showed that PBX1 was both necessary and sufficient to drive myeloma proliferation, and analyses of transcriptomics and chromatin binding demonstrated a network of genes that are regulated by PBX1 binding to promoters and enhancers. This network includes the additional transcription factors FOXM1 and E2F1/2, which regulate the cell cycle, as well as its own transcription (see figure). These data are consistent with the findings that proliferation is a significant contributor to poor outcomes in myeloma.[6] FOXM1 is also a transcriptional regulator of NEK2, a gene that has been associated with drug resistance and poor outcomes,[7] and it is also downregulated upon silencing of FOXM1 or PBX1.

Although identifying a network of genes that drive myeloma proliferation is important from a prognostic standpoint, developing therapies to target these networks could have a significant impact on patient outcomes. In fact, therapeutic targeting of the dysregulated cell proliferation pathways is a major unmet need in multiple myeloma, for which the most effective therapies target normal plasma cell biology rather than the genomic aberrations that drive progression.[8] Targeting of such pathways will become a critical area of therapeutic development in myeloma, particularly for patients with biologically aggressive disease that is less sensitive to standard therapies, including those with amp1q. Trasanidis et al use an inhibitor of FOXM1 and a novel PBX1 inhibitor that they recently developed[9] and demonstrate that pharmacological inhibition of this pathway can inhibit myeloma growth/survival both in vitro and in vivo. Importantly, the activity of the PBX1 inhibitor appears to be dependent on the presence of +1q. Moreover, this compound's activity is not limited to myeloma, as cells from several solid tumors that harbor 1q gains are also sensitive. This fact suggests that the transcriptional remodeling associated with +1q that occurs in myeloma very likely occurs in other tumors with this copy number alteration. Although the PBX1 inhibitor is in early stages of development, it offers a promising opportunity to neutralize and target a key regulator of proliferation in +1q myeloma and other cancers, as well as to sensitize tumors to other therapies. One is left to wonder whether this could also include newer immunotherapeutic approaches, as the genes that are repressed by PBX1 are associated with interferon responses. It is also important to investigate whether the context of 1q copy number (gain vs amp) and/or co-occurring cytogenetic abnormalities such as t(4;14), which are known to influence outcomes among patients with +1q,[10] affect myeloma cells' susceptibility to inhibition of this pathway. PBX1 appears to be one of the most active genomic "gadgets" yet to be discovered in the (ch1)Q branch, and perhaps a double agent has left the door open for targeted therapy in +1q myeloma.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Ward et al, page 1999

Always be prepared for success

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In this issue of *Blood*, Ward et al[1] report the promising efficacy and manageable toxicity profile from an investigator-initiated phase 1 trial combining brentuximab vedotin and lenalidomide in patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) who had received or were ineligible for autologous stem cell transplantation (ASCT).

For patients with relapsed or refractory DLBCL, second-line treatment algorithms have historically categorized patients based on their fitness for potential high-dose chemotherapy and ASCT, or, to state this more succinctly, either curative or palliative intent. Patients who were ineligible for or relapsed after ASCT potentially benefit from additional therapy; however, results were often transient and toxic. Thankfully, this paradigm is shifting.

Brentuximab vedotin, an antibody drug conjugate targeting CD30, and lenalidomide, an immunomodulatory drug targeting cereblon, are both Food and Drug Administration approved for various hematologic malignancies. As single-agent therapy for patients with relapsed DLBCL, both drugs have resulted in modest efficacy, with brentuximab vedotin achieving an overall response rate (ORR) of 44% and complete response

Results from recent clinical trials of targeted agents in patients with relapsed DLBCL not eligible for stem cell transplant

	N	ORR (%)	95% CI	CR (%)	Median DOR (mo)	Median PFS (mo)	Median OS (mo)
Brentuximab lenalidomide[1]	37	57	39.6-72.5	35	13.1	10.2	14.3
Rituximab lenalidomide ibrutinib[6]	39	44	28-60	28	15.9	5.5	9.5
Tafasitamab lenalidomide[5]	80	57.5	45.9-68.5	40	43.9	11.6	33.5
Polatuzumab bendamustine rituximab[7]	106	41.5	NR	38.7	9.5	6.6	12.5
Loncastuximab[8]	145	48.3	39.9-56.7	24.1	10.3	4.9	9.9

CR, complete response; DOR, duration of response; NR, nonresponder; OS, overall survival; PFS, progression-free survival.

rate (CRR) of 17%,[2] and lenalidomide achieving an ORR of 28% and CRR of 15%.[3] Based on the nonoverlapping drug targets, manageable toxicities, and single-agent efficacy, the combination of brentuximab vedotin and lenalidomide was evaluated by Ward and colleagues.

In total, 37 patients with DLBCL were enrolled with 3 median prior lines of therapy, of which 46% were refractory to initial therapy and 54% were refractory to most recent therapy. Patients were treated in 3 dose levels, identifying the maximum tolerated dose of the combination to be 1.2 mg/kg of brentuximab vedotin IV every 21 days and 20 mg of lenalidomide orally. Contrary to the commonly used lenalidomide dosing strategy of a dosing period followed by a rest period, the investigators on this trial used a continuous dosing strategy, which may have contributed to the 84% of patients requiring growth factor support and with 55% requiring lenalidomide dose reductions. Toxicities were generally similar to previous data with either brentuximab vedotin and lenalidomide, with only 10% of patients discontinuing therapy because of an adverse event.

The combination of brentuximab vedotin and lenalidomide resulted in an ORR of 57% (95% confidence interval [CI]: 39.6% to 72.5%) and CRR of 35% (95% CI: 20.7% to 52.6%), which compares favorably to the historical single-agent efficacy. Responses generally occurred quickly with a median time to response of 1.4 months and were durable with a median duration of response of 13.1 months for all patients who responded. The median progression-free survival was 10.2 months, and median overall survival was 14.3 months. To confirm these results, a randomized phase 3 study comparing the CD20 targeting monoclonal antibody rituximab and lenalidomide with and without brentuximab vedotin is currently enrolling (NCT04404283).[4]

With the caveats of this being a relatively small phase 1 trial, the efficacy results are comparable to several other clinical trials recently conducted in similar patients with relapsed DLBCL (see table). Lenalidomide-based combinations, with dosing of 25 mg for 21 days on followed by 7 days off, have impressive efficacy when combined with the CD19 targeting monoclonal antibody tafasitamab,[5] and with the BTK inhibitor ibrutinib and rituximab (although improved efficacy was observed in the nongerminal center subtype).[6] Antibody drug conjugates including the CD79B targeting polatuzumab vedotin in combination with bendamustine and rituximab[7] and the CD19 targeting loncastuximab tesirine have also had promising activity.[8]

With all of these intriguing combinations, including lenalidomide and/or antibody drug conjugates, what is the treating physician to do? How to choose a particular therapy for a particular patient? Ideally, a large, randomized study would be conducted to directly compare various treatment options in the same population, but with multiple novel treatments emerging for patients with relapsed DLBCL, or potentially moving to first-line therapy, such a trial would be prohibitive and potentially out of date prior to completion.

The legendary clinical investigator, Emil J. Freireich, who recently died at age 93, helped develop essential concepts, including combination chemotherapy, platelet transfusions, and plasmapheresis, but also was known to turn a phrase (or ten).[9,10] Freireich's law number 2 is "Always be prepared for success. Failure creates few problems." This sage advice speaks volumes regarding the current situation for treatment of patients with relapsed DLBCL: we have several recent successful trials, with a high probability of many more to come. We must be prepared to catch this growing wind in our sails whichever way it blows, to help treating physicians to select the optimal therapy for each patient.

Ward and colleagues have taken an important first step along these lines by conducting a detailed analysis of both tumor tissue and dynamic changes in the peripheral blood immune cell subsets. Actionable baseline clinical factors that may be predictive for response with brentuximab vedotin and lenalidomide were not identified in this trial, perhaps because of the relatively small sample size or assays used. However, the intention of the investigators was laudable and should represent an expected norm for future such trials.

As a field, we should work to harmonize our approaches for patients with DLBCL, starting with plans for the analysis of baseline and dynamic factors, including tumor tissue, immune cell subsets, circulating tumor DNA, and microbiome samples that could be compared across trials. These data would not replace the need for future randomized trials but

could allow for greater cross-trial comparisons and eventually improved precision in treatment selection.

In conclusion, the combination of brentuximab vedotin and lenalidomide is impressive in this phase 1 trial, and a confirmatory randomized phase 3 trial is now ongoing. The results of this and other similar trials highlight a large and increasingly urgent need: let's prepare for success.

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HEMATOPOIESIS AND STEM CELLS

Comment on Loeffler et al, page 2011

Asymmetric division: the choice of fate for huHSCs

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In this issue of *Blood*, Loeffler et al[1] provide evidence that human hematopoietic stem cells (hHSCs) can undergo asymmetric cell division (ACD) to generate 2 cells with distinct functional fates, and that lysosome asymmetric inheritance determines these fates. These findings answer the long-standing question of ACD in HSC biology and uncover unexpected factors that determine cell fate that may be used for developing new protocols for HSC expansion.

Remaining a stem cell or committing to differentiation is the most important fate-determining event in the lifetime of the HSC. This process maintains the balance between the pool of HSCs and progenitors and ensures homeostasis of the hematopoietic system. During development, stem cells use ACD to create cellular diversity and maintain adequate numbers of both stem cells and differentiated cells. Stem cells can adapt their mode of division and divide symmetrically (generating 2 stem cells or 2 progenitors) or asymmetrically, to meet the regenerative need of tissues,[2] the failure of which can cause long-term tissue exhaustion or tumor development (see figure panel A). Although hHSC are believed to divide asymmetrically, definite evidence has been lacking.

ACD is the unequal partitioning of cellular components during cell division that enables daughter cells to have distinct fates. ACD is controlled by the asymmetric reorganization of the cytoskeleton, which results in cellular polarity, with an asymmetric accumulation of factors that determine the fate of the cell. The orientation of the mitotic spindle along the polarity axis ensures unequal partitioning of these factors between daughter cells, with the level of fate-determining factors received deciding the future identity of each daughter cell (see figure panel B).[2]

Demonstrating ACD requires the linking of factors that alter cell fate to their asymmetric distribution. Identifying these linking factors has been a challenging task in HSCs because of the limited knowledge of the factors that determine HSC identity. HSCs are retrospectively defined by their ability to generate mature cells, making assessment of HSC fate dependent on the behavior of the progeny. ACD was first suggested in HSCs when paired daughter cells generated clones of distinct size and myeloid lineage potential in vitro.[3] In murine HSCs, cellular factors, including lysosomes, can asymmetrically segregate and alter HSC fate.[4,5] In hHSCs, studies have shown that the surface markers CD53 and CD62L are unequally partitioned, which can correlate with the potential lineage of daughter cells.[6] The endosomal protein Ap2a2[7] and myosin II[8] can both alter hHSC fate and may be asymmetrically inherited. None of the aforementioned studies linked asymmetric inheritance of these components to hHSC fate.

In a tour de force, Loeffler et al were able to link asymmetric lysosomal inheritance to hHSC fate ex vivo, demonstrating that hHSCs can use ACD to generate daughter cells with distinct fates. They used a long-term quantitative single-cell imaging technique to quantify factors inherited by paired daughter cells during