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Anatomy of a crime: how IL7R and NRAS join forces to drive T-cell acute lymphoblastic leukemia

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Disclosure of Conflicts of Interest

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In this issue of *Haematologica*, Winer, Li, et al (1) demonstrate that MYC is crucial to the oncogenic cooperation between *IL7R* and *NRAS* driving T-cell acute lymphoblastic leukemia (T-ALL) development (2). They further show that the kinase PLK-1 may contribute to MYC protein stability and that MYC-modulating drugs can be of therapeutic value against T-ALL driven by activating mutations in both *IL7R* and *NRAS*.

Why the relevance of diving deeper into the mechanisms underlying this collaboration? Roughly 10% of T-ALL patients display *IL7R* gain-of-function mutations (mut*IL7R*), and a much larger fraction (some 50 to 80% of the cases) express IL7R and may benefit from IL7 produced in the leukemia milieu (3-7). IL7R-mediated signaling (because of IL7, high IL7R levels or mutational activation of the receptor or downstream effectors) can promote T-ALL establishment and maintenance, and resistance to glucocorticoids (5). *RAS* activating mutations in general occur in around 2% of the cases, and *NRAS* alterations are infrequent. However, full appreciation of the importance of RAS signaling in T-ALL must consider other lesions, including inactivating mutations in the RAS-MEK-ERK pathway negative regulator *NF1* or *RasGRP1* overexpression. Interestingly, MEK-ERK pathway can be activated also by IL7/IL7R in T-ALL, contributing to IL7R-mediated resistance to glucocorticoids (8).

To understand why *IL7R* or *NRAS* alone cannot drive T-ALL in a transplant mouse model, whereas their combination is clearly leukemogenic (2), Winer, Li, and collaborators now analyzed the transcriptome of immature mouse thymocytes transduced with mutant *IL7R* (a particular type 1a *IL7R* activating mutation, hereafter referred to as mut*IL7R*) (3, 5), mutant *NRAS* (coding for NRAS G13D, which leads to NRAS activation; hereafter referred to as mut*NRAS*) or their combination. They found no evidence of Myc activation (as measured by the upregulation of Myc target genes) by mut*IL7R* alone, whereas mut*NRAS* activated Myc and, importantly, this effect was augmented by the combination of mut*IL7R* and mut*NRAS*. In agreement, Myc itself was upregulated by mut*NRAS* and mut*NRAS*+mut*IL7R*, but not by

mut*IL7R* alone. The fact that mut*IL7R* was unable to activate Myc is intriguing, as MYC is a downstream target of IL7R-mediated signaling in human thymocytes, and in zebrafish models of T-ALL (9). The reasons for these discrepancies are unclear. They may relate to the stage of disease development at which the analyses were conducted: D1 cells, being *p53*-null, are one step closer to transformation than healthy thymocytes, and the analyses in zebrafish and human T-ALLs focused on fully transformed cells.

These considerations apart, the authors provide convincing evidence linking RAS signaling and MYC. They show that MYC overexpression phenocopied mutant *NRAS* in its ability to collaborate with mut*IL7R* to drive T-ALL. Both mut*IL7R*+mut*NRAS* and mut*IL7R*+*MYC* led to CD4+CD8+ T-ALL, with relatively similar expression of TCR Vα and predominance of αβ over γδ T-cells. Whether this resemblance extends to the transcriptional profile was not addressed. Nonetheless, these findings align with previous studies showing that mut*IL7R* and *MYC* collaborate to drive T-ALL in zebrafish (9). The relevance of MYC in mut*IL7R*+mut*NRAS*-driven leukemias was further exposed by experiments showing that *Myc* deletion decreased leukemia burden *in vivo* and that *Myc* silencing decreased the fitness of D1 cells and primary thymocytes transduced with the combination of the two oncogenes.

Durum's team further combined RNA-sequencing and mass-spectrometry to show that Bcl-2 transcript and protein were upregulated. Contrary to Myc, Bcl-2 upregulation was due to mut*IL7R* and not mut*NRAS*, and the combination of the two oncogenes did not potentiate mut*IL7R* effects. That IL7-IL7R-mediated signaling upregulates Bcl-2 is well known, although (opposite to the illustration in the final figure of the paper) this is unlikely mediated by STAT5 in T-ALL (8, 10).

Contrary to Bcl-2, Myc was upregulated exclusively at the protein level, and the authors propose that NRAS collaborated with IL7R essentially by stabilizing Myc. How activated NRAS, and its combination with mutant IL-7R, promoted Myc stabilization may involve two

mechanisms. RAS-MEK-ERK signaling is known to phosphorylate Myc at Serine 62 (S62), and this phosphorylation contributes to Myc stability. Indeed, Myc S62 was upregulated by mut*IL7R* and even more so by mut*NRAS*, although not further increased by the combination. On the other hand, AKT-dependent Myc Threonine 58 (T58) phosphorylation was upregulated by each oncogene alone and by their combination. This should mark Myc for degradation, which obviously was not the case. So, there should be a mechanism counterbalancing the effects of Myc T58-phosphorylation. Winer, Li et al noticed that PLK-1 (which contributes to MYC stabilization by preventing its proteasomal degradation) was upregulated by NRAS and its combination with IL7R. Thus, they used Volasertib, a PLK-1 inhibitor, to test the impact on Myc expression. Volasertib not only downregulated Myc protein but also, surprisingly, *Myc* transcript levels, suggesting that PLK-1 may upregulate Myc via different mechanisms. In the absence of PLK-1 genetic manipulation and given that pharmacological inhibitors often have off-target effects, it may be that Volasertib impacted Myc expression, particularly at the transcript level, by PLK-1-independent mechanisms. Nonetheless, the authors provide good evidence that PLK-1 and MYC are likely involved in a positive-feedback loop that partakes in leukemia development promoted by the combination of mut*IL7R* and mut*NRAS*.

Evidently, the question that remained was whether these findings have translational potential. Using their transplant mouse model of mut*IL7R*+mut*NRAS* T-ALL, Winer, Li et al show that Volasertib diminished Myc levels and leukemia burden *in vivo*. There was no benefit in combining Volasertib with the Bcl-2 inhibitor Venetoclax. This is surprising, given the importance of Bcl-2 for IL-7R-mediated viability in T-ALL, and how MYC (which is downregulated by Volasertib *in vivo*) and BCL2 synergize to promote cancer development. The authors also tested JQ-1, a BET bromodomain BRD4 inhibitor, which downregulates

MYC and *IL7R* transcription. JQ-1 demonstrated similar *in vivo* effects to Volasertib, yet another demonstration of the importance of MYC in these leukemias.

Overall, the studies by Winer, Li and collaborators not only allow a better understanding of how oncogenic *IL7R* cooperates with *RAS* signaling in driving T-ALL (**Figure 1**), but also pave the way for preclinical studies testing the value of PLK1 and/or MYC inhibitors in human T-ALL patient samples, and patient-derived xenograft models, with IL7R and RAS pathway mutations.

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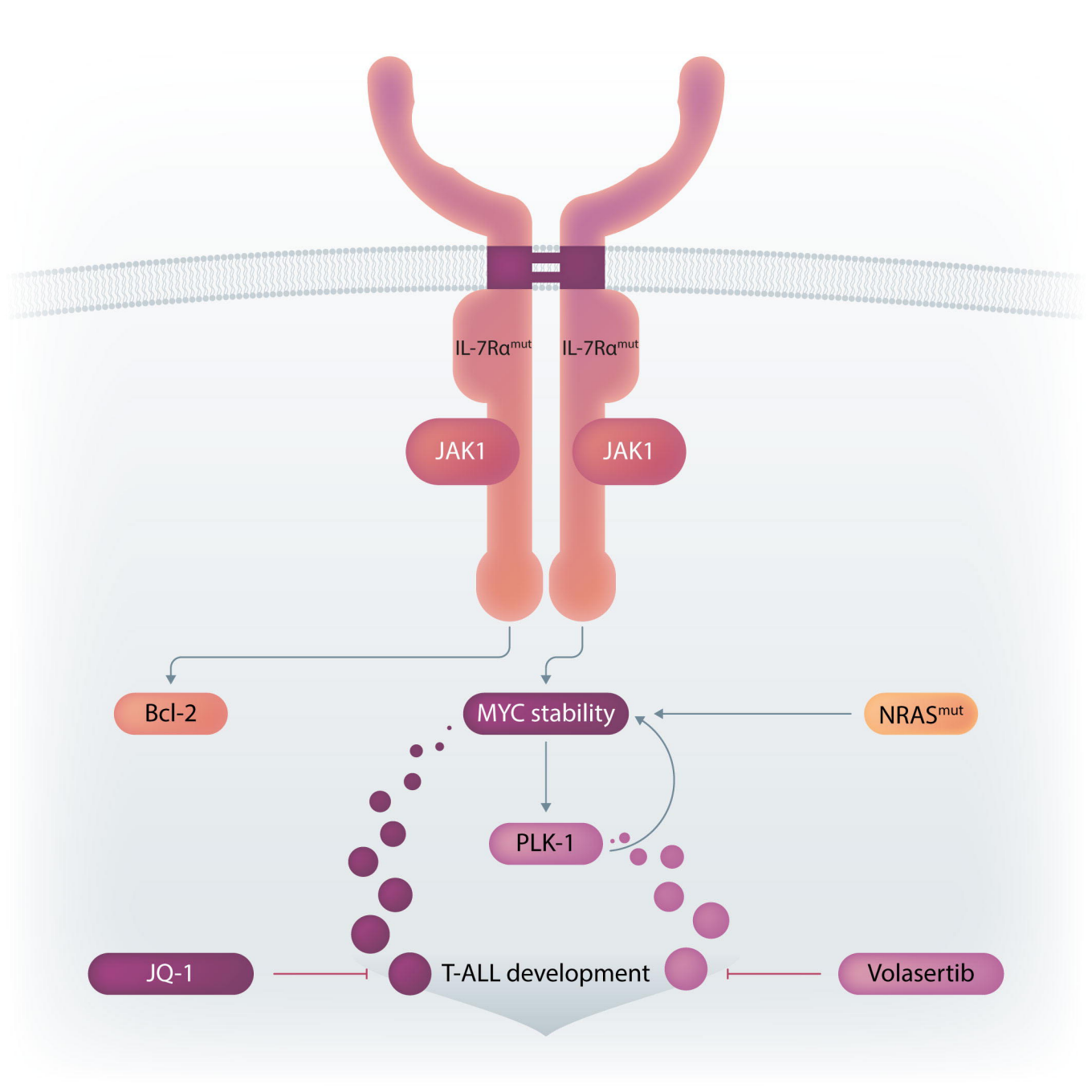
Figure Legend

Figure 1. Winer, Li et al demonstrate that gain-of-function (i.e. activating) mutations in *IL7R* and *NRAS* cooperate to drive T-ALL in mice mainly due to the ability to activate MYC, in particular via NRAS-dependent upregulation of MYC protein levels. NRAS likely promotes MYC protein stabilization by at least two mechanisms: directly, by phosphorylation of MYC and indirectly, via transcriptional activation of PLK-1, whose increased expression prevents MYC proteasomal degradation. Bcl-2 is also upregulated (essentially due to IL7R-mediated signaling), although its exact role in the cooperative oncogenic effects of *IL7R* and *NRAS* is unclear. The use of a chemical inhibitor of PLK-1 (Volasertib) or of a drug that downregulates *MYC* (and *IL7R*) transcription (JQ-1) diminishes leukemia burden *in vivo*. Pre-clinical studies evaluating the value of these drugs against human T-ALL with *IL7R* and *RAS* signaling pathway mutations are warranted. Original cartoon created by Marta Fernandes. Final version by somersault18:24.


IL-7Rα^mut
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JAK1
JAK1
Bcl-2
MYC stability
NRAS^mut
PLK-1
JQ-1
T-ALL development
Volasertib