preventive vaccines and immunotherapy treatments.

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

REFERENCES

1. Vietzen H, Furlano PL, Cornelissen JJ, et al. HLA-E-restricted immune responses are crucial for the control of EBV infections and the prevention of PTLD. *Blood*. 2023; 141(13):1560-1573.
2. Nourse JP, Jones K, Dua U, et al. Fulminant infectious mononucleosis and recurrent Epstein-Barr virus reactivation in an adolescent. *Clin Infect Dis*. 2010;50(6): e34-e37.
3. Opat S, Hearps AC, Thia K, et al. Adaptive reprogramming of NK cells in X-linked lymphoproliferative syndrome. *Blood*. 2018; 131(6):699-702.
4. Diepstra A, Niens M, Vellenga E, et al. Association with HLA class I in Epstein-Barr-virus-positive and with HLA class III in Epstein-Barr-virus-negative Hodgkin's lymphoma. *Lancet*. 2005;365(9478): 2216-2224.
5. McAulay KA, Higgins CD, Macsween KF, et al. HLA class I polymorphisms are associated with development of infectious mononucleosis upon primary EBV infection. *J Clin Invest*. 2007;117(10):3042-3048.
6. Jones K, Nourse JP, Morrison L, et al. Expansion of EBNA1-specific effector T cells in posttransplantation lymphoproliferative disorders. *Blood*. 2010;116(13):2245-2252.
7. Jones K, Wockner L, Brennan RM, et al. The impact of HLA class I and EBV latency-II antigen-specific CD8(+) T cells on the pathogenesis of EBV(+) Hodgkin lymphoma. *Clin Exp Immunol*. 2016;183(2): 206-220.
8. Gandhi MK, Hoang T, Law SC, et al. EBV-associated primary CNS lymphoma occurring after immunosuppression is a distinct immunobiological entity. *Blood*. 2021;137(11):1468-1477.
9. Jorgensen PB, Livbjerg AH, Hansen HJ, Petersen T, Hollsberg P. Epstein-Barr virus peptide presented by HLA-E is predominantly recognized by CD8(bright) cells in multiple sclerosis patients. *PLoS One*. 2012;7(9):e46120.
10. Mbiribindi B, Pena JK, Arvedson MP, et al. Epstein-Barr virus peptides derived from latent cycle proteins alter NKG2A + NK cell effector function. *Sci Rep*. 2020;10(1):19973.

https://doi.org/10.1182/blood.2022019180

LYMPHOID NEOPLASIA

Comment on Stong et al, page 1574

Highs and lows of t(4;14) in multiple myeloma

Bruno Paiva and Maria-Jose Calasanz | Clinica Universidad de Navarra

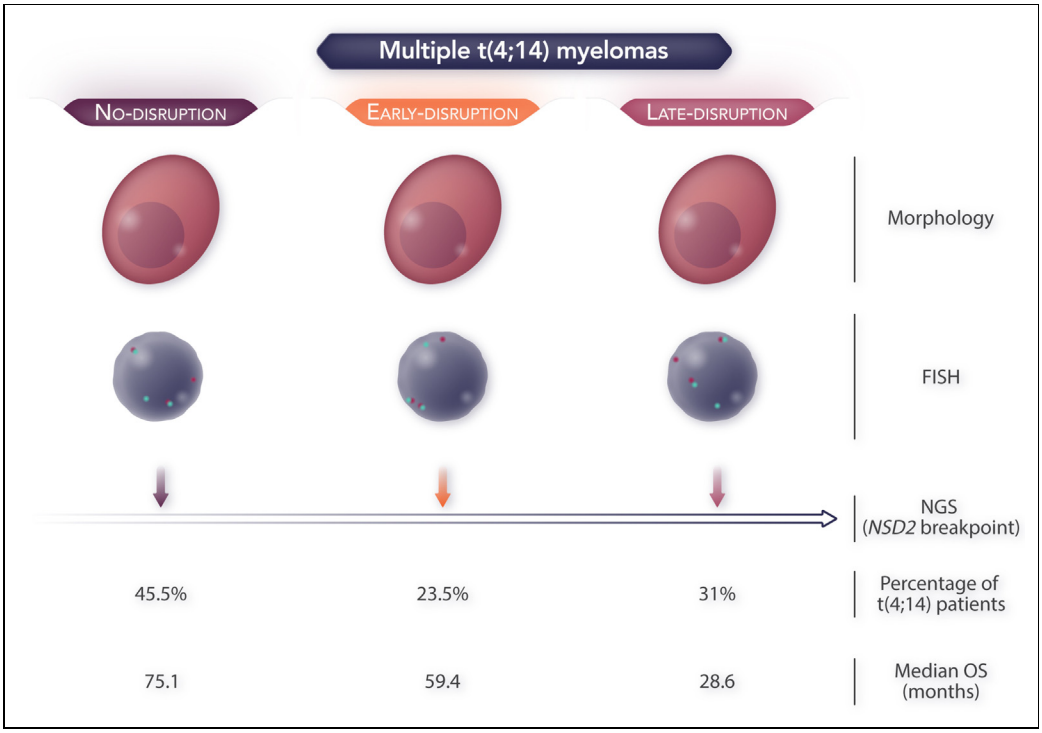
In this issue of *Blood*, Stong et al show that true high-risk t(4;14) multiple myeloma (MM) patients can be identified by using the coordinates of the translocation breakpoints in the *NSD2* gene.[1] The authors provide an elegant and detailed characterization of a single genetic alteration that improves our understanding of disease biology and prediction of clinical outcomes (see figure).

Among the first reports showing the presence of t(4;14) in MM were those published in 1997 by Chesi et al[2] and Richelda et al.[3] One year later, Chesi et al[4] demonstrated that the t(4;14) was an interesting example of an IgH translocation that simultaneously dysregulates 2 genes with oncogenic potential: *FGFR3* and *MMSET*, which is currently named *NSD2*. In 2001, Fonseca et al showed that the t(4;14) was strongly associated with chromosome 13 abnormalities.[5] This finding was confirmed in the comprehensive analysis performed by Stong et al,[1] which further uncovered a constellation of copy number alterations and somatic mutations that were enriched in t(4;14) patients. Most interestingly, *FGFR3* mutations were exclusive to these and absent in non-t(4;14) patients, but such mutations had no impact in survival.[1]

In 2001 and 2003, Rasmussen et al[6] and Keats et al[7] concluded that, in MM, t(4;14) is an adverse prognostic factor irrespective of *FGFR3* expression. Notably, Stong et al[1] confirmed this finding and uncovered that expression of *NSD2* was also unrelated to poor outcome. In 2013, Walker et al[8] performed whole genome sequencing and identified breakpoint locations upstream of the *NSD2* gene or within the coding sequence. Other groups have suggested a potential association between expression of *NSD2* truncated isoforms (resulting from breakpoint locations within the coding sequence) and a poor prognosis, but the study from Stong et al, performed in the largest cohort of 258 t(4;14) newly diagnosed MM patients (153 discovery and 105 independent replication), showed unequivocally that only those with a breakpoint within the *NSD2* gene and downstream of the translation start site (coined as "late disruption"; 31%) have a dismal overall survival.[1] Patients with a breakpoint between the transcription and translation start site ("early disruption"; 23.5%) and upstream ("no disruption"; 45.5%) of the *NSD2* gene displayed progressively longer survival.[1] Importantly, risk stratification according to the 3 breakpoint regions was superior to that achieved with previously identified *NSD2* truncated isoforms.[1] Thus, an *NSD2* breakpoint analysis is the way forward to identify high-risk t(4;14) patients.

The authors have probably generated the largest dataset on t(4;14) MM, which includes whole genome and RNA sequencing data. The latter were used to analyze fusion *NSD2* transcripts, which confirmed in most patients the correlation between the no disruption or early disruption and full-length fusion transcripts, as well as between late disruption and truncated fusion transcripts.[1] Further investigation from this group using data from RNA sequencing will be an important sequel of this article, hopefully identifying novel therapeutic targets for t(4;14) MM. The identification of true high-risk t(4;14) may prove extremely useful for the initial use of targeted therapy for this genetic risk group. The median overall survival of patients with no disruption, early disruption, and late disruption t(4;14) was 75.1, 59.4 and 28.6 months, respectively.[1]

The discovery and independent replication cohorts included patients receiving numerous induction regimens and transplant-based and nontransplant approaches, as well as maintenance of fixed vs continuous duration. Thus, although targeted therapies are eagerly awaited for this and other genetic subgroups, future analyses should address



The presence of multiple t(4;14) myelomas is illustrated in this schematic figure. Tumor cells from patients with t(4;14) may look similar under the microscope during morphologic and cytogenetic assessments (using fluorescence in situ hybridization [FISH]). Yet, identifying the coordinates of the translocation breakpoints in the *NSD2* gene using next-generation sequencing (NGS), could uncover the presence of no, early, and late disruption subgroups with different median overall survival (OS). Professional illustration by Somersault18:24.

whether the dismal survival of patients with late disruption t(4;14) can be improved with the nuances of current treatment approaches, including the use of anti-CD38 monoclonal antibodies upfront. In such analyses, that would probably require an even larger series of patients with t(4;14), it will be interesting to investigate whether the multiparameter definition of cytogenetic risk proposed by the same authors[9] is able to improve outcome predictions in each of the newly defined t(4;14) molecular subgroups.

Both the late and early disruption *NSD2* breakpoint, as well as del(17p), del(1p), and 1qAmp, were significantly associated with inferior overall survival in a multivariate analysis, although age or the International Staging System (ISS) evaluation were not.[1] These data, along with prior publications by some of the same authors that identified del(17p) with cancer clonal fraction of 0.55 or higher and 1qAmp as high-risk features, "makes a scientific case to discuss modifications of the revised ISS (R-ISS) criteria to define high-risk MM."[1] Interestingly, a second revision of the R-ISS (R2-ISS) was recently proposed, which includes chromosome 1q gain/amplification, that outperforms the R-ISS.[10] The authors might be correct in their claim, but new staging systems must prove superiority to the R2-ISS, and should be easily performed worldwide. Unfortunately, next-generation sequencing or polymerase chain reaction-based approaches to characterize high-risk t(4;14) are not performed routinely. Many groups support a progressive replacement of karyotyping and fluorescence in situ hybridization by targeted sequencing; the study from Stong et al nicely shows that such a replacement is not only about studying more genetic alterations using a single assay, but also detailed characterization of selected abnormalities for improved risk stratification in MM. A targeted sequencing panel should therefore analyze the coordinates of the translocation breakpoints in the *NSD2* gene. The authors should be commended for showing why and how this should be done.

Conflict-of-interest disclosure: B.P. reports honoraria for lectures from and membership on advisory boards with Adaptive, Amgen, Becton Dickinson, Bristol-Myers Squibb-Celgene, Creative BioLabs, GSK, Janssen, Kite Pharma, Roche, Sanofi, and Takeda; unrestricted grants from Celgene, EngMab, Roche, Sanofi, and Takeda; and consultancy for Bristol-Myers Squibb-Celgene, Janssen, Sanofi, and Takeda. M.-J.C. declares honoraria for lectures from and membership on advisory boards with Janssen, Jazz Pharmaceuticals, Astellas, Novartis, Amgen, and Bristol-Myers Squibb-Celgene. ■

REFERENCES

1. Stong N, Ortiz M, Towfic F, et al. The location of the t(4;14) translocation breakpoint within the NSD2 gene identifies a subset of patients with high-risk NDMM. *Blood*. 2023;141(13):1574-1583.
2. Chesi M, Nardini E, Brents LA, et al. Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat Genet*. 1997;16(3):260-264.
3. Richelda R, Ronchetti D, Baldini L, et al. A novel chromosomal translocation t(4;14)(p16.3; q32) in multiple myeloma involves the fibroblast growth-factor receptor 3 gene. *Blood*. 1997;90(10):4062-4070.
4. Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. *Blood*. 1998;92(9):3025-3034.
5. Fonseca R, Oken MM, Greipp PR. The t(4;14)(p16.3;q32) is strongly associated with chromosome 13 abnormalities in both multiple myeloma and monoclonal gammopathy of undetermined significance. *Blood*. 2001;98(4):1271-1272.

6. Rasmussen T, Hudlebusch HR, Knudsen LM, Johnsen HE. FGFR3 dysregulation in multiple myeloma: frequency and prognostic relevance. *Br J Haematol*. 2002;117(3):626-628.

7. Keats JJ, Reiman T, Maxwell CA, et al. In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression. *Blood*. 2003;101(4):1520-1529.

8. Walker BA, Wardell CP, Johnson DC, et al. Characterization of IGH locus breakpoints in multiple myeloma indicates a subset of translocations appear to occur in pregerminal center B cells. *Blood*. 2013;121(17):3413-3419.

9. Perrot A, Lauwers-Cances V, Tournay E, et al. Development and validation of a cytogenetic prognostic index predicting survival in multiple myeloma. *J Clin Oncol*. 2019;37(19):1657-1665.

10. D'Agostino M, Cairns DA, Lahuerta JJ, et al. Second revision of the International Staging System (R2-ISS) for Overall Survival in Multiple Myeloma: a European Myeloma Network (EMN) Report Within the HARMONY Project. *J Clin Oncol*. 2022:JCO2102614.

https://doi.org/10.1182/blood.2022018007

LYMPHOID NEOPLASIA

Comment on Zhang et al, page 1584

The cat-and-mouse game of BTK inhibition

Ana Portelinha and Hans-Guido Wendel | Memorial Sloan Kettering Cancer Center

In this issue of *Blood*, Zhang et al[1] report the preclinical efficacy of a new Bruton tyrosine kinase (BTK) degrader molecule (NRX-0492, a close relative of the clinical compounds NX-2127 and NX-5948)[2] to overcome BTK inhibitor resistance. The authors show that NRX-0492 degrades wild-type and C481 mutant BTK, resulting in significant single-agent activity against chronic lymphatic leukemia (CLL) patient-derived xenografts in vivo.[1] A closely related compound, NX-2127,[2] is now in clinical trials in B-cell malignancies (NCT04830137).

BTK is a required component of B-cell receptor signaling that stimulates the proliferation of malignant B cells in diseases such as CLL and mantle cell lymphoma. BTK itself is not a target of oncogenic or activating mutations; however, BTK is required to transmit growth signals and to sustain the malignant B cells. Ibrutinib is the first-in-class BTK inhibitor and has dramatically changed the treatment of CLL patients.[3] Ibrutinib covalently binds to a cysteine residue (C481) in the active site of BTK, and resistance mutations such as C481S abolish ibrutinib binding, thereby restoring normal BTK functions.[4] This has prompted the development of second-generation, noncovalent BTK inhibitors that retain activity against C481 mutant disease. Genetic mutagenesis studies in our laboratory predicted BTK mutations that could interfere with noncovalent BTK inhibitors, and these have recently been confirmed in CLL patient specimens.[5,6] Especially noteworthy is a gatekeeper residue T474 that is analogous to the T315 gatekeeper in imatinib-resistant BCR-ABL (breakpoint cluster region of the Abelson gene). Mutation of T474 impairs the binding of different noncovalent BTK inhibitors.[5]

The degrader molecules (NX-2127, NX-5948, NRX-0492) use a noncovalent BTK inhibitor moiety as a "hook" that is linked to a "harness" and recruits the E3 ligase adaptor cereblon.[7] This approach has been applied to other targets, including transcription factors, BTK, and other kinases, with the goal of triggering ubiquitylation and proteasomal degradation. The compounds discussed here act on wild-type and C481S mutant BTK at sub-nanomolar concentrations and cause rapid degradation, leading to responses in patient-derived xenografts in vivo that the authors describe as comparable to those of alternate BTK inhibitors.

How are these compounds superior to noncovalent BTK inhibitors? The key difference lies in the requirement for prolonged and near-complete target occupancy for regular kinase inhibitors. By contrast, the degraders trigger loss of the BTK protein and do not need to occupy all or most BTK molecules for a prolonged time. This leads to different pharmacodynamic properties and results in prolonged target inhibition beyond the drug's clearance time. The relevance of this observation is demonstrated by the lackluster clinical activity of the noncovalent BTK inhibitor vecabrutinib that has been attributed to impaired target occupancy.[8] To what extent this difference may translate into superior clinical activity and how it may affect unwanted side effects and toxicities of regular kinase inhibitors remains to be seen. A surprising finding is that the degrader shows binding to BTK proteins with the T474I gatekeeper mutation. This is unexpected, because a noncovalent inhibitor is used as the "hook" and the T474 residue impairs access of these compounds to the BTK binding pocket.[5] The therapeutic effect of this experimental binding is not fully explored in the present study. However, recent abstracts submitted to the 2022 American Society of Hematology meeting appear to confirm the activity BKT degrader molecules in cells harboring mutations that impair covalent inhibitor binding in vitro and even in patients. On the other hand, one would not expect that BTK degraders will be able to overcome mechanisms of resistance that bypass the cellular effects of BTK loss, such as activation of phospholipase C gamma-mediated signaling.[4] The new targeting mechanism further depends on an intact protein degradation machinery, and this may provide cancer cells a potential escape mechanism to the class of compounds.

In summary, the study reports on the sub-nanomolar efficacy and in vivo activity of a new, orally bioavailable BTK degrader with advantages in target occupancy that overcome ibrutinib resistance related to the C481 mutation and that, intriguingly, may even retain activity against BTK forms that are resistant to noncovalent inhibitors. Hence, BTK degraders ring the opening bell for a new round in the cat-and-mouse game of BTK-directed therapeutics.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■