have been associated with more profound responses, including bone marrow remissions.[2] Nevertheless, despite the promise of FLT3 as a therapeutic target, significant challenges remain.

Among these challenges is the development of therapeutic resistance to FLT3 inhibitors with concurrent disease progression, which has been increasingly noted as these agents have been used off-protocol or on clinical study in recent years.[8] Investigators have found that a common mechanism of resistance following use of FLT3 TKI therapy in *ITD* disease is the development of kinase domain activating point mutations, a particularly difficult obstacle in the management of individual patients with advanced AML, and in the over-arching goal of developing effective and durable therapies for *FLT3*-mutant AML.

In the current edition of *Blood*, Zimmerman and colleagues attempt to investigate the feasibility of effective inhibition of FLT3 through use of crenolanib, a TKI with suspected type I properties. Type I TKIs can bind the active or inactive conformations of receptor tyrosine kinases, unlike type II inhibitors, which only bind the inactive form (see figure). Point mutations within the kinase domain are thought to destabilize the inactive conformation of FLT3. With either type I or type II inhibition, downstream signaling of the FLT3 receptor is aborted, leading to suppression of signaling through key pathways and thereby promoting differentiation and apoptosis of leukemic cells. Most FLT3 inhibitors investigated to date, with predominantly type II properties, have minimal activity against *FLT3-TKD* AML. This in turn selects for a persistence of *TKD* clones or emergence of new ones, which in part explains the development of therapeutic resistance increasingly seen after FLT3 inhibitor therapy.

In this study, the investigators report several important observations. First, crenolanib was a potent inhibitor of both *FLT3-ITD* and *FLT3-TKD* mutations. It was noted to effectively suppress *FLT3-ITD* leukemic cells, as demonstrated in *ITD* cell lines, such as MOLM-13 and MV4-11, as well as in a xenograft model. They also found that cells expressing either point mutations alone or combined with *ITD* mutations were more responsive to crenolanib than to the type II FLT3 inhibitor sorafenib. It is important to note that the profound efficacy of crenolanib against *FLT-D835* and *FLT3-ITD* mutant AML has been previously reported by other groups,[9,10] although the current report expands and confirms these observations through study of both in vitro and in vivo models. They further observed that simultaneous treatment with sorafenib and crenolanib led to even greater anti-leukemic activity, suggesting that dual type I and type II inhibition may increase efficacy by concurrently targeting the active and inactive kinase conformations of FLT3. Simultaneous inhibition may also suppress the outgrowth or emergence of resistant disease, which frequently demonstrate *TKD* mutations. Of note, the efficacy of crenolanib was also evaluated in a limited number of TKI-resistant primary samples, which had acquired D835H/Y mutations, and found to be more profound than that of sorafenib.

These findings, along with those reported by others, suggest that crenolanib may have a future role as a clinically active agent against AML with activation loop mutations, such as those affecting D835. Whether crenolanib can be incorporated into frontline therapeutic approaches for patients with *FLT3-ITD* and/or *TKD* mutations or whether it will best serve in recapturing response in patients who have developed secondary activating point mutations requires further study. There are currently multiple phase II studies evaluating crenolanib in the relapsed and refractory setting. In addition, combined type I and type II TKI therapy is an intriguing concept to help enhance efficacy and perhaps suppress the emergence of therapeutic resistance. However, given the individual toxicities and pharmacokinetic properties of such agents, optimizing the tolerability of combined TKI therapy, such as with sorafenib and crenolanib, may be challenging. In summary, the recent introduction of potent and targeted agents, such as quizartinib and crenolanib, for *FLT3*-mutant disease increases hope for a new era of effective and durable therapies for this frequently lethal subtype of AML.

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

REFERENCES

1. Zimmerman EI, Turner DC, Buaboonnam J, et al. Crenolanib is active against models of drug-resistant FLT3-ITD-positive acute myeloid leukemia. *Blood*. 2013; 122(22):3607-3615.

2. Fathi AT, Chabner BA. FLT3 inhibition as therapy in acute myeloid leukemia: a record of trials and tribulations. *Oncologist*. 2011;16(8):1162-1174.

3. Nakao M, Yokota S, Iwai T, et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. *Leukemia*. 1996;10(12):1911-1918.

4. Abu-Duhier FM, Goodeve AC, Wilson GA, Care RS, Peake IR, Reilly JT. Identification of novel FLT-3 Asp835 mutations in adult acute myeloid leukaemia. *Br J Haematol*. 2001;113(4):983-988.

5. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*. 2002;99(12):4326-4335.

6. Gale RE, Green C, Allen C, et al; Medical Research Council Adult Leukaemia Working Party. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood*. 2008;111(5):2776-2784.

7. Mizuki M, Fenski R, Halfter H, et al. Flt3 mutations from patients with acute myeloid leukemia induce transformation of 32D cells mediated by the Ras and STAT5 pathways. *Blood*. 2000;96(12):3907-3914.

8. von Bubnoff N, Engh RA, Aberg E, Sänger J, Peschel C, Duyster J. FMS-like tyrosine kinase 3-internal tandem duplication tyrosine kinase inhibitors display a nonoverlapping profile of resistance mutations in vitro. *Cancer Res*. 2009;69(7):3032-3041.

9. Galanis A, Rajkhowa T, Muralidhara C, Ramachandran A, Levis M. Crenolanib is a highly potent, selective, FLT3 TKI with activity against D835 mutations [abstract]. *Blood*. 2012;120. Abstract 1341.

10. Smith CC, Lasater E, Mccreery M, et al. Crenolanib (CP-868,596) is a potent and selective type I FLT3 inhibitor that retains activity against AC220 resistance-causing FLT3 kinase domain mutations [abstract]. *Blood*. 2012;120. Abstract 141.

● ● ● LYMPHOID NEOPLASIA

Comment on Perry et al, page 3599

T-cell LPD of the GIT: first do no harm

Jonathan Said[1] [1]UNIVERSITY OF CALIFORNIA, LOS ANGELES

In this issue of *Blood*, Perry et al describe cases of an indolent T-cell lymphoproliferative disorder (LPD) of the gastrointestinal tract (GIT) that

mimics inflammatory bowel disease or peripheral T-cell lymphoma (PTCL).[1] The lesions involved all sites in the GIT, and mucosal infiltrates consisted of small lymphoid cells with minimal atypia. The cases were phenotypically heterogeneous but predominantly $CD8^+/CD4^-$, and all had clonal T-cell receptor (TCR) gene rearrangements.

The intestinal epithelium is the largest surface in the body exposed to antigens. The mucosal T cells are essential to maintaining the barrier function of the gut, preventing penetration by commensal and pathogenic bacteria via antigen recognition and cytokines, and sustaining epithelial health as well as selective immune tolerance.[2] Mucosal T cells include naive T cells located in the gut-associated lymphoid tissue, which includes the Peyer's patches. Lamina propria and intraepithelial lymphocytes (IEL) have potent cytolytic and immunoregulatory capacities and >70% are $CD8^+$ cells. Unlike circulating T cells, $CD4^-$ $CD8^-$ "double negatives" account for >10%, and $CD4^+$ αβ T cells are sparse. IEL also include greater numbers of γδ T cells. For purposes of simplification, IEL have been divided into just 2 types: a and b.[3] Type a includes TCR αβ T cells that mainly recognize antigens presented by conventional major histocompatibility complex classes I and II. Type b includes TCR αβ and γδ T cells, which typically express CD8αα homodimer and detect and respond to ligands and products not restricted by conventional major histocompatibility complex but instead are representative of products of the molecular stress response.

Given this complexity, it is not surprising that a spectrum of T-cell LPD is encountered in the GIT (see table). Cases of enteropathy-associated T-cell lymphoma (EATL) are likely derived from type b cells, and although usually αβ, may be γδ or T-cell silent. Type I EATL is related to celiac disease, and refractory celiac disease type II may be an early form of EATL.[4] Type II EATL has distinctive histologic features and phenotype, and is generally unrelated to celiac disease. The time may have come for a change in nomenclature to reflect this distinction.

Although the majority of intestinal T-/natural killer (NK)-cell lymphomas are aggressive, there are exceptional NK- or T-cell proliferations that may persist and require minimal if any therapeutic intervention. One example is NK-cell enteropathy that presents with vague abdominal symptoms and extensive mucosal lesions mimicking NK-cell lymphoma.[5] Similarly, although PTCL is considered highly aggressive by the World Health Organization,[6] indolent cases have been documented, usually in extranodal sites with low clinical stage, and comprising small cells with mild nuclear atypia and low proliferation rate.[7] Primary cutaneous $CD4^+$ small/medium T-cell lymphoma, a provisional entity by the World Health Organization,[6] is another indolent lesion that may be indistinguishable from what has previously been termed cutaneous pseudo-T-cell lymphoma.[8] Indolent $CD8^+$ lymphoid proliferation of the ear or face is also slow-growing and remains localized.[9]

At a recent international workshop on peripheral T- and NK-cell lymphomas and their mimics, organized by the European Association for Haematopathology and the Society for Hematopathology, there was a spectrum of cases from patients with extranodal T-cell infiltrates, some of which had been treated aggressively and were considered indolent and indeterminate for malignancy by an expert panel.[10] DNA sequencing has identified recurrent genetic alterations in some T-cell proliferations and is paving the way to an objective molecular classification of T-cell proliferations. Until that time, astute clinicopathologic studies such as those reported by Perry et al are critical in drawing attention to anomalous entities such as low-grade LPD of the GIT, which can easily be misinterpreted as inflammatory bowel disease or lymphoma.

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

REFERENCES

1. Perry AM, Warnke RA, Hu Q, et al. Indolent T-cell lymphoproliferative diseases of the gastrointestinal tract. *Blood.* 2013;122(22):3599-3606.

2. van Wijk F, Cheroutre H. Intestinal T cells: facing the mucosal immune dilemma with synergy and diversity. *Semin Immunol.* 2009;21(3):130-138.

T-cell LPD of the GIT

Type	Clinical	Histology	Phenotype	Outcome
EATL type I	Overt or silent gluten-sensitive enteropathy	Polymorphous medium to large cells including Hodgkin-like cells	$CD4^-$, $CD8^{-/+}$ TCR αβ (usually) or γδ Variable expression of CD30	Aggressive
EATL type II	Worldwide, not clearly linked to celiac disease	Monomorphic with epitheliotropism	TCR γδ (usually) or αβ $CD8^+$, $CD56^+$	Aggressive
Extranodal NK-/T-cell lymphoma nasal type	Upper aerodigestive tract and small bowel most often involved	Medium to large cells	NK/cytotoxic T cells, EBV^+	Aggressive
PTCL-NOS	No history of celiac disease	No significant epitheliotropism	Variable More often TCR silent	Aggressive
NK-cell enteropathy	Vague gastrointestinal symptoms, can involve entire GIT	Medium to large irregular cells do not invade glandular epithelium	$CD56^+$ Cytotoxic granules, cytoplasmic $CD3^+$	Indolent chronic relapsing course
Indolent T-cell lymphoproliferative disease of the GIT	Diarrhea, abdominal pain	Superficial infiltrate of small uniform lymphoid cells	Mostly $CD8^+$ cytotoxic T cell	Indolent chronic relapsing course

EBV, Epstein-Barr virus; NOS, not otherwise specified.

3. Hayday A, Theodoridis E, Ramsburg E, Shires J. Intraepithelial lymphocytes: exploring the Third Way in immunology. *Nat Immunol.* 2001;2(11):997-1003.

4. Malamut G, Meresse B, Cellier C, Cerf-Bensussan N. Refractory celiac disease: from bench to bedside. *Semin Immunopathol.* 2012;34(4):601-613.

5. Mansoor A, Pittaluga S, Beck PL, Wilson WH, Ferry JA, Jaffe ES. NK-cell enteropathy: a benign NK-cell lymphoproliferative disease mimicking intestinal lymphoma: clinicopathologic features and follow-up in a unique case series. *Blood.* 2011;117(5): 1447-1452.

6. In: Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues.* Lyon: IARC; 2008

7. Hayashi E, Takata K, Sato Y, et al. Distinct morphologic, phenotypic, and clinical-course characteristics of indolent peripheral T-cell lymphoma. *Hum Pathol.* 2013;44(9):1927-1936.

8. Cetinözman F, Jansen PM, Willemze R. Expression of programmed death-1 in primary cutaneous CD4-positive small/medium-sized pleomorphic T-cell lymphoma, cutaneous pseudo-T-cell lymphoma, and other types of cutaneous T-cell lymphoma. *Am J Surg Pathol.* 2012;36(1): 109-116.

9. Petrella T, Maubec E, Cornillet-Lefebvre P, et al. Indolent CD8-positive lymphoid proliferation of the ear: a distinct primary cutaneous T-cell lymphoma? *Am J Surg Pathol.* 2007;31(12):1887-1892.

10. Attygalle AD, Cabecadas J, Gaulard P, et al. Peripheral T- and NK-cell lymphomas and their mimics; taking a step forward—report on the lymphoma workshop of the XVI meeting of the European Association for Haematopathology and the Society for Hematopathology in Lisbon 2012. *Histology*. In press.

MYELOID NEOPLASIA

Comment on Papaemmanuil et al, page 3616

The importance of subclonal genetic events in MDS

Rafael Bejar[1] and Omar Abdel-Wahab[2] [1]UNIVERSITY OF CALIFORNIA, SAN DIEGO; [2]MEMORIAL SLOAN-KETTERING CANCER CENTER

In this issue of *Blood*, Papaemmanuil et al present results from the largest single targeted sequencing effort in myelodysplastic syndrome (MDS) to date both in terms of number of patients studied (738 patients, of which 603 had MDS) and genes sequenced (111 genes).[1]

Recently, comprehensive mutational profiling has identified the importance of somatic mutations in predicting outcome[2,3] and characterizing clonal hierchical events in patients with MDS.[4-6] Data from Papemmanuil et al confirm and extend prior observations about the correlation of mutations with clinical phenotype in MDS and present a clearer picture of the stepwise transformation events most commonly seen in MDS (see figure).

Given the relatively recent discovery of many recurrently mutated genes in MDS and the need for validation of prior mutational correlative data, a number of clinically important observations are now solidified with the publication of this work. First, it is clear that the total number of mutations present in a patient with MDS has important prognostic value, even independent of currently clinically used prognostic information.[1,2] Second, of the most commonly mutated genes in MDS, mutations in *ASXL1, SRSF2, RUNX1,* and *TP53* are once again associated with worsened outcome and decreased leukemia-free survival.[2,3] Comprehensively sequencing such a large cohort of patients also further strengthens our knowledge of the mutational combinations that are likely critical in MDS pathogenesis. For instance, by carefully the examining mutational co-occurrences, Papaemmanuil et al have identified that each of the mutated spliceosomal genes has a unique spectrum of mutational overlap. This might account for the divergent phenotypes associated with each of the spliceosomal mutations despite their mutual exclusivity,[3,7] a hypothesis that will be excellent to test functionally.

In addition to these observations, Papaemmanuil et al also examined the mutational allele frequency of each of the commonly mutated genes to estimate the temporal order of mutation acquisition in MDS and understand the impact of such subclonal mutations (defined as mutations not present in the entire proportion of malignant cells) on disease course. The authors found that the detection of mutations in genes associated with adverse outcome had significant impact even when not present in the major clone. This finding simplifies the clinical interpretation of poor prognostic mutations and emphasizes the need for sensitive detection methods to identify them in practice. This is reminiscent of the recently appreciated importance of subclonal genetic events in chronic lymphocytic leukemia (CLL),[8] another leukemia characterized by a highly diverse clinical course and a frequent clinical practice of monitoring disease without initiation of therapy. Unlike this recent work in CLL, or prior work in MDS,[5,6] which used sequencing of all coding exomes or the entire genome to track cancer clones, Papaemmanuil et al used targeted sequencing of the most frequently mutated genes at a much higher depth to achieve a similar goal. These data suggest that mutations impacting RNA splicing and DNA methylation occur early in disease progression with mutations affecting histone post-translational modifications and DNA demethylation following, and kinase activating mutations (such as *KIT* and *NRAS*) occurring even later in disease progression.[4] Indeed, we previously identified that mutations in *NRAS* can be present at surprisingly low mutation allele burdens (0.02-0.1%) and are associated with adverse overall survival even when present at 0.5% mutant allele burden.[9]

Despite the large number of patients studied and extent of sequencing performed, it is clear that there is still much to be learned about clonal dynamics and molecular prognostication in MDS. For example, surprisingly, 22% of the 738 patients included here had no identifiable somatic genetic abnormality (mutation or cytogenetic alteration). Secondly, prior work using whole genome sequencing (WGS) to define the clonal architecture and events in the progression of MDS to acute myeloid leukemia identified the presence of dozens to hundreds of mutations in a founding clone followed by outgrowth of ⩾1 subclone each marked by the acquisition of an increasing number of new mutations.[5,6] Comparing this WGS approach with a targeted sequencing approach, it appeared that using variant allele frequencies of recurrently mutated genes alone did not fully recapitulate clonal architecture identified by WGS of the same