

In myeloma, several of these immunotherapies are now in the clinic and have improved outcomes. These include daratumumab, an anti-CD38 antibody, and elotuzumab, a monoclonal antibody targeting SLAMF7.² More recently, BCMA has been identified as a key target in myeloma and used as a platform for different immunotherapy formats.³ Belantamab mafodotin was the first BCMA-targeting agent approved for myeloma. BCMA CAR-T has shown remarkable activity, with the first, idecabtagene vicleucel, approved for clinical use.⁴ BCMA bispecific T-cell engagers have also shown activity in myeloma and several are in clinical trials. Other targets, GPRC5D and FcRH5, have also shown promise.⁵

However, none of these treatments is currently curative. Loss of therapeutic activity eventually happens through various mechanisms, including antigen loss through shedding or even genetic deletions,⁶ as well as interference by soluble ligands.⁷ Therefore, the identification of additional targets is important for salvage therapy following relapse from existing immunotherapy. The use of combinations of targets may also provide broader antigen coverage that may improve outcomes.

In this context, the current work is important for a few reasons. The authors identified a new immunotherapeutic target, SEMA4A, that has higher expression than BCMA and SLAMF7 in myeloma cells and is rapidly internalized, making it a good target for ADCs. ADCs are worth further exploration in myeloma because they do not rely on the immune system of the patients, which tend to be impaired from treatment and disease. SEMA4A may also be a good target because it has minimal shedding and seems to be essential to the survival of myeloma cells, making it less likely to be genetically deleted. In addition, the techniques used by the authors can be replicated in other cancers. Their vector-based scoring system to prioritize candidate proteins is flexible, allowing for adjustment of the parameters used and the weightage assigned to each parameter. In the current study, the 3 parameters used included high expression in myeloma cells, low expression in normal tissues, and the size of ectodomain as a surrogate for the number of unique B-cell epitopes. Despite the use of this scoring system, SEMA4A is also

expressed in normal plasma cells and may be a marker of late B-cell differentiation. It is also expressed to some extent on monocytes. Although it is true that plasma cell and monocyte depletion may not lead to significant short-term toxicities but there may be long-term immunosuppression, including inadequate immune response to vaccination, particularly with long-term continuous use or persistence in the case of CAR-T. This has been highlighted by current studies of response to COVID vaccination in patients with myeloma treated with daratumumab and BCMA targeting therapies.⁸

The identification of membrane antigens or epitopes that differentiate malignant from normal cells remains an important challenge.⁹ Most, if not all, antibody-based immunotherapeutic targets are antigens that are also expressed on normal cellular counterparts. Therefore, although effective in eradicating the blood cancer cells, these immunotherapies also significantly affect normal immune cells, resulting in significant immunosuppression with increased risk of infections. We may need to look beyond proteins but also cancer-specific modifications on the proteins.¹⁰

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

REFERENCES

1. Anderson GSF, Ballester-Beltran J, Giotopoulos G, et al. Unbiased cell surface proteomics identifies SEMA4A as an

effective immunotherapy target for myeloma. *Blood*. 2022;139(16):2471-2482.

2. Maples KT, Johnson C, Lonial S. Antibody treatment in multiple myeloma. *Clin Adv Hematol Oncol*. 2021;19(3):166-174.
3. Shah N, Chari A, Scott E, Mezzi K, Usmani SZ. B-cell maturation antigen (BCMA) in multiple myeloma: rationale for targeting and current therapeutic approaches. *Leukemia*. 2020;34(4):985-1005.
4. Rasche L, Wäsch R, Munder M, Goldschmidt H, Raab MS. Novel immunotherapies in multiple myeloma – chances and challenges. *Haematologica*. 2021;106(10):2555-2565.
5. Swan D, Routledge D, Harrison S. The evolving status of immunotherapies in multiple myeloma: the future role of bispecific antibodies. *Br J Haematol*. 2021; 196(3):488-506.
6. Da Vià MC, Dietrich O, Truger M, et al. Homozygous BCMA gene deletion in response to anti-BCMA CAR T cells in a patient with multiple myeloma. *Nat Med*. 2021;27(4):616-619.
7. Chen H, Li M, Xu N, et al. Serum B-cell maturation antigen (BCMA) reduces binding of anti-BCMA antibody to multiple myeloma cells. *Leuk Res*. 2019;81:62-66.
8. Van Oekelen O, Gleason CR, Agte S, et al; PVI/Seronet team. Highly variable SARS-CoV-2 spike antibody responses to two doses of COVID-19 RNA vaccination in patients with multiple myeloma. *Cancer Cell*. 2021;39(8):1028-1030.
9. Teoh PJ, Chng WJ. CAR T-cell therapy in multiple myeloma: more room for improvement. *Blood Cancer J*. 2021;11(4):84.
10. Boligan KF, Mesa C, Fernandez LE, von Gunten S. Cancer intelligence acquired (CIA): tumor glycosylation and sialylation codes dismantling antitumor defense. *Cell Mol Life Sci*. 2015;72(7):1231-1248.

DOI 10.1182/blood.2022015481

© 2022 by The American Society of Hematology

LYMPHOID NEOPLASIA

Comment on Antoszewski et al, page 2483

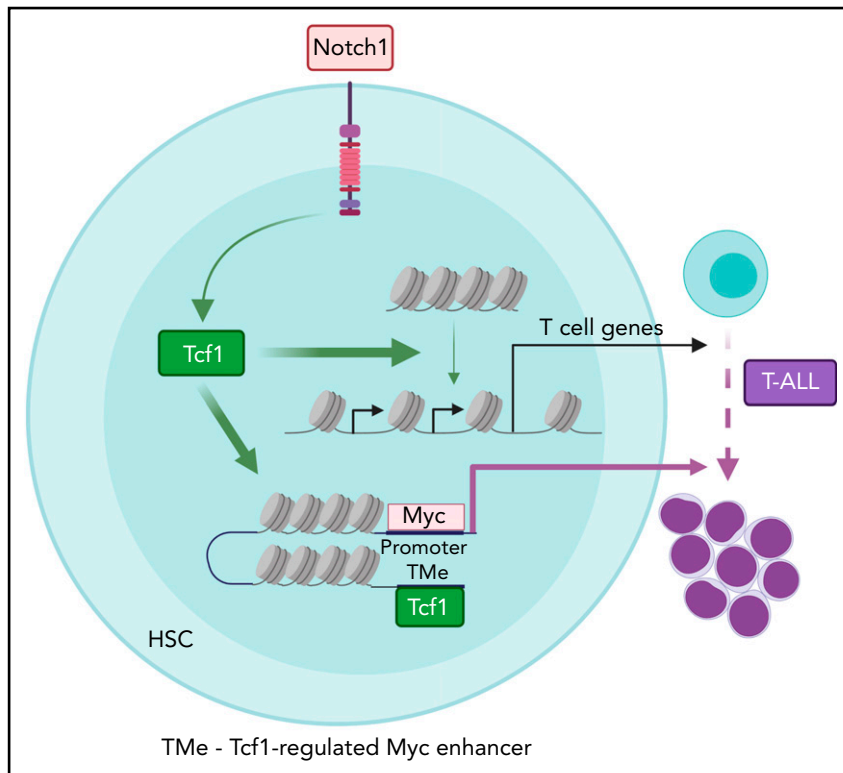
Firing up chromatin to forge T-ALL

Mark Y. Chiang | University of Michigan School of Medicine

In this issue of *Blood*, Antoszewski et al report that the transcription factor T-cell factor 1 (Tcf1) remodels chromatin in Notch-activated early hematopoietic progenitor cells to promote transformation through oncogenes like MYC.¹

In 1957, Francis Crick put forth the central dogma of information flowing from DNA to RNA. Since then, we have learned that this process, called transcription, is tightly regulated in highly complicated structures. In the current

model of transcription, sequence-specific transcription factors bind DNA, forming macromolecular complexes that displace nucleosomes at promoter and enhancer elements. Next, chromosomal looping brings enhancers into proximity of the



Model depicting the genome-scale actions of T-cell factor 1 (Tcf1) in promoting chromatin accessibility for T-cell development and leukemogenesis. Physiological Notch signaling directs Tcf1 to displace nucleosomes, paving the way for additional transcription factors to bind and induce T-cell differentiation genes. In contrast, supraphysiological Notch signaling directs Tcf1 to open a newly appreciated myelocytomatosis protooncogene (Myc) enhancer (TMe) that transforms Notch actions from physiological to oncogenic. HSC, hematopoietic stem cell. See the visual abstract in the article by Antoszewski et al that begins on page 2483.

transcriptional start site. These enhancer-promoter interactions are restricted within giant folds of chromatin called topologically associating domains (TADs). Finally, RNA polymerase binds and initiates transcription. Since these transcriptional structures are so intricate, developing cancer cells often find it easier to transform from normal cells by putting these structures into overdrive rather than creating new ones from scratch. A classic example are the structures created by supraphysiological Notch signaling in T-cell acute lymphoblastic leukemia (T-ALL). During normal T-cell development, ligands on stromal cells activate Notch1 receptors on the surface of hematopoietic precursors to promote T-cell commitment and robust expansion (see figure).² But unfortunate events like chromosomal translocations or mutations can raise Notch signaling to hyperactive levels, inducing overexpression of oncogenic target genes like *MYC* through native promoter-enhancer structures.^{3,4} Normal T-cell precursors are transformed into malignant T-ALL, a devastating outcome.

T-ALL is an aggressive hematological malignancy, accounting for ~15% of pediatric ALL and ~25% of adult T-ALL cases. Adult T-ALL show only ~50% long-term survival, whereas children fare better but are at high risk for long-term toxicities. To improve outcomes, investigators initially sought to inhibit Notch signaling with drugs. However, early clinical trials showed that pan-Notch inhibitors were too toxic. Because of this, investigators turned to identifying target genes of Notch in hopes of finding more attractive therapeutic targets. Although these studies revealed T-ALL drivers important for diverse cancerous functions, it remained unclear which target genes of Notch can remodel chromatin to set the stage for T-ALL transformation. Notch itself does not have this function. With the emergence of protein-protein interaction inhibitors, protein degraders, and covalent inhibitors in the modern era, transcriptional regulators are no longer thought of as “undruggable.” In this context, Antoszewski et al reveal the key role played by the transcription factor Tcf1, which is encoded by the Notch target gene

Tcf7, in shaping chromatin architecture to facilitate initiation of Notch-induced T-ALL.

Antoszewski et al zeroed in on Tcf1 because it has “pioneer” activity, which is the ability to open repressive chromatin to allow other transcription factors to bind.⁵ During early T-cell development, Notch signaling directly induces Tcf1 expression, which establishes an epigenetic landscape that promotes expression of genes important for T-cell fate.⁵⁻⁷ To test whether Tcf1 is also important for T-ALL initiation, Antoszewski et al conditionally deleted *Tcf7* in a genetically engineered mouse model of Notch-induced T-ALL. T-ALL initiation was abrogated. In contrast, deletion of the gene encoding β -catenin, a well-known Tcf1 cofactor in the Wnt pathway, had less impressive effects. Thus, Tcf1 primarily functions in a β -catenin/Wnt-independent manner. To determine the mechanism, Antoszewski et al applied a comprehensive suite of genomic tools to analyze chromatin 3D architecture, chromatin accessibility, enhancer activity, and gene expression in Notch-activated mouse bone marrow hematopoietic progenitor cells. *Tcf7* loss in these rare cells reversed many Notch-induced changes in chromatin accessibility and T-cell specific gene expression. *Tcf7* loss also reversed some effects of Notch on TAD boundaries and enhancer-promoter interactions, which often correlated with effects on gene expression and enhancer activity. Next, Antoszewski et al focused on a Notch-dependent distal *Myc* enhancer region that is known to drive T-ALL transformation and maintenance. *Tcf7* loss not only rendered this region inaccessible in Notch-activated cells but also another enhancer ~14 kb more distal, which they nicknamed “TMe” for Tcf1-regulated *Myc* enhancer. To establish the importance of this enhancer, they deleted the TMe in their mouse models. Strikingly, TMe deletion disabled Notch-induced T-cell leukemogenesis while preserving Notch-induced T-cell development.

The Antoszewski et al study merges high quality and elegant mouse modeling with comprehensive and technically challenging differential analysis of chromatin structure in rare hematopoietic progenitor cells. These data combined with previous literature strongly implicate *Tcf7* as an important Notch target gene that

shapes the chromatin environment for other transcription factors to bind and intensify oncogene expression. Unfortunately, the lack of a high-quality Tcf1 antibody for chromatin immunoprecipitation sequencing makes it difficult to separate direct vs indirect effects as downstream targets of Tcf1, like Gata3, have chromatin remodeling activity.⁸ It also remains unclear whether transforming cells stimulated by weaker Notch signals, such as through Notch ligand-receptor interactions, access oncogenic regulatory regions through similar mechanisms. Given its importance as a primary regulator of chromatin accessibility in early T cells,⁵ Tcf1 likely plays a role even in such cases. It is also unclear whether Tcf1 maintains chromatin architecture in established T-ALL or becomes superseded by other factors like Gata3.⁸ Other groups showed that Notch signaling itself has limited activity in this regard in cancer.^{9,10} Finally, it is unclear how Notch1/Tcf1 mechanisms at native cis-elements interact with a dysregulated sea of aberrancies in transcriptional regulators, DNA methylation, and mutations in promoters, enhancers, and insulators during human T-ALL initiation and maintenance. Future studies will be needed.

In an impressively ambitious and resource-heavy undertaking, Antoszewski et al rewards us with a genome-scale understanding of how Notch and Tcf1 transform chromatin into a nurturing environment for the development of T-cell leukemia. We have learned that Notch signaling directs Tcf1 to evict nucleosomes from repressed chromatin to recruit oncogenic transcription factors that fire up enhancers important for T-cell commitment and expansion (see figure). We have even seen that a previously unrecognized MYC enhancer (TME) can separate oncogenic from physiological Notch functions, which might be key to targeting Notch without intolerable side effects. By dissecting the interactions at these oncogenic enhancers, we might find safe ways to quench their activities and reprogram malignant T cells back to their normal selves.

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

REFERENCES

1. Antoszewski M, Fournier N, Ruiz Buendía GA, et al. Tcf1 is essential for initiation of

oncogenic Notch1-driven chromatin topology in T-ALL. *Blood*. 2022;139(16):2483-2498.

2. Radtke F, Wilson A, Stark G, et al. Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity*. 1999;10(5):547-558.
3. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*. 2004;306(5694):269-271.
4. McCarter AC, Wang Q, Chiang M. Notch in leukemia. *Adv Exp Med Biol*. 2018;1066:355-394.
5. Johnson JL, Georgakilas G, Petrovic J, et al. Lineage-determining transcription factor TCF-1 initiates the epigenetic identity of T cells. *Immunity*. 2018;48(2):243-257.e10.
6. Weber BN, Chi AW, Chavez A, et al. A critical role for TCF-1 in T-lineage specification and differentiation. *Nature*. 2011;476(7358):63-68.

7. Germar K, Dose M, Konstantinou T, et al. T-cell factor 1 is a gatekeeper for T-cell specification in response to Notch signaling. *Proc Natl Acad Sci USA*. 2011;108(50):20060-20065.
8. Belver L, Yang AY, Alberio R, et al. GATA3-controlled nucleosome eviction drives MYC enhancer activity in T-cell development and leukemia. *Cancer Discov*. 2019;9(12):1774-1791.
9. Kloetgen A, Thandapani P, Ntziachristos P, et al. Three-dimensional chromatin landscapes in T cell acute lymphoblastic leukemia. *Nat Genet*. 2020;52(4):388-400.
10. Petrovic J, Zhou Y, Fasolino M, et al. Oncogenic Notch promotes long-range regulatory interactions within hyperconnected 3d cliques. *Mol Cell*. 2019;73(6):1174-1190.e12.

DOI 10.1182/blood.2021015364

© 2022 by The American Society of Hematology

LYMPHOID NEOPLASIA

Comment on Wilson et al, page 2499

CNS prophylaxis in DLBCL: first do no harm

Craig A. Portell | University of Virginia

In this issue of *Blood*, Wilson et al¹ present a retrospective analysis of the timing of intravenous high-dose methotrexate (HD-MTX) during first-line treatment of diffuse large B-cell lymphoma (DLBCL). They found that intravenous HD-MTX given at the end of treatment (EOT) was equally effective for central nervous system (CNS) prophylaxis and less toxic than MTX given earlier (ie, between cycles of anthracycline-based immunochemotherapy).

Secondary progression of DLBCL into the CNS after initial treatment with standard anthracycline-based immunochemotherapy is a devastating event. Several groups have identified risk factors for secondary CNS progression and have developed the CNS International Prognostic Index (CNS-IPI)² that identifies high-, intermediate-, and low-risk groups. Because the high-risk group has an ~10% risk of progression in the CNS at 2 years, strategies to prevent CNS progression have been explored.³ The blood-brain barrier prevents many treatments from entering the CNS space, and MTX has long been used as a method for treating and preventing CNS progression. It has been speculated that CNS progression occurs early in the course of treatment, and thus, should be intercalated into standard immunochemotherapy.³ However, guidance has largely come from expert opinion developed

from subgroup analyses of prospective and retrospective studies. Rigorous evaluations focused on the utility of CNS prophylaxis are lacking.

Thus, Wilson et al performed a wide-ranging retrospective analysis of the timing of CNS prophylaxis with intravenous HD-MTX occurring during or after immunochemotherapy. Their study includes 1384 patients with DLBCL or high-grade B-cell lymphoma not otherwise specified who were diagnosed between 2007 and 2020 at 47 centers in Europe, Australia, and North America. It should be noted that roughly half the patients (46.1%) also received intrathecal MTX, with more patients in the group that received MTX after immunochemotherapy than in the group that received MTX during immunochemotherapy. Ultimately, there was no significant difference in CNS progression rates when HD-MTX was given