portant questions both about the nature of the evidence and about its sufficiency, a topic that has been the subject of inquiry and discussion in the patient-safety community for well over a decade.[1] As noted in the editorial, replications have confirmed substantial effects regarding the use of a surgical checklist, but rigorous randomized trials have not been carried out and are unlikely to be. In contrast to the relatively simple act of providing a new drug or procedure, implementing the surgical checklist calls for performance of a diverse array of 20 or more actions, which can, and should, vary from one institution to another.

An even more important barrier to performing a randomized trial is that implementation of the checklist almost always requires major culture change. Although culture can (and should) be measured, because of its unique nature in a given operating suite (even among individual rooms), the more relevant comparison after implementation of a checklist is with the prior condition, a before-versus-after study, not with other organizations with very different cultures. The key culture change facilitated by the surgical checklist is the development of highly functioning teams, the value of which is well supported by evidence from many venues in and out of health care.

Weiser and Krummel reemphasize the key learnings from all checklist replication studies: success requires great effort directed toward the implementation process and strong leadership, a point also made by Haynes et al., who in addition note that the 9% reduction in mortality that Urbach et al. report could be a significant trend if mortality was followed for a longer time period.

Robblee notes a potential benefit from implementing the surgical checklist that has been underappreciated in the literature: the identification of near misses, which are defined as errors or malfunctions that might well have caused harm if they had not been intercepted. If these events are analyzed, the underlying process failures (so-called latent failures) can often be identified and the process redesigned to prevent the errors from recurring. Indeed, if and when performance of the surgical checklist is fully institutionalized as an integral part of a culture of everyday teamwork in the operating room, it may turn out that one of its major benefits will be identifying opportunities for process improvement.

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Since publication of his article, the author reports no further potential conflict of interest.

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Ibrutinib Resistance in Chronic Lymphocytic Leukemia

TO THE EDITOR: Ibrutinib, an inhibitor that binds covalently to C481 of Bruton's tyrosine kinase (BTK), has produced remarkable responses in patients with relapsed and refractory chronic lymphocytic leukemia (CLL).[1-4] However, 5.3% of patients have disease progression, and the mechanism of resistance is largely unknown. Herein we describe the mechanism of resistance in such a case.

A 49-year-old woman had a diagnosis of CLL established in 2000. After the failure of multiple treatments, she began receiving ibrutinib at a dose of 560 mg daily in 2010 as part of a phase 1, dose-escalation study of ibrutinib in B-cell cancers.[1] By month 11, a partial response was achieved with an absolute lymphocyte count of 4530 cells per cubic millimeter. Computed tomography at month 18 showed a marked but incomplete reduction of lymphadenopathy. At month 21, a rapidly rising lymphocyte count and progressive lymphadenopathy were noted. Despite a dose escalation to 840 mg daily, CLL progressed during the next 4 weeks (for details, see the Supplementary Appendix, available with the full text of this letter at NEJM.org). Peripheral-blood samples were collected before ibrutinib administration (day −52), while the patient was having a response to the drug (day 472), when progressive disease was first noted (day 589), and before dose escalation (day 616). Figure S1 in the Supplementary Appendix shows the dates of sample collection in relation to the patient's absolute lymphocyte count over the treatment course.

RNA sequencing revealed a thymidine-to-adenine mutation at nucleotide 1634 of the BTK complementary DNA (cDNA) (GenBank accession number, NM_000061.2), leading to a substitution of serine for cysteine at residue 481 (C481S). The mutation was detected in the samples collected

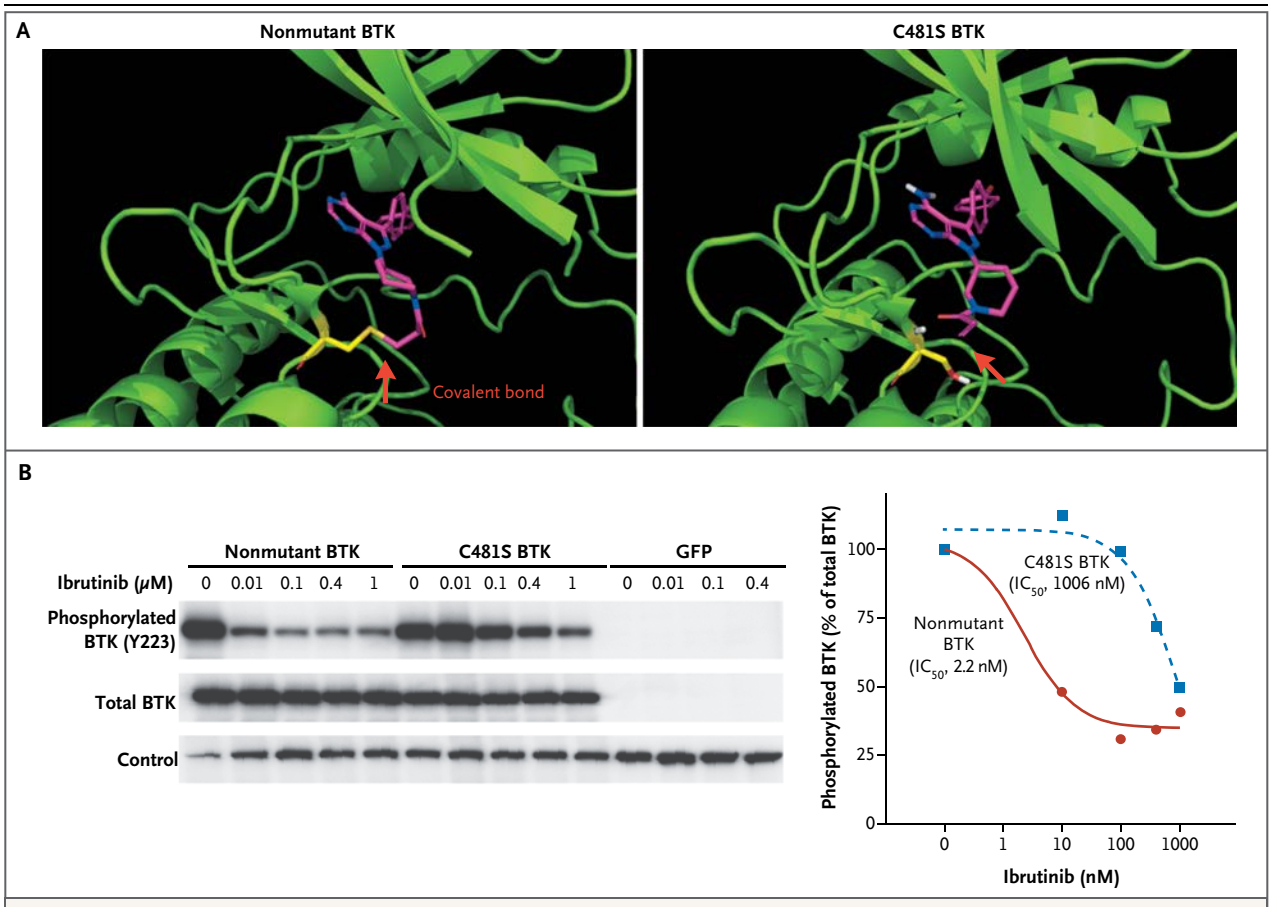

Figure 1. Effect of C481S Mutation of Bruton's Tyrosine Kinase (BTK) on Ibrutinib Binding and the Ability of Ibrutinib to Inhibit BTK Phosphorylation.

Panel A shows structural modeling of nonmutant and mutant BTK with ibrutinib. The red arrows indicate the covalent bond between ibrutinib (purple and blue) and BTK (green and yellow) before and after the mutation. Panel B shows the inhibition of nonmutant BTK or C481S BTK phosphorylation by ibrutinib in HEK 293 cells. The half-maximal inhibitory concentration (IC_{50}) of ibrutinib for inhibition of BTK phosphorylation was analyzed and plotted with GraphPad Prism. GFP denotes green fluorescent protein.

when progressive disease was first noted (88% of reads) and before dose escalation (92% of reads) but not in those collected before ibrutinib administration or while the patient was having a response (Fig. S2A in the Supplementary Appendix). No other genetic changes were identified that correlated with the patient's clinical course in the same manner as the BTK mutation. Sanger sequencing of cDNA verified that the mutation was detected only in the samples collected during relapse (Fig. S2B in the Supplementary Appendix). A more sensitive, allele-specific polymerase-chain-reaction assay (1% analytic sensitivity) detected the mutation in the genomic DNA of samples collected during relapse but not in those collected before ibrutinib administration or while the patient was having a response (Fig. S3 in the Supplementary Appendix).

Ibrutinib binds covalently to the sulfhydryl group of C481 of BTK in the active site, resulting in irreversible inhibition of its kinase activity.[5] Structural modeling suggested that the C481S mutation would disrupt this covalent binding, changing irreversible binding to reversible binding (Fig. 1A). Fluorescently tagged ibrutinib labeled the nonmutant BTK, and the covalent binding that was formed withstood electrophoresis, whereas reversible binding to the C481S or C481A mutant of BTK did not. This showed biochemically the critical role of cysteine in covalent-bond formation (Fig. S4 in the Supplementary Appendix).

Phosphorylation of BTK (pY223) reflects BTK

kinase activity. Introduction of the recombinant nonmutant and C481S BTK constructs into HEK 293 cells showed that phosphorylation of C481S BTK at Y223 became significantly less sensitive to ibrutinib inhibition than the nonmutant BTK did (half-maximal inhibitory concentration, 1006 nM vs. 2.2 nM) (Fig. 1B).

Taken together, our data indicate that the C481S mutation disrupts the covalent binding between BTK and ibrutinib. The impaired binding leads to a loss of inhibition of BTK enzymatic activity that ultimately results in ibrutinib resistance in the patient. Consistent with the findings reported in the *Journal* by Woyach et al.,[6] our studies confirm that BTK is a relevant pharmacologic target of ibrutinib from a genetic perspective.

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CORRECTIONS

Case 12-2014: A 59-Year-Old Man with Fatigue, Abdominal Pain, Anemia, and Abnormal Liver Function (April 17, 2014; 370:1542-50). In Table 2 (page 1547), the mode of inheritance for erythropoietic protoporphyria should have been "Autosomal recessive or X-linked," rather than "Autosomal dominant." The article is correct at NEJM.org.

Case 11-2014: A Man with Traumatic Injuries after a Bomb Explosion at the Boston Marathon (April 10, 2014;370:1441-51). In the legend for Figure 1 (page 1443), the phrase "taken on admission," should be added after "Plain radiographs of the chest (Panel A) and pelvis (Panel B) . . . ," and the phrase "taken after the surgical amputation" should be added after ". . . contrast-enhanced multidetector CT (MDCT) with volume rendering . . . ." The article is correct at NEJM.org.

The Renormalization of Smoking? E-Cigarettes and the Tobacco "Endgame" (January 23, 2014;370:293-5). In Figure 2 (page 294), the bars for use of cigarettes and use of electronic cigarettes should have been shown side by side, rather than stacked, since some students may have been included in both categories. The article is correct at NEJM.org.