

LYMPHOID NEOPLASIA

Comment on Thompson et al, page 1951

# CLL: deep dive for residual cells by NGS matters

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**Thompson et al show that a substantial fraction of patients with chronic lymphocytic leukemia (CLL) in complete remission still harbors leukemic cells by using a next-generation sequencing (NGS) technique for detection of minimal residual disease (MRD). This NGS-based technology is able to detect 1 CLL cell out of 1 million leukocytes, thus having the potential of identifying patients with CLL at high risk for clinical relapse and allowing preparation of therapeutic rescue strategies as early as possible.<sup>1</sup>**

In CLL, impressive long-term remissions, including complete remissions with undetectable minimal residual disease (U-MRD), can be induced by chemoimmunotherapy based on fludarabine, cyclophosphamide, and rituximab (FCR).<sup>2,3</sup> Nevertheless, patients with CLL with specific high-risk features, such as an unmutated IGHV status, unfortunately still relapse after this aggressive chemotherapy despite some testing negative for MRD by classic flow cytometry-based assays.

The work by Thompson and colleagues tries to provide a better understanding for patients that are at true risk for a clinical relapse while tested negative for MRD in their blood or bone marrow by flow cytometry. Although the latter assay

can only detect 1 positive CLL cell out of 10 000 normal cells (U-MRD4), an NGS-based technique lowers the detection sensitivity to the order of 1 positive cell out of 1 million cells (U-MRD6). Therefore, patients with undetectable disease by flow cytometry are rendered positive by this highly sensitive NGS-based method. In 62 patients with undetectable disease by flow cytometry (U-MRD4) at end of treatment with FCR, only 1 of 4 patients was documented to be MRD negative by NGS (U-MRD6). This significant difference between techniques is even more apparent for patients with CLL with an unmutated IGHV, a group considered to be at high risk for relapse. Only 13% of patients considered MRD negative by flow cytometry had undetectable disease by

NGS. This observation has a major clinical impact because, unfortunately, most of these patients will relapse sooner or later. The median progression-free survival (PFS) was significantly shorter for patients with detectable disease by NGS at end of treatment.

The data on MRD assessment by NGS in this paper by Thompson et al were based on patients that were treated with a chemoimmunotherapy (FCR) that is nowadays often supplanted by novel targeted drugs like the Bruton tyrosine kinase inhibitor ibrutinib or the BCL2 inhibiting drug venetoclax.<sup>4,5</sup> Although 27.4% of patients showed undetectable disease by NGS after therapy with FCR, frontline therapy with venetoclax plus obinutuzumab is able to induce MRD-negative disease in a higher proportion (ie, 42% of patients) at this sensitivity level, as has been recently shown in the context of the CLL14 trial of the German CLL Study Group. In this trial, the former standard for elderly nonfit patients with CLL (ie, chlorambucil plus obinutuzumab) showed U-MRD in only 7% of patients, resulting in significantly shorter PFS compared with venetoclax plus obinutuzumab (hazard ratio 0.35). Therefore, in this trial, U-MRD by NGS has been shown to be associated with a survival advantage in a head-to-head comparison (see table).

The question arises whether NGS-based assays are ready for daily practice and might guide treatment decisions in the near future. Currently, a molecular relapse would not necessitate an immediate intervention, especially not after a time-limited

### Outcome including U-MRD in frontline CLL

Outcome	FCR3 (N = 408)	CLB/Obinutuzumab5 (N = 216)	Venetoclax/Obinutuzumab5 (N = 216)	Ibrutinib/Venetoclax6 (N = 80)
ORR, %	90	71	85	100
CR, %	44	23	50	96
U-MRD4 (PB), %	63	35	76	n.d.
U-MRD6 (PB), %	n.d.	7	42	n.d.
U-MRD4 (BM), %	44	17	57	69
U-MRD6 (BM), %	n.d.	n.d.	n.d.	n.d.
Median PFS (mo)	56.8 (at median follow-up of 5.9 y)	n.r. (at month 24, 64% were progression free)	n.r. (at month 24, 88% were progression free)	n.r. (at month 12, 98% were progression free)
Median OS (mo)	n.r. (at median follow-up of 5.9 y)	n.r. (at month 24, 93% were alive)	n.r. (at month 24, 92% were alive)	n.r. (at month 12, 99% were alive)

BM, bone marrow; CLB, chlorambucil; CR, complete remission; n.d., not detected; n.r. = not reached; ORR, overall response rate; OS, overall survival; PB, peripheral blood.

frontline chemotherapy with FCR. Many treatment options for such patients, like ibrutinib or venetoclax plus rituximab, are available and approved at a clinical relapse. Not many laboratories would be able to fulfill the sophisticated standards needed to provide reliable MRD data at such a high sensitivity level, making sample shipping to experienced reference centers mandatory. However, MRD-guided stopping and reinitiation of therapy could be of major interest despite these hurdles, when prescribing more and more continuous or prolonged treatments with novel drugs to our patients. A time-limited treatment with a nonchemotherapy oral drug combination would be the future goal for the majority of our patients with CLL. Although a combination of ibrutinib and venetoclax can induce U-MRD in up to 69% of patients based on flow cytometry (U-MRD4), NGS-based assays might even better guide us in the future to define the subpopulation of patients with residual disease that would benefit from a prolonged combination therapy or an extended maintenance phase.<sup>6</sup> Similar MRD-tailored treatment concepts in the frontline and relapsed setting have been introduced also by other research groups.<sup>7,8</sup> The better and more sensitive our MRD detection tool is, the better and more precise our treatment strategy will be for the individual patient with CLL. NGS-based MRD assessment is currently also developed for molecular monitoring of other leukemias (eg, chronic myeloid leukemia). Therefore, the data by Thompson et al in this issue of *Blood* are an important step forward that might guide us in the near future to treat our patients with different types of leukemia as much as possible, but as little as necessary.

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## MYELOID NEOPLASIA

Comment on Park et al, page 1960

# Killing the minotaur in its amazing maze

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**In this issue of *Blood*, Park et al describe a novel KLF4-DYRK2-mediated pathway that affects survival and self-renewal of leukemic stem cells (LSCs) in chronic myeloid leukemia (CML).<sup>1</sup>**

In ancient Greek mythology, the minotaur, a curious and ferocious creature with the head and tail of a bull and the body of a man, dwelt in a maze on the Greek island of Crete, constructed by the architect Daedalus. The labyrinth shielded the minotaur, who sustained himself by devouring young men and women before being slaughtered by the hero Theseus.

The LSC, by analogy, has evolved an ever-growing maze of signal transduction pathways leading to LSC survival, self-renewal and, consequently, therapy resistance. Eradication of the LSC is the goal of many researchers working in this field, although this concept may be controversial, at least in CML.<sup>2,3</sup> Novel complexities and intricate connections between pathways have been discovered in this labyrinth, yet, as the ancient Greeks would say, the song of the single hero killing the minotaur has not yet been sung.

The remarkable efficacy of tyrosine kinase inhibitors (TKIs) in controlling CML and inducing deep molecular remissions have now led clinicians, scientists, and patients to the question of whether treatment may safely be stopped. However, studies have shown that only around 40% of patients maintain a complete molecular remission

after withdrawal of TKI therapy,<sup>4</sup> maintaining a so called "treatment-free remission." The remaining 60% of patients relapse. Factors influencing the risk of recurrence may include immune surveillance of the CML stem cell clone, efficient sheltering of the CML stem cell by the bone marrow microenvironment or other protective niches, or CML stem cell-intrinsic signal transduction pathways.

Several pathways, including p53,<sup>5</sup> c-MYC, and many others, mediate the survival of CML LSCs and their resistance to TKIs. CML LSCs are independent of the oncoprotein BCR-ABL1, which causes the disease. Stabilization of p53 and downregulation of c-Myc, but not BCR-ABL1 itself, has been shown to lead to efficient elimination of CML LSCs, while not affecting normal hematopoietic stem cells.<sup>5</sup> Follow-up studies have shed light on other pathways and strategies for increasing levels of p53 to efficiently eradicate LSCs in CML.<sup>6,7</sup> Indeed, p53-activating agents may be an effective way of inducing cure in CML patients.<sup>8</sup>

In the article by Park et al, the authors reveal a novel pathway that leads to decreased survival and self-renewal in CML via activation of p53 and depletion of c-Myc. They show that loss of the zinc-finger