expressing the highest levels of CD38 more significantly inhibited T-cell proliferation than CD38-negative Tregs. Furthermore, both helper and cytotoxic T cells were induced in daratumumab-treated patients, with interferon-γ and $CD8^+$/Treg ratios increased in responders at week 8 following treatment. Most importantly, HLA-DR^+CD8^+ T cells, effector memory $CD8^+$ T cells, and clonal T cells based on T-cell repertoire analysis were significantly increased during drug treatment and response. Conversely, effector memory $CD8^+$ T cells returned to baseline levels at relapse.

These novel effects of daratumumab on multiple immune populations indicate that daratumumab overcomes immunosuppression by targeting Bregs, Tregs, and MDSCs, which are elevated at diagnosis and increase the risk of disease progression and relapse. These cells express higher levels of CD38 and are therefore more sensitive to treatment than helper and effector T cells, which express lower levels or lack CD38. However, CD38 levels alone may not be the sole determinant of sensitivity to daratumumab, because CD38-negative Tregs were also reduced in responsive patients. These results suggest the potential benefits of combining daratumumab with other therapies targeting Tregs, including immune checkpoint inhibitors or immunomodulatory drugs, to further trigger a shift to positive vs negative regulators of anti-MM immune response. Importantly, these studies suggest that daratumumab may have even greater clinical activity when used in earlier stages of disease (ie, smoldering MM), when the patient immune repertoire is preserved and not impacted by therapy. Finally, long-lived and/or MM-initiating cells from which MM PCs are derived are $CD38^{high}CD19^-$ PCs, suggesting that MM stem cells express high levels of CD38 and may also be susceptible to daratumumab therapy. Ongoing studies will define the impact of daratumumab, alone and in combination with other immune and targeted agents.

An overdue era of immune therapy in MM has begun, and the prospect of triggering long-term memory anti-MM immunity in patients at early stages of disease offers great potential for prolonged survival and potential cure.

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● ● ● LYMPHOID NEOPLASIA

Comment on Herling et al, page 395

Synergy: karyotypes and mutations in CLL

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In this issue of *Blood*, Herling et al present the first large, prospective clinical trial that integrates cytogenetic, next-generation sequencing (NGS), clinical, and laboratory data into a prognostic analysis.[1] They demonstrate that karyotypic complexity is an independent prognostic factor of survival in chronic lymphocytic leukemia (CLL). They are also the first to show that mutations in *KRAS* or *POT1* affect treatment response and survival after chemoimmunotherapy. Their results provide a strong rationale for incorporating the results of karyotypic and NGS analyses in clinical trial design and in routine practice.

Until relatively recently, the genomic landscape of CLL was considered to be characterized mainly by gains and deletions (del) of chromosomal material, rather than by translocations, unlike other hematologic malignancies. Because most peripheral blood (PB) CLL cells are in the G0/G1 phase of the cell cycle, under standard culture conditions most samples fail to undergo cell division. Stimulation with conventional B-cell mitogens, such as lipopolysaccharide, pokeweed mitogen, or phorbol 12-myristate 13-acetate, yields metaphases in fewer than half of cases. Under these conditions, the most commonly identified chromosomal abnormalities are trisomy of chromosome 12, followed by del in the long arms of chromosomes 13 and 11, and the short arm of chromosome 17; translocations are uncommon.[2]

In a landmark study published in 2000, Döhner et al demonstrated, by fluorescence in situ hybridization (FISH) analysis performed on interphase (nondividing) nuclei, that ~80% of CLL cases contained at least 1 of 4 recurrent chromosomal gains or del, most commonly del(13)(q14.1) (55% of cases; the site of the microRNA *15a* and *16-1* genes), followed by trisomy 12 (16% of cases), del(11)(q22-23) (18% of cases; the site of the ataxia telangiectasia mutated [*ATM*] gene), and del(17)(p13.1) (7% of cases; the site of the tumor protein *p53* [*TP53*] tumor suppressor gene).[3] Further, these abnormalities were useful for risk stratification; patients with del(13q) had a good prognosis, whereas patients with del(17p) had a particularly poor prognosis characterized by rapid disease progression, treatment resistance, and poor survival. Based

on their results, and on the high yield and relative ease of performing FISH analysis on interphase nuclei compared with karyotypic analysis performed on metaphases, FISH analysis using a panel of probes to the common recurrent abnormalities has become part of the routine clinical evaluation at the time of CLL diagnosis. Most centers no longer perform metaphase analysis on PB CLL cells. However, FISH has significant limitations. It detects only those abnormalities to which the probes are directed; it cannot detect new abnormalities. FISH also fails to detect cytogenetic complexity (3 or more clonal cytogenetic abnormalities), which correlates strongly with poor outcome.

Over the past decade, a variety of methods have been developed that reliably and reproducibly stimulate PB CLL cells to divide in culture.[4,5] Using different combinations of cytokines, CpG oligodeoxynucleotides, and mitogens, analyzable metaphases can be obtained in up to 90% of PB CLL samples. From these studies, it has become clear that karyotypic complexity and translocations, both balanced and unbalanced, are not uncommon in CLL. Further, the presence of translocations and cytogenetic complexity are associated with shorter treatment-free and overall survival.

Herling et al have analyzed the data obtained from a cohort of 161 CLL patients with significant comorbidity treated in a 3-arm phase 3 trial that compared frontline chlorambucil (Clb) to Clb plus rituximab, or Clb plus obinutuzumab.[6] They performed chromosome banding on stimulated cultures, FISH, and NGS analysis using an 85 gene panel on PB samples obtained before treatment. They correlated these data with clinical and laboratory parameters. Karyotypic analysis demonstrated chromosomal abnormalities in 68.8% of patients, 31.2% with translocations, and 19.5% with complex karyotypes. They identified mutations in 76.4% of patients, most commonly in the *NOTCH1*, *SF3B1*, *ATM*, *TP53*, *BIRC3*, *POT1*, *XPO1*, and *KRAS* genes.[7] They made several novel observations. First, they found an increased frequency of *KRAS* mutations, most of them known to be activating, in patients who failed to respond to chemoimmunotherapy. The authors raise the possibility that these patients may benefit from treatment with small molecule inhibitors that target components of the mitogen-activated protein kinase pathway, an important B-cell receptor signaling pathway. Second, they found that mutations in *POT1*, reported previously in only 1 other study of CLL,[8] were associated with poor survival. *POT1* is a shelterin protein that is critical for telomere capping and telomerase recruitment. It is conceivable that mutations in *POT1* could lead to genomic instability and drive tumorigenesis. Third, they identified previously unreported mutations in 6 genes involved in the DNA damage response, which may also contribute to genomic instability in CLL.

Finally, Herling et al made the important observation that karyotypic complexity and *TP53* abnormalities (del or mutation) independently portend a poor prognosis. Previous studies have demonstrated that both types of abnormalities are associated with a poor outcome. However, this is the first study with sufficient power to show that the effects of karyotypic complexity and *TP53* aberrations on prognosis in patients treated with chemoimmunotherapy are additive. A recent study demonstrated that karyotypic complexity, which is more common than *TP53* aberrations, is also associated with poor prognosis in patients treated with the Bruton tyrosine kinase inhibitor, ibrutinib.[9] It is likely that in the relatively near future, we will assess karyotypic complexity using sequencing technologies. For now, as new agents that selectively target signaling pathways become available for frontline therapy, the effects of karyotypic complexity can be evaluated only by including metaphase analysis on stimulated PB CLL cells in large prospective clinical trials.

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● ● ● LYMPHOID NEOPLASIA

Comment on Kastritis et al, page 405

Defining ultrahigh-risk AL amyloidosis with VWF

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In this issue of *Blood*, Kastritis et al report the results of using von Willebrand factor antigen (VWF:Ag) to define an ultrahigh-risk group of patients with immunoglobulin amyloid light-chain (AL) amyloidosis.[1] The accurate identification of these patients could help design future clinical trials desperately needed to define the best treatment of these patients.

One of the most remarkable achievements in medicine I got to witness is the advances made in AL amyloidosis. The diagnosis that once struck fear and trepidation into patients and clinicians is now viewed more as a worthy foe. In just 1 decade, the median