the US Intergroup (#NCT03737981 and #NCT03701282) will use a triple combination therapy of ibrutinib, venetoclax, and obinutuzumab. In the setting of a clinical trial, it is quite conceivable that a combination of a BTKi together with a CD20 antibody, such as performed by Burger, for a fixed time period of therapy might also achieve a beneficial clinical benefit.

Moving forward, how does the landmark study of Burger and colleagues and the already published North American intergroup study[6] inform the field? In no setting outside of clinical trials should rituximab be administered together with ibrutinib as part of a continuous treatment regimen for CLL. Although the primary end point of the important trial by Burger and colleagues was negative, both this answer and the secondary end points inform the field of CLL research moving forward. Targeting CD20 with therapeutic antibodies as part of future trials should not be abandoned, but adaptation to better molecules, preclinical rationale, and end points with modern therapy should be accounted for.

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LYMPHOID NEOPLASIA

Comment on Opstal-van Winden et al, page 1130

Genetic susceptibility to breast cancer in lymphoma survivors

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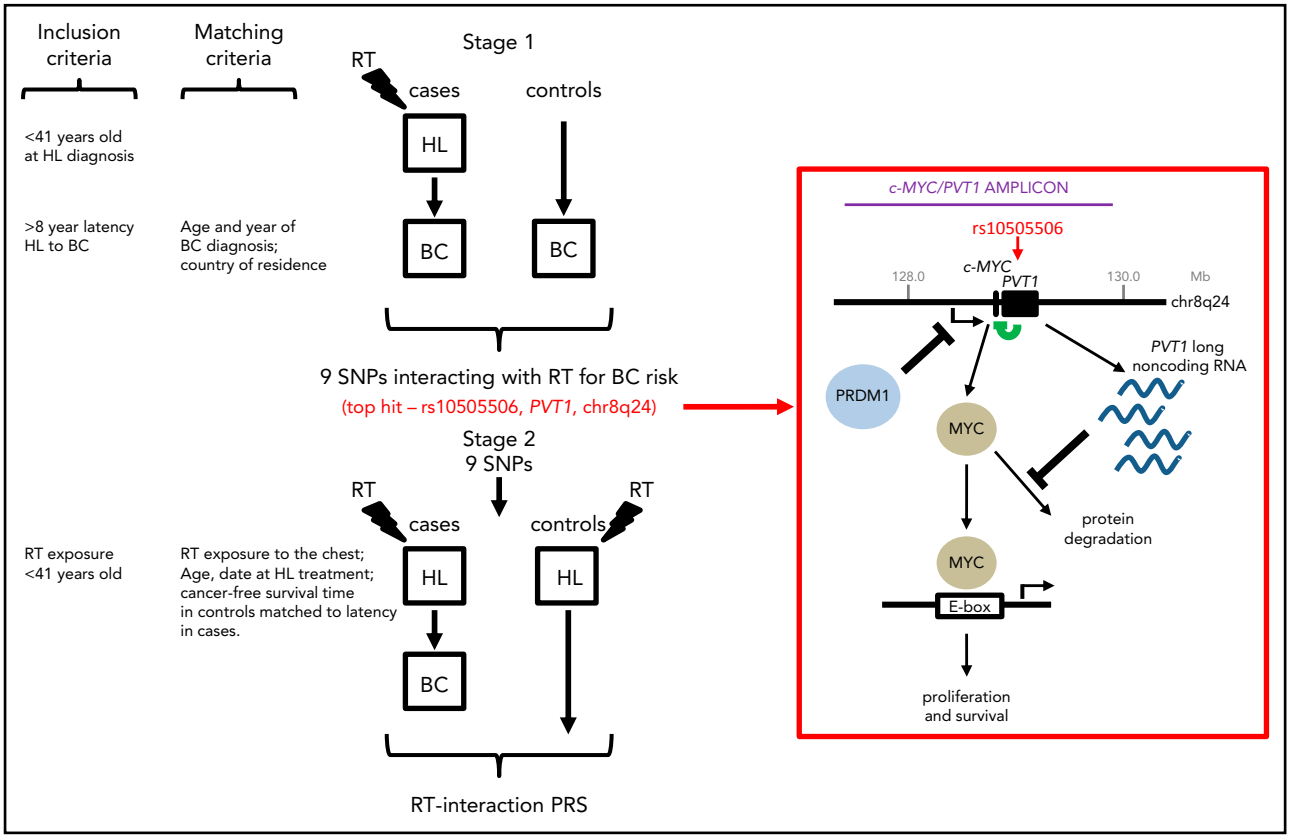
In this issue of *Blood*, Opstal-van Winden and colleagues report the results of a genome-wide association study to identify constitutional genetic variants (single nucleotide polymorphisms; SNPs) associated with risk of developing radiation-induced breast cancer in Hodgkin lymphoma survivors.[1] Therapy-induced cancer is a potentially lethal complication of treatment of a first primary cancer, and breast cancer is one of the most common therapy-induced cancers in long-term survivors of Hodgkin lymphoma treated with radiotherapy. Travis et al[2] estimated the cumulative absolute risks of breast cancer following a ≥40 Gy dose at age 25 to be 11% and 29% at 20 and 30 years, respectively. Data from a large cancer registry showed relative risk of breast cancer to be ~6 times higher in Hodgkin lymphoma survivors compared with the general population.[3] As such, the prospective identification of individuals at high risk of radiogenic breast cancer could facilitate improvements in the clinical management of Hodgkin lymphoma patients, reducing subsequent cancer risk and improving outcomes.

Numerous patient- and exposure-related factors modify radiogenic breast cancer risk, including age at exposure, cumulative radiation dose, radiation field size, radiation field location (mediastinal or mantle), and early menopause.[4] Evidence also suggests a role for constitutional genetics as a determinant of individual risk,[5] with the prevailing model suggesting that the genetic contribution to radiogenic breast cancer risk is polygenic and determined by coinheritance of multiple low-penetrance genetic variants in numerous genes.

Based on this premise, Opstal-van Winden and colleagues used an innovative 2-phase approach to identify genetic variants associated with risk of radiogenic breast cancer specifically in Hodgkin lymphoma survivors. Their approach was designed to first identify variants interacting with radiation for breast cancer risk and then use these to generate a polygenic risk score (PRS) for breast cancer in Hodgkin lymphoma survivors while simultaneously eliminating variants associated with Hodgkin lymphoma risk (see figure). Carefully defined inclusion criteria were used to maximize the frequency of likely radiogenic cases while simultaneously minimizing the frequency of "sporadic" second primary breast cancer cases without a radiation etiology. Specifically, the authors restricted their study to cases exposed to radiation at a



Study design used to develop a PRS for radiogenic breast cancer. Opstal-van Winden and colleagues used a 2-stage approach to identify SNPs interacting with radiotherapy (RT) for breast cancer (BC) risk (top) and subsequently used these to develop a PRS for radiogenic breast cancer in Hodgkin lymphoma (HL) survivors (bottom). Inclusion and matching criteria (left) were used to maximize the power to identify genetic variants associated with risk of radiogenic breast cancer. A SNP (rs10505506) localized to *PVT1* significantly associates with risk of radiogenic breast cancer in Hodgkin lymphoma survivors (boxed in red). The *PVT1* long noncoding RNA negatively regulates MYC-driven oncogenic transcriptional activity by inhibiting protein degradation. The *PVT1* promoter also negatively regulates *MYC* transcription directly (green). MYC expression drives transcription of downstream target genes via binding to E-box elements in promoters, promoting tumor cell proliferation and survival. *c-MYC* and *PVT1* are coamplified in primary breast cells exposed to ionizing radiation (purple bar shows approximate location of amplicon identified by Wade and colleagues[6]). A risk allele for radiogenic cancer in Hodgkin lymphoma survivors was identified in the *PRDM1* gene.[5] PRDM1 is a negative regulator of *c-MYC* transcription, but *PRDM1* upregulation in response to ionizing radiation is attenuated in cells carrying the risk variant, leading to elevated *c-MYC* expression and acquisition of a pro-proliferative phenotype. Additional work is required to determine functionality of the rs10505506 variant, although it is plausible this also operates via the MYC pathway. Approximate amplicon, gene, and SNP locations are based on GRCh37.

young age and with a latency of at least 8 years between lymphoma and subsequent breast cancer (see figure). Furthermore, the use of stringent case-control matching was designed to minimize the contribution of established risk factors (such as age at exposure) while simultaneously maximizing the power to discern genetic variants associated with risk of radiogenic breast cancer. This approach was used to generate a PRS based on 9 SNPs that showed a statistically significant interaction with radiotherapy for breast cancer risk. Critically, the PRS retained predictive power irrespective of exposure to gonadotoxic therapy, despite this not being included in the matching criteria. Radiation exposure to the ovaries or systemic alkylating chemotherapy can ablate ovarian function, can reduce estrogen production, and is associated with a significant reduction in radiogenic breast cancer risk in Hodgkin lymphoma survivors.[4] Likewise, the PRS retained predictive power irrespective of age at radiation exposure, suggesting applicability to all Hodgkin lymphoma survivors. The study by Opstal-van Winden and colleagues focused on "common" SNPs with a minor allele frequency of >1% in the general population. It is likely that rarer SNPs also contribute to individual risk of radiogenic breast cancer, but identification will require large studies that either directly genotype these variants (using next-generation sequencing, for example) or that use accurate methods for imputing nongenotyped low-frequency variants. Large studies with enhanced statistical power will also be required to discern gene-gene interactions and to overcome stringent statistical thresholds associated with multiple testing in genome-wide studies.

As well as predicting individual risk, the identification of susceptibility variants informs on the molecular mechanisms driving the development of radiogenic cancer. In this regard, it is noteworthy that the most statistically significant of the 9 SNPs identified by Opstal-van Winden and colleagues localizes to the *PVT1* long noncoding RNA gene on chromosome 8q24 (rs10505506), adjacent to the *c-MYC* proto-oncogene (see figure). High-level somatic copy number gain of this locus, capturing both *c-MYC* and *PVT1*, is significantly more common in radiogenic breast cancer compared with disease without a radiation etiology.[6,7] Furthermore, focal high-level amplification of this locus was reported in primary breast epithelial cells and an immortalized nontransformed breast cell line following exposure to ionizing radiation,[6] suggesting *c-MYC/PVT1* amplification to

be an early somatic event in radiation-driven breast transformation. *c-MYC* and *PVT1* are almost always amplified together in human tumors, and the *PVT1* long noncoding RNA was recently identified as a key regular of MYC-driven oncogenic transcriptional activity by augmenting protein stability[8] (see figure). There is also evidence that the *PVT1* promoter directly regulates *MYC* transcription, independent of long noncoding RNA expression.[9] Knockout of *PVT1* reduces MYC protein levels and attenuates tumorigenic potential of cells.[8] Other data also suggest a role for dysregulated MYC in radiation-induced breast transformation. Specifically, Best et al[5] identified a SNP in the *PRDM1* gene associated with radiogenic cancer risk (predominantly breast cancer) in pediatric Hodgkin lymphoma survivors. PRDM1 is a negative regulator of *c-MYC* transcription, but PRDM1 upregulation in response to ionizing radiation is attenuated in cells carrying the risk variant, leading to elevated *c-MYC* expression and acquisition of a pro-proliferative phenotype.[5] Collectively, these studies provide compelling evidence that dysregulated MYC is a common feature of radiogenic breast cancer during the early stages of transformation. As such, it is plausible that the *PVT1* SNP identified by Opstal-van Winden et al also operates via MYC to affect risk of radiogenic breast cancer, although additional work is required to determine functionality of the rs10505506 variant.

Pending independent validation, the data presented by Opstal-van Winden and others[5] could aid the development of personalized risk-adapted strategies for the clinical management of Hodgkin lymphoma patients, including alternative treatments and posttherapy surveillance for therapy-induced breast cancer. Such approaches could prove important in pediatric and young adult Hodgkin lymphoma patients where the risk of radiogenic breast cancer is particularly high and associated with premature death.

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MYELOID NEOPLASIA

Comment on Christen et al, page 1140

More than a fusion gene: the *RUNX1-RUNX1T1* AML

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In this issue of *Blood*, Christen et al investigated the largest cohort to date of 331 patients with acute myeloid leukemia (AML) and t(8;21).[1]

These patients have AML with specific morphologic features such as dysplasia in granulopoiesis (90% of patients) and eosinophilia and are mostly classified as AML with maturation (90%; formally called French-American-British [FAB] M2) or AML without maturation (10%; formally called FAB M1).[2] This subtype of AML is also diagnosed by immunophenotyping that shows the coexpression of CD19 or PAX5 and CD56. The cytogenetics show a typical pattern of loss of the sex chromosome and del9q. These characteristics resulted in *RUNX1-RUNX1T1*–mutated AML being designated as a separate World Health Organization (WHO) entity within the category of AML with recurrent genetic abnormalities. The diagnosis is made irrespective of bone marrow blast cell counts.[3] *RUNX1-RUNX1T1*–mutated AML also demonstrates secondary cooperating mutations in *KIT*, *KRAS* or *NRAS*, and *ASXL1* as well as in *ASXL2*.[4,5] *RUNX1-RUNX1T1* was one of the first fusion genes to be used for minimal residual disease (MRD) monitoring.[6] Based on these diagnostic definitions, the best clinical practice to follow after standard chemotherapy needed to be determined, including the meaningfulness of allogeneic transplantation in first complete molecular remission (CMR).[7,8]

Today, large sequencing studies including exome sequencing or whole-genome sequencing (WES) are possible. In their article, Christen et al provide a comprehensive characterization of this specific WHO entity in 331 patients based on a screening that included 66 recurrently mutated genes. They found that 95% of patients had at least 1 additional mutation, with a mean of 2.2 driver mutations per patient. Recurrently mutated genes affecting the RAS/RTE signaling pathway were present in nearly two-thirds of patients and other epigenetic regulators in nearly half the patients. Several previously unexpected genes were found to be mutated. Data using deep sequencing (45 000×) in 62 samples from patients in complete remission demonstrated persistent mutations in 12 samples, including 5 patients who were quantitative polymerase chain reaction–negative for *RUNX1-RUNX1T1* at the time of the analysis. In multivariate analysis, *JAK2*, *FLT3*-ITD^{high}, and *KIT*^{high} were identified as significant negative prognostic factors. Furthermore, it was demonstrated that one-third of patients