

As depicted in the diagram (see figure), mouse recipients with *ATG16L1* deficiency in intestinal epithelium had augmented gut-GVHD with enhanced intestinal epithelial necroptosis after allo-HCT, which could be recapitulated in an ex vivo organoid culture system.¹ The death of the cultured organoid mediated by necroptosis was associated with an IFN signature, as measured with RNA-Seq analysis.¹ Blockade of the IFN signaling pathway by the JAK2 inhibitor Ruxolitinib or blockade of necroptosis pathway by RIPK1 inhibitor GSK547 markedly reduced necroptosis and increased survival of cultured intestinal organoids derived from *ATG16L1*-deficient mice.¹

Matsuzawa-Ishimoto et al were also able to set up intestinal organoid cultures from fresh as well as frozen human intestinal tissues. Coculture with donor T cells or TNF- α induced death of the cultured human organoids. Remarkably, organoid cell death was markedly increased when intestinal tissues were from patients with *ATG16L1*^{T300A/T300A} risk alleles, and blockade of IFN signaling or necroptosis greatly increased the organoid cell survival¹ (see figure). Therefore, the GVHD protective role of intestinal epithelial cell expression of *ATG16L1* in mouse and human can be both demonstrated by ex vivo organoid cultures. More importantly, the organoid cultures allow for screening new drugs with both mouse and human tissues for targeting the same inflammatory pathways. This approach allows for linked observations from ex vivo assays to in vivo mouse models or vice versa.

Finally, GVHD and other inflammatory diseases have tissue-specific pathogenesis.³ Ex vivo organoid culture systems can use different tissues, including intestine, liver, lung, heart, and skin.¹⁰ Therefore, ex vivo organoid culture systems can serve as a platform for testing pathogenic or protective lymphocytes and drug candidates. The combination of mouse models and ex vivo organoid culture systems may ultimately serve as a platform for personalized medicine.

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

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LYMPHOID NEOPLASIA

Comment on Tausch et al, page 2402

Genetics in the era of targeted CLL therapy

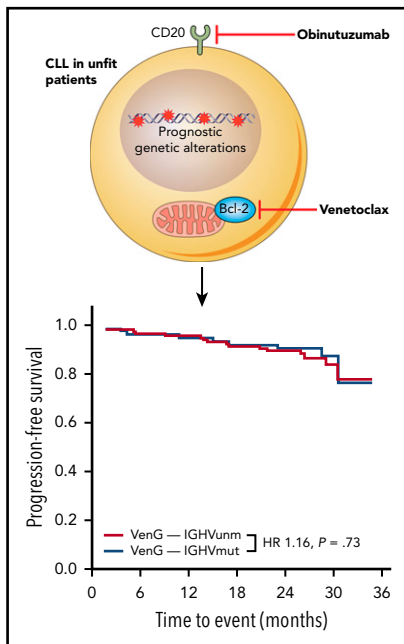
Tatjana Stankovic and Marwan Kwok | University of Birmingham

In this issue of *Blood*, Tausch and colleagues demonstrated the clinical benefit of venetoclax and obinatumab for *IGHV*-unmutated chronic lymphocytic leukemia (CLL) and for all other CLL genetic subgroups with the exception of *del(17p)* and mutated *TP53*, within a phase 3 trial.¹

We are heading into a new era in our battle against CLL. Knowledge of CLL biology acquired from basic science research has led to transformative treatments that are revolutionizing the CLL therapeutic landscape. Chemoimmunotherapy, which until a few years ago was the mainstay of CLL treatment, is increasingly being replaced by chemo-free treatment options. Chemoimmunotherapy is effective in a proportion of CLL patients but much less so among those with high-risk genetic features, including deletions of 17p or 11q and *TP53*, *ATM*, *SF3B1*, and *NOTCH1* mutations, as well as unmutated *IGHV*. In these patients, small molecule inhibitors targeting B-cell receptor signaling (eg, ibrutinib) and the antiapoptotic protein Bcl-2 (eg, venetoclax) are producing response rates that were unimaginable with previous chemoimmunotherapy, and often with less toxicity. It remains unclear, however, whether these excellent treatment responses translate into long-term,

durable remissions for all CLL genetic subgroups and whether genetic stratification in this new era of CLL targeted therapies still matters.

In this important study, Tausch and colleagues have provided us with some answers to these pertinent questions. Here, the clinical impact of genetics is studied in the context of the CLL14 trial, which establishes the combination between immunotherapy, anti-CD20 antibody, obinatumab, and the Bcl-2 inhibitor, venetoclax, as a new standard of care for previously untreated, elderly, and less fit CLL patients with comorbidities.² In contrast to chemoimmunotherapy, where unmutated *IGHV* and adverse genetics are predictive for unfavorable outcome, durable remissions were observed with this combination regardless of *IGHV* mutational status (see figure), *del(11q)*, trisomy 12, or mutations of *ATM*, *SF3B1*, *BIRC3*, and *NOTCH1*. Similar observations have



A novel combination of an anti-CD20 antibody obinutuzumab (G) and the Bcl-2 inhibitor venetoclax (Ven) provides a new solution for unfit CLL patients. This combination resulted in durable remissions on the CLL14 trial regardless of *IGHV* mutational status, therefore overcoming unmutated *VH* status as an indicator of poor prognosis. HR, hazard ratio; *IGHV*mut, *IGHV* mutated; *IGHV*nm, *IGHV* unmutated.

been reported with ibrutinib within the RESONATE and RESONATE-2 trials with the exception that in the RESONATE study patients with *SF3B1* mutations showed a trend toward inferior progression-free survival.^{3,4}

Tausch and colleagues also observed high rates of response and minimal residual disease negativity in patients with *del*(17p) and/or *TP53*-mutant CLL following combined venetoclax/obinutuzumab treatment, but these responses were often not durable. Shorter progression-free survival has also been reported in *TP53*-defective CLL treated with venetoclax and rituximab within the MURANO study,⁵ and with B-cell receptor signaling inhibitors.^{3,6} The p53 protein mediates apoptosis in response to DNA damaging agents but the loss of p53 dependent apoptosis cannot explain the limited anti-CLL effects of targeted therapies. One potential explanation is that *TP53*-defective CLL cells

are more genomically unstable owing to the pivotal role p53 plays in the DNA damage response during DNA replication. Indeed, clonal evolution and genomic complexity frequently precede disease progression in patients treated with venetoclax or ibrutinib.⁷⁻⁹

Venetoclax-based therapies remain expensive. The greatest clinical significance of the current study is that it helps us define CLL treatments according to genetic subgroups. In patients with *IGHV*-mutated CLL with no genetic aberrations other than *del*(13q), there may be continued justification for the use of chemoimmunotherapy providing there are no significant tolerability issues. Patients with *IGHV*-unmutated CLL and those with *del*(11q), trisomy 12, *ATM*, *NOTCH1*, *SF3B1*, and *BIRC3* mutations are the individuals who stand to benefit the most from venetoclax with obinutuzumab over chemoimmunotherapy. Finally, patients with *del*(17p) and *TP53* mutations also derive some benefit from venetoclax/obinutuzumab compared with chemoimmunotherapy, but should ideally be enrolled in clinical trials exploring novel therapies and therapeutic combinations.

Finding the best way to manage *TP53*-defective CLL undoubtedly continues to be a pressing task. It remains to be determined whether prolonged venetoclax treatment and a higher depth of response could prevent disease relapse and whether the use of ibrutinib and venetoclax in combination could overcome resistance to either agent. It will also be of interest to establish how effective chimeric antigen receptor T cells and other immunotherapies are and whether better understanding of the subclonal dynamics, resistance mechanisms, and the pathways on which *TP53*-defective CLL rely for their survival can improve clinical outcomes in this genomically unstable CLL subgroup.

Over the past decade, treatments have been developed that are highly successful in targeting pathogenic processes common to all CLL cells. To secure durable disease control, or even a cure, future

research efforts will need to take the therapeutic vulnerabilities of resistant CLL subclones into consideration. Clearly, the arrival of targeted therapies has not consigned CLL genetics to irrelevance. Quite the opposite, Tausch et al have shown us that genetics continues to influence treatment outcome, and provides us with important pointers for future research.

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