic diagram of treatment regimens of murine TLX1-transgenic tumors. 1 million TLX1-transgenic T-ALL cells were injected into lethally irradiated recipients together with resove syngeneic bone marrow cells. Recipients were treated with placebo, doxycycline (DOX), GSI, or DOX + GSI. To shut down TLX1 expression in the transgenic mouse model, mice were fed placebo (5% sucrose) or doxycycline (2g/L in 5% sucrose) in drinking water starting two days before transplantation. Starting at 3 weeks after transplant, mice were treated with DMSO or the Notch inhibitor GSI (10umol/kg DBZ, EMD chemicals) for a total of 3 cycles. During each cycle, DMSO or GSI was injected daily for three days followed by 4 days of rest. (B) Peripheral blood T-lymphoblasts [$CD4^+$ $CD8^+$ Double positivbe (DP) cells] at 7 weeks after transplantation according to treatment regimen. (C) Kaplan-Meier curve showing survival of T-ALL mice according to treatment regimen.

alleles collaborated to induce T-ALL in mice [50]. ZMIZ1 and activated *NOTCH1* were co-expressed in ~20% of patients across diverse oncogenomic subsets [50]. Inhibition of ZMIZ1 in T-ALL cell lines slowed proliferation and overcame resistance to NOTCH inhibitors [50]. We identified *MYC* and *IL7R* as critical downstream target genes. However, the mechanism by which Zmiz1 interacts with the Notch pathway remains unclear.

CONCLUSION

Recent studies have revealed several collaborative pathways that functionally interact with the *NOTCH1* pathway. As a result, clinical trials are underway combining GSI with agents that target collaborative pathways such as mTOR inhibitors [51,52]. However, none of the known collaborative pathways have been found to be sufficient to confer resistance [45,53]. The mechanism of many of these pathways remains elusive. Thus,

there is a critical need to identify and understand the signaling networks that functionally amplify *NOTCH1* signals. Therapeutic agents that target these networks will be required to increase the effectiveness of T-ALL therapy, including NOTCH inhibitors. In the absence of these agents, advancing the treatment of T-ALL will be difficult.

REFERENCES

1. Weng AP, Ferrando AA, Lee W, Morris JP 4th, Silverman LB, Sanchez-Irizarry C, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004; 306: 269-271.
2. Thompson BJ, Buonamici S, Sulis ML, Palomero T, Vilimas T, Basso G, et al. The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. J Exp Med. 2007; 204: 1825-1835.
3. O'Neil J, Grim J, Strack P, Rao S, Tibbitts D, Winter C, et al. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. J Exp Med. 2007; 204: 1813-1824.
4. Chiang MY, Xu ML, Histen G, Shestova O, Roy M, Nam Y, et al. Identification of a conserved negative regulatory sequence that influences the leukemogenic activity of NOTCH1. Mol Cell Biol. 2006; 26: 6261-6271.
5. Malecki MJ, Sanchez-Irizarry C, Mitchell JL, Histen G, Xu ML, Aster JC, et al. Leukemia-associated mutations within the NOTCH1 heterodimerization domain fall into at least two distinct mechanistic classes. Mol Cell Biol. 2006; 26: 4642-51.
6. Gordon WR, Vardar-Ulu D, Histen G, Sanchez-Irizarry C, Aster JC, Blacklow SC. Structural basis for autoinhibition of Notch. Nat Struct Mol Biol. 2007; 14: 295-300.
7. Palomero T, Lim WK, Odom DT, Sulis ML, Real PJ, Margolin A, et al. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. Proc Natl Acad Sci U S A. 2006; 103: 18261-18266.
8. Sharma VM, Calvo JA, Draheim KM, Cunningham LA, Hermance N, Beverly L, et al. Notch1 contributes to mouse T-cell leukemia by directly inducing the expression of c-myc. Mol Cell Biol. 2006; 26: 8022-8031.
9. Weng AP, Millholland JM, Yashiro-Ohtani Y, Arcangeli ML, Lau A, Wai C, et al. c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. Genes Dev. 2006; 20: 2096-2109.
10. Wendorff AA, Koch U, Wunderlich FT, Wirth S, Dubey C, Brüning JC, et al. Hes1 is a critical but context-dependent mediator of canonical Notch signaling in lymphocyte development and transformation. Immunity. 2010; 33: 671-684.
11. Medyouf H, Gusscott S, Wang H, Tseng JC, Wai C, Nemirovsky O, et al. High-level IGF1R expression is required for leukemia-initiating cell activity in T-ALL and is supported by Notch signaling. J Exp Med. 2011; 208: 1809-1822.
12. Wouters BJ, Jordà MA, Keeshan K, Louwers I, Erpelinck-Verschueren CA, Tielemans D, et al. Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. Blood. 2007; 110: 3706-3714.
13. González-García S, García-Peydró M, Martín-Gayo E, Ballestar E, Esteller M, Bornstein R, et al. CSL-MAML-dependent Notch1 signaling controls T lineage-specific IL-7R{alpha} gene expression in early human thymopoiesis and leukemia. J Exp Med. 2009; 206: 779-791.
14. Chan SM, Weng AP, Tibshirani R, Aster JC, Utz PJ. Notch signals positively regulate activity of the mTOR pathway in T-cell acute lymphoblastic leukemia. Blood. 2007; 110: 278-286.
15. Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M, et al. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med. 2007; 13: 1203-1210.
16. Deangelo DJ, Stone RM, Silverman LB, Stock W, Attar EC, Fearen I, et al. A phase I clinical trial of the notch inhibitor MK-0752 in patients with T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) and other leukemias J Clin Oncol, 2006 ASCO Annual Meeting Proceedings. 2006; 24: 6585.
17. Krop I, Demuth T, Guthrie T, Wen PY, Mason WP, Chinnaiyan P, et al. Phase I pharmacologic and pharmacodynamic study of the gamma secretase (Notch) inhibitor MK-0752 in adult patients with advanced solid tumors. J Clin Oncol. 2012; 30: 2307-2313.
18. Tolcher AW, Messersmith WA, Mikulski SM, Papadopoulos KP, Kwak EL, Gibbon DG, et al. Phase I study of RO4929097, a gamma secretase inhibitor of Notch signaling, in patients with refractory metastatic or locally advanced solid tumors. J Clin Oncol. 2012; 30: 2348-2353.
19. Chiang MY, Xu L, Shestova O, Histen G, L'heureux S, Romany C, et al. Leukemia-associated NOTCH1 alleles are weak tumor initiators but accelerate K-ras-initiated leukemia. J Clin Invest. 2008; 118: 3181-3194.
20. Yatim A, Benne C, Sobhian B, Laurent-Chabalier S, Deas O, Judde JG, et al. NOTCH1 nuclear interactome reveals key regulators of its transcriptional activity and oncogenic function. Mol Cell. 2012; 48: 445-458.
21. Marçais A, Jeannet R, Hernandez L, Soulier J, Sigaux F, Chan S, et al. Genetic inactivation of Ikaros is a rare event in human T-ALL. Leuk Res. 2010; 34: 426-429.
22. Sun L, Goodman PA, Wood CM, Crotty ML, Sensel M, Sather H, et al. Expression of aberrantly spliced oncogenic ikaros isoforms in childhood acute lymphoblastic leukemia. J Clin Oncol. 1999; 17: 3753-3766.
23. Beverly LJ, Capobianco AJ. Perturbation of Ikaros isoform selection by MLV integration is a cooperative event in Notch(IC)-induced T cell leukemogenesis. Cancer Cell. 2003; 3: 551-564.
24. Dumortier A, Jeannet R, Kirstetter P, Kleinmann E, Sellars M, dos Santos NR, et al. Notch activation is an early and critical event during T-Cell leukemogenesis in Ikaros-deficient mice. Mol Cell Biol. 2006; 26: 209-220.
25. Wang H, Zou J, Zhao B, Johannsen E, Ashworth T, Wong H, et al. Genome-wide analysis reveals conserved and divergent features of Notch1/RBPJ binding in human and murine T-lymphoblastic leukemia cells. Proc Natl Acad Sci U S A. 2011; 108: 14908-14913.
26. Della Gatta G, Palomero T, Perez-Garcia A, Ambesi-Impiombato A, Bansal M, Carpenter ZW, et al. Reverse engineering of TLX oncogenic transcriptional networks identifies RUNX1 as tumor suppressor in T-ALL. Nat Med. 2012; 18: 436-440.
27. Grossmann V, Kern W, Harbich S, Alpermann T, Jeromin S, Schnittger S, et al. Prognostic relevance of RUNX1 mutations in T-cell acute lymphoblastic leukemia. Haematologica. 2011; 96: 1874-1877.
28. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature. 2012; 481: 157-163.
29. Giambra V, Jenkins CR, Wang H, Lam SH, Shevchuk OO, Nemirovsky O, et al. NOTCH1 promotes T cell leukemia-initiating activity by RUNX-mediated regulation of PKC-Î¸ and reactive oxygen species. Nat Med. 2012; 18: 1693-1698.
30. King B, Trimarchi T, Reavie L, Xu L, Mullenders J, Ntziachristos P, et al. The Ubiquitin Ligase FBXW7 Modulates Leukemia-Initiating Cell Activity by Regulating MYC Stability. Cell. 2013; 153: 1552-1566.

31. Margolin AA, Palomero T, Sumazin P, Califano A, Ferrando AA, Stolovitzky G. ChIP-on-chip significance analysis reveals large-scale binding and regulation by human transcription factor oncogenes. Proc Natl Acad Sci U S A. 2009; 106: 244-249.

32. Girard L, Hanna Z, Beaulieu N, Hoemann CD, Simard C, Kozak CA, et al. Frequent provirus insertional mutagenesis of Notch1 in thymomas of MMTVD/myc transgenic mice suggests a collaboration of c-myc and Notch1 for oncogenesis. Genes Dev. 1996; 10: 1930-1944.

33. Hoemann CD, Beaulieu N, Girard L, Rebai N, Jolicoeur P. Two distinct Notch1 mutant alleles are involved in the induction of T-cell leukemia in c-myc transgenic mice. Mol Cell Biol. 2000; 20: 3831-3842.

34. Berquam-Vrieze KE, Nannapaneni K, Brett BT, Holmfeldt L, Ma J, Zagorodna O, et al. Cell of origin strongly influences genetic selection in a mouse model of T-ALL. Blood. 2011; 118: 4646-4656.

35. Welcker M, Orian A, Jin J, Grim JE, Harper JW, Eisenman RN, et al. The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. Proc Natl Acad Sci U S A. 2004; 101: 9085-9090.

36. Yada M, Hatakeyama S, Kamura T, Nishiyama M, Tsunematsu R, Imaki H, et al. Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. EMBO J. 2004; 23: 2116-2125.

37. Inuzuka H, Shaik S, Onoyama I, Gao D, Tseng A, Maser RS, et al. SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. Nature. 2011; 471: 104-109.

38. Wertz IE, Kusam S, Lam C, Okamoto T, Sandoval W, Anderson DJ, et al. Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature. 2011; 471: 110-114.

39. Fu L, Kim YA, Wang X, Wu X, Yue P, Lonial S, et al. Perifosine inhibits mammalian target of rapamycin signaling through facilitating degradation of major components in the mTOR axis and induces autophagy. Cancer Res. 2009; 69: 8967-8976.

40. Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, Raimondi SC, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell. 2002; 1: 75-87.

41. O'Neil J, Calvo J, McKenna K, Krishnamoorthy V, Aster JC, Bassing CH, et al. Activating Notch1 mutations in mouse models of T-ALL. Blood. 2006; 107: 781-785.

42. Mansour MR, Sanda T, Lawton LN, Li X, Kreslavsky T, Novina CD, et al. The TAL1 complex targets the FBXW7 tumor suppressor by activating miR-223 in human T cell acute lymphoblastic leukemia. J Exp Med. 2013; 210: 1545-1557.

43. Sanda T, Lawton LN, Barrasa MI, Fan ZP, Kohlhammer H, Gutierrez A, et al. Core transcriptional regulatory circuit controlled by the TAL1 complex in human T cell acute lymphoblastic leukemia. Cancer Cell. 2012; 22: 209-221.

44. Gutierrez A, Sanda T, Grebliunaite R, Carracedo A, Salmena L, Ahn Y, et al. High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood. 2009; 114: 647-650.

45. Medyouf H, Gao X, Armstrong F, Gusscott S, Liu Q, Gedman AL, et al. Acute T-cell leukemias remain dependent on Notch signaling despite PTEN and INK4A/ARF loss. Blood. 2010; 115: 1175-1184.

46. Aster JC, Blacklow SC, Pear WS. Notch signalling in T-cell lymphoblastic leukaemia/lymphoma and other haematological malignancies. J Pathol. 2011; 223: 262-273.

47. De Keersmaecker K, Real PJ, Gatta GD, Palomero T, Sulis ML, Tosello V, et al. The TLX1 oncogene drives aneuploidy in T cell transformation. Nat Med. 2010; 16: 1321-1327.

48. Rakowski LA, Lehotzky EA, Chiang MY. Transient responses to NOTCH and TLX1/HOX11 inhibition in T-cell acute lymphoblastic leukemia/lymphoma. PLoS One. 2011; 6: e16761.

49. Uren AG, Kool J, Matentzoglu K, de Ridder J, Mattison J, van Uitert M, et al. Large-scale mutagenesis in p19(ARF)- and p53-deficient mice identifies cancer genes and their collaborative networks. Cell. 2008; 133: 727-741.

50. Rakowski LA, Garagiola DD, Li CM, Decker M, Caruso S, Jones M, et al. Convergence of the ZMIZ1 and NOTCH1 pathways at C-MYC in acute T lymphoblastic leukemias. Cancer Res. 2013; 73: 930-941.

51. Diaz-Padilla I, Hirte H, Oza AM, Clarke BA, Cohen B, Reedjik M, et al. A phase Ib combination study of RO4929097, a gamma-secretase inhibitor, and temsirolimus in patients with advanced solid tumors. Invest New Drugs. 2013; .

52. Cullion K, Draheim KM, Hermance N, Tammam J, Sharma VM, Ware C, et al. Targeting the Notch1 and mTOR pathways in a mouse T-ALL model. Blood. 2009; 113: 6172-6181.

53. Tosello V, Ferrando AA. The NOTCH signaling pathway: role in the pathogenesis of T-cell acute lymphoblastic leukemia and implication for therapy. Ther Adv Hematol. 2013; 4: 199-210.

Cite this article

Pinnell NE, Chiang MY (2013) Collaborating Pathways that Functionally Amplify NOTCH1 Signals in T-Cell Acute Lymphoblastic Leukemia. J Hematol Transfus 1(1): 1004.