REFERENCES

1. Palladini G, Sachchithanantham S, Milani P, et al. A European collaborative study of cyclophosphamide, bortezomib, and dexamethasone in upfront treatment of systemic AL amyloidosis. *Blood.* 2015;126(5):612-615.

2. Cibeira MT, Sanchorawala V, Seldin DC, et al. Outcome of AL amyloidosis after high-dose melphalan and autologous stem cell transplantation: long-term results in a series of 421 patients. *Blood.* 2011;118(16):4346-4352.

3. Palladini G, Milani P, Foli A, et al. Oral melphalan and dexamethasone grants extended survival with minimal toxicity in AL amyloidosis: long-term results of a risk-adapted approach. *Haematologica.* 2014;99(4):743-750.

4. Kastritis E, Wechalekar AD, Dimopoulos MA, et al. Bortezomib with or without dexamethasone in primary systemic (light chain) amyloidosis. *J Clin Oncol.* 2010;28(6):1031-1037.

5. Mikhael JR, Schuster SR, Jimenez-Zepeda VH, et al. Cyclophosphamide-bortezomib-dexamethasone (CyBorD) produces rapid and complete hematologic response in patients with AL amyloidosis. *Blood.* 2012;119(19):4391-4394.

6. Venner CP, Lane T, Foard D, et al. Cyclophosphamide, bortezomib, and dexamethasone therapy in AL amyloidosis is associated with high clonal response rates and prolonged progression-free survival. *Blood.* 2012;119(19):4387-4390.

7. Jaccard A, Comenzo RL, Hari P, et al. Efficacy of bortezomib, cyclophosphamide and dexamethasone in treatment-naïve patients with high-risk cardiac AL amyloidosis (Mayo Clinic stage III). *Haematologica.* 2014;99(9):1479-1485.

8. Palladini G, Milani P, Foli A, et al. Melphalan and dexamethasone with or without bortezomib in newly diagnosed AL amyloidosis: a matched case-control study on 174 patients. *Leukemia.* 2014;28(12):2311-2316.

9. Venner CP, Gillmore JD, Sachchithanantham S, et al. A matched comparison of cyclophosphamide, bortezomib and dexamethasone (CVD) versus risk-adapted cyclophosphamide, thalidomide and dexamethasone (CTD) in AL amyloidosis. *Leukemia.* 2014;28(12):2304-2310.

IMMUNOBIOLOGY

Comment on Yi et al, page 620

Wipping p53 into subservience in B-cell development

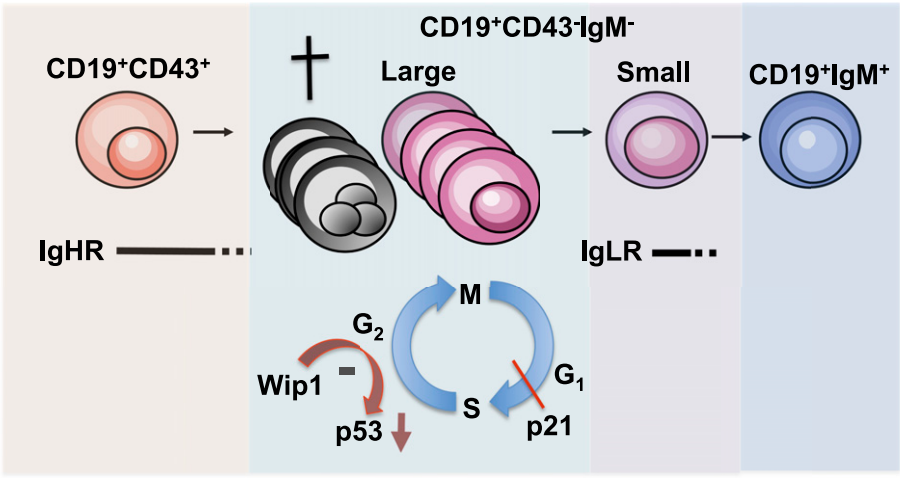
Mikael Sigvardsson LINKÖPING UNIVERSITY

In this issue of *Blood*, Yi et al reveal an important role for the protein phosphatase Wip1 (PPM1D) in the regulation of B-cell homeostasis.[1] Mice deficient in the *Wip1* gene display increased apoptosis in the pre–B-cell compartment and a reduction in peripheral B-cell numbers, a phenotype exacerbated with age and upon serial transplantations of bone marrow (BM) cells.[1] Even though Wip1 has the ability to modulate multiple signaling pathways in the cell, the restoration of B-cell numbers upon deletion of the *p53* gene[1] suggests that an autoregulatory loop between p53 and Wip1 is of importance to maintain normal production of B lymphocytes.

The development of mature B lymphocytes from hematopoietic stem cells in the BM is a complex process in which proliferation and expansion need to be coordinated with DNA recombination events as well as the selection of functional progenitor cells (see figure). In order to better understand the molecular interplay underlying the homeostatic expansion of B-lymphoid cells, Yi and colleges explored the functional role of the p53-activated serine/threonine protein phosphatase Wip1[2] in the formation and expansion of B-lymphoid cells.[1] *Wip1* was originally identified as a p53 target gene,[2] and subsequent experiments revealed that this protein acts in an autoregulatory loop to reduce the functional activity of p53.[3] The current report reveals that in the absence of Wip1, the formation of the pre–B-cell compartment is impaired.[1] The early pre–B-cell stage represents one of the major windows for cell proliferation in early B-cell development, as coordinated interleukin-7 and pre–B-cell receptor signaling drives the proliferation of cells carrying a functional immunoglobulin (Ig) heavy-chain gene,[4] and it is therefore reasonable that targeting of this developmental stage impairs the formation of mature B-lineage cells. p53 can block cell-cycle progression by induction of p21 expression; however, deletion of p21 could not rescue B-cell development in Wip1-deficient mice, arguing against the idea that the loss of pre–B cells would be due to a disruption of cell-cycle progression.[1] Analysis of the cell-cycle status of defined B-cell compartments supported this notion, because no significant differences in G_1-S-G_2 composition could be detected. Rather, the phenotype appeared to be related to an increased apoptosis in the pre–B-cell compartment, a phenotype that could be rescued by loss of p53 function, indicating a need for harnessing p53 to control apoptosis in early B-cell development.

p53 is activated by DNA damage, and it is tempting to speculate that Ig rearrangements, known to induce double-strand breaks and a DNA damage response, would result in increased p53 activity that needs to be modulated by Wip1. However, while a CDK/cyclin A–mediated degradation of RAG-2 restricts Ig recombination to the G_1 phase of the cell cycle,[5] Wip1 has been suggested to act mainly in G_2 phase, where the protein is able to potently release a cell-cycle block.[6] Furthermore, DNA damage generated during G_1 phase would likely result in sustained p21 expression and a cell-cycle block at the G_1-S transition. Hence, the p53 response modulated by Wip1 is unlikely to be related to the Ig recombination process per se but rather to other DNA-damaging events occurring during the replication process.

The reduction in pre–B-cell numbers in Wip1-deficient mice could not be compensated for by peripheral expansion of mature B cells because the reduction in cell numbers was consistent in blood, lymph nodes, and spleen,[1] possibly indicating an additional need of modulated p53 activity in peripheral cells. Even though Wip1 deficiency results in impaired T-cell development[7] and an expanded peripheral pool of neutrophils[8] that could potentially impact the peripheral expansion of the mature B-cell compartments, Yi et al use BM chimera experiments to demonstrate that Wip1 deficiency impacts B-cell development in a cell-autonomous manner.[1] The impact of reduced Wip1 activity was further exacerbated upon serial transplantation or aging, suggesting an involvement of immature long-lived progenitor compartments. In line with this notion, the exacerbated phenotype was associated with reduced numbers of pre-pro– and pro–B cells that was not observed to the same extent in young adult mice.[1] In further support of a role for Wip1 in early progenitors, recently published data suggest that regulation of p53 activity by Wip1 impacts



Stage-specific expansion in the early B-cell compartment. The figure displays a schematic drawing of early B-cell development indicating stage-specific recombination events of the immunoglobulin heavy-chain (IgHR) and light-chain (IgLR) genes and the expansion or deletion (apoptosis indicated by a cross) of early pre–B cells. The lower part of the figure indicates the specific stages of the cell cycle (G_1 [gap I], S [synthesis], G_2 [gap II], and M [mitosis]), as well as the cell-cycle block in G_1 induced by p21 and the ability of Wip1 to modulate the activity of p53 in G_2 phase.

long-lived hematopoietic progenitors in the BM.[9]

Although there exists an apparent need to harness p53 activity to achieve normal B-cell production, there is likely a need to balance rather than abolish the activity of this protein, because loss of p53 function is a common event in human malignancies. Of special interest in the context of this report is the finding that a mutated or deleted *TP53* gene is one of the strongest independent predictors of inferior treatment outcome in childhood B-lineage acute lymphoblastic leukemia.[10] In a situation where the functional dose of a transcription factor like p53 is of critical importance, the use of an autoregulatory loop such as that created by the induction of *Wip1* transcription by p53 presents an elegant solution to preserve a high output of normal cells while still preventing uncontrolled malignant proliferation of progenitor cells. Therefore, the extended insight to Wip1 function provided by this report is an important contribution to our understanding of regulatory events in early B-lymphocyte development.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Yi W, Hu X, Chen Z, et al. Phosphatase Wip1 controls antigen-independent B-cell development in a p53-dependent manner. *Blood.* 2015;126(5):620-628.

2. Fiscella M, Zhang H, Fan S, et al. Wip1, a novel human protein phosphatase that is induced in response to ionizing radiation in a p53-dependent manner. *Proc Natl Acad Sci USA.* 1997;94(12):6048-6053.

3. Takekawa M, Adachi M, Nakahata A, et al. p53-inducible wip1 phosphatase mediates a negative feedback regulation of p38 MAPK-p53 signaling in response to UV radiation. *EMBO J.* 2000;19(23):6517-6526.

4. Ochiai K, Maienschein-Cline M, Mandal M, et al. A self-reinforcing regulatory network triggered by limiting IL-7 activates pre-BCR signaling and differentiation. *Nat Immunol.* 2012;13(3):300-307.

5. Lee J, Desiderio S. Cyclin A/CDK2 regulates V(D)J recombination by coordinating RAG-2 accumulation and DNA repair. *Immunity.* 1999;11(6):771-781.

6. Shaltiel IA, Aprelia M, Saurin AT, et al. Distinct phosphatases antagonize the p53 response in different phases of the cell cycle. *Proc Natl Acad Sci USA.* 2014;111(20):7313-7318.

7. Schito ML, Demidov ON, Saito S, Ashwell JD, Appella E. Wip1 phosphatase-deficient mice exhibit defective T cell maturation due to sustained p53 activation. *J Immunol.* 2006;176(8):4818-4825.

8. Liu G, Hu X, Sun B, et al. Phosphatase Wip1 negatively regulates neutrophil development through p38 MAPK-STAT1. *Blood.* 2013;121(3):519-529.

9. Chen Z, Yi W, Morita Y, et al. Wip1 deficiency impairs haematopoietic stem cell function via p53 and mTORC1 pathways. *Nat Commun.* 2015;6:6808.

10. Krentz S, Hof J, Mendioroz A, et al. Prognostic value of genetic alterations in children with first bone marrow relapse of childhood B-cell precursor acute lymphoblastic leukemia. *Leukemia.* 2013;27(2):295-304.

● ● ● LYMPHOID NEOPLASIA

Comment on Qin et al, page 629

TSLPR: a new CAR in the showroom for B-ALL

David Marc Davies[1] and John Maher[1,2,3] [1]KING'S COLLEGE LONDON; [2]BARNET HOSPITAL; [3]KING'S COLLEGE HOSPITAL NHS FOUNDATION TRUST

In this issue of *Blood*, Qin et al demonstrate the ability of chimeric antigen receptor (CAR)-engineered T cells targeted against thymic stromal lymphopoietin receptor (TSLPR) to eradicate disease in several models of B-cell acute lymphoblastic leukemia (B-ALL) that overexpress this protein.[1]

Chimeric antigen receptors are genetically delivered fusion proteins that redirect the specificity of polyclonal T cells or natural killer cells against a chosen cell surface molecule. Constructs consist of a targeting moiety such as a peptide, ligand derivative, or antibody-derived single-chain variable fragment (scFv) that is coupled in series to a hinge/spacer, membrane-spanning element, and signaling endodomain (see figure, part A). Target antigen is engaged in its native conformation, rather than as a processed peptide displayed within the groove of a human leukocyte antigen (HLA) molecule. Consequently, CARs recognize target cells irrespective of a patient's HLA haplotype. Furthermore, their function is unhindered by a common immune evasion strategy that is deployed in acute lymphoblastic leukemia (ALL) and other malignancies, namely, the selective downregulation of some HLA allo-specificities. In so-called "first-generation" CARs, the endodomain generally contains CD3ζ alone, thereby providing signals that mimic those naturally provided by the T-cell receptor/CD3 complex. Second- and third-generation receptors are distinct in that they respectively contain either 1 or 2 additional costimulatory motifs, commonly