EDITORIAL

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

João T. Barata

Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Correspondence: J.T. Barata
joao_barata@medicina.ulisboa.pt

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In this issue of *Haematologica*, Winer *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, and a much larger fraction (some 50-80% 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 *et al.* have 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 up-regulated 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-ALL 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 $\alpha\beta$ over $\gamma\delta$ 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.

Winer *et al.* further combined RNA-sequencing and mass-spectrometry to show that Bcl-2 transcript and protein were up-regulated.[1] Contrary to Myc, Bcl-2 up-regulation 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 up-regulates Bcl-2 is well known, although (in contrast to the illustration in Figure 8 of the paper) this is unlikely to be mediated by STAT5 in T-ALL.[8,10]

Contrary to Bcl-2, Myc was up-regulated exclusively at the protein level, and the authors propose that NRAS collabo-

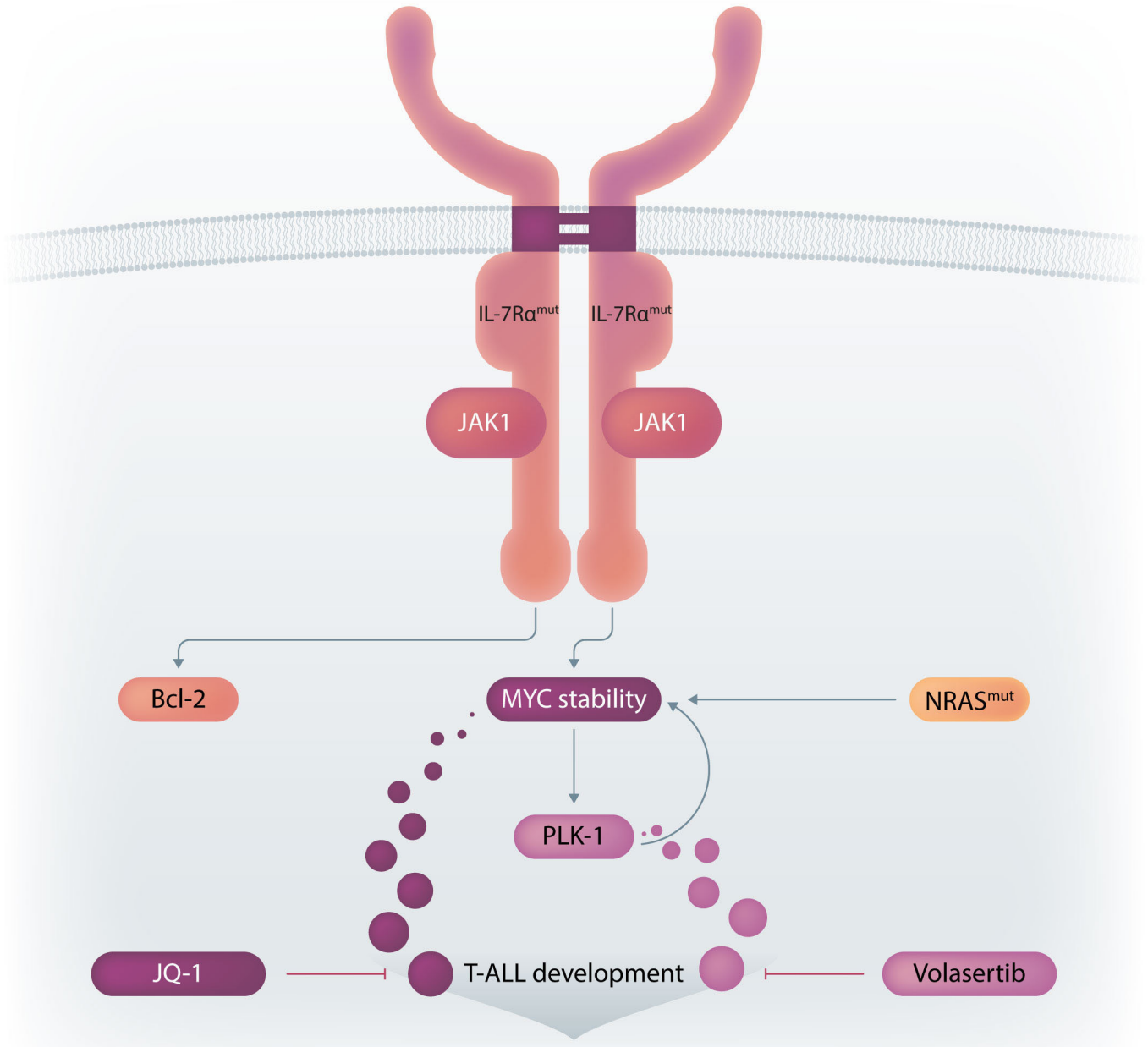

Figure 1. Winer *et al.* demonstrate that gain-of-function (i.e., activating) mutations in *IL7R* and *NRAS* cooperate to drive T-cell acute lymphoblastic leukemia (T-ALL) in mice mainly due to the ability to activate MYC, in particular via NRAS-dependent up-regulation of MYC protein levels. NRAS likely 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 up-regulated (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 down-regulates *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. Final version by somersault18:24.

rated 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 up-regulated 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 up-regulated 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 *et al.* noticed that PLK-1 (which contributes to MYC stabilization by preventing its proteasomal degradation) was up-regulated 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 down-regulated Myc protein but also, surprisingly, *Myc* transcript levels, suggesting that PLK-1 may up-regulate 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 *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 down-regulated by volasertib *in vivo*) and BCL2 synergize to promote cancer development. The authors also tested JQ-1, a BET bromodomain BRD4 inhibitor, which down-regulates *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 *et al.* 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 PLK-1 and/or MYC inhibitors in human T-ALL patient samples, and patient-derived xenograft models, with IL7R and RAS pathway mutations.

Disclosures

No conflicts of interest to disclose.

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