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Cyclophosphamide for CLL: to be or not CYP2B activated?

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In this issue of Blood, Johnson et al1 report their findings of the hepatic cytochrome P450 (CYP) enzyme gene polymorphisms in patients with chronic lymphocytic leukemia (CLL) treated with fludarabine and cyclophosphamide (FC) within a clinical trial. Patients with at least one *6 allele had significantly lower rates of complete response and also less toxicity than patients with *1/*1 alleles, as well as a trend of shorter progression-free survival (PFS). Albeit indirect, this is the first indication in a controlled clinical trial of a significant impact of cyclophosphamide (CPA) bioactivation in CLL and a step toward tailored therapy of cancer.

When combined, FC exerts synergism owing to inhibition by fludarabine of the repair of CPA-induced point mutations.2 The British LFR CLL4 trial,3 which compared frontline therapy of CLL with chlorambucil, fludarabine, or FC, is one of the 3 important controlled trials4-5 that established FC as the standard chemotherapy in fit CLL patients and led to the CLL8 trial, which subsequently established rituximab-FC as the standard immuno-chemotherapy6 and was the first shown to prolong survival in CLL.

CPA is a pro-drug that requires bioactivation by hydroxylation to 4-hydroxy-CPA (4OH-CPA) by hepatic CYP enzymes, in particular CYP2B6 (see figure). 4OH-CPA is in equilibrium with its tautomter aldophosphamide, which, by off-cleavage of the toxic metabolite acrolein, turns into phosphoramidite mustard, the active compound. CYP2B6 mainly expresses *1/*1 alleles (wild type), but is quite polymorphic, with several known single nucleotide polymorphisms (SNPs) that lead to variant alleles, particularly the *6 allele, which occurs in approximately one-third of whites. In such individuals, the enzyme levels are reduced but may be more active in CPA hydroxylation than the *1/*1 allele enzyme.7,8

Johnson and colleagues1 performed real-time polymerase chain reaction for the CYP2B6 SNP genotypes in 455 (roughly all with available DNA) of the 777 patients in the CLL4 study. The 428 patients with the *1/*1 (265), *1/*6 (134) or *6/*6 (29) genotypes were selected for further investigation, of whom 206 had been assigned to chlorambucil, 103 to fludarabine, and 119 to FC (77 *1/*1 and 42 with at least one *6 allele, respectively). Clinical or other genomic characteristics did not differ among these treatment- or enzyme-variant subgroups, or among those with and without available DNA, and the main trial findings were the same in this cohort as in the original entire CLL4 cohort.

Among the patients assigned to FC, the patients expressing at least one *6 allele (*6 carriers) achieved a complete remission (CR) or a CR + nodal partial remission significantly less often than *1/*1 carriers, also confirmed by analysis of specific response indicators like white blood cell count and lymphadenopathy. In addition, a trend was found of shorter PFS among the *6 carriers than among the *1/*1 carriers (P = .055). Of note, in multivariate analysis of biological variables and response, only TP53 deletion/mutation and CYP2B6 variant had an independent influence on the likelihood of achieving CR. Furthermore, some typical FC-related adverse events and in-hospital days were also less frequent among *6 carriers. None of these differences were observed among the 309 patients assigned to either CLB or F. These findings clearly indicate a negative impact of the *6 allele on conversion of CPA to its active form.

That the response to CPA may vary with CYP2B3 variants is not a new observation, but has been suggested in lymphoma, myeloma, breast cancer, and stem cell transplantation, but often in retrospective studies with divergent
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A new view of integrin αIIbβ3 bound to membrane

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Integrins are a group of heterodimeric (α/β) transmembrane receptors crucial for mediating a variety of cell adhesion–dependent physiological and pathological processes such as tissue formation, blood clotting, immune responses, and tumor metastasis.1 Discovered 3 decades ago, integrins have been extensively studied with >50,000 citations found in PubMed. A central topic of the integrin research has been focused on understanding how integrin/ligand interaction (integrin affinity) is regulated. The answer to this question is crucial not only for understanding diverse integrin-mediated biological processes but also for diagnosing/treating various integrin-related diseases. A powerful way to address this question is to obtain detailed 3D structures of inactive integrin vs active integrin. In early 2000, the first groundbreaking crystal structure of integrin αvβ3 ectodomain was reported,2 which revealed a surprising bent conformation in which the ligand-binding head domain pointed down toward the membrane surface and the 2 legs were straight, parallel, and adjacent (see figure, panel A). A subsequent low-resolution cryo-electron microscopy (EM) model of intact integrin αIIbβ33 in detergent indicated a different ectodomain orientation, especially with the head pointing away from membrane, suggesting that the transmembrane-cyttoplasmic domain may influence the global architecture of the receptor. Nevertheless, the same unusual bent fold was observed in several other integrin ectodomains including αIIbβ3 (for review, see Campbell and Humphries5). These ectodomain structures, together with a series of biochemical, biophysical, and cell biology studies, led to a widely accepted model that integrins are activated via a switchblade-like conformational transition.6 Meanwhile, structures of integrin αIIbβ3 transmembrane-cyttoplasmic or cytoplasmic heterodimer were also pursued to understand how these segments control the resting, inactive state of the receptor.5 Nuclear Magnetic Resonance (NMR) analysis indicated that integrins contain a conserved α/β transmembrane interface but highly flexible cytoplasmic tail conformation. Notably, the membrane-proximal αIIb region was found to adopt helical,6 reverse turn,7 or disordered conformation.8 The C-terminal β3 cytoplasmic tail was also found to adopt variable conformations including helix, β-strand, or loop.6,8 Such structural variations may be caused by multiple factors such as different membrane-mimetic conditions, truncation of protein constructs, or binding to different partners/regulators. They may also dictate different functional or intermediate states of the receptor, which remains to be further investigated.

Given the uncertainties in structure and orientation of isolated integrin domains, Choi et al decided to pursue the structural investigation of full-length integrin. They purified intact inactive integrin αIIbβ3: the major platelet integrin that has been extensively studied for understanding the integrin structure/function. Because determining the structure of full-length integrin is still technically limited for x-ray crystallography and NMR, the authors used transmission EM to analyze the entire αIIbβ3 that was embedded in membrane-like nanodisc. By fitting the crystal structures of individual subdomains into an EM map, they were able to obtain the 3D reconstruction model of the ectodomain at 20.5Å resolution. The results were surprising: they found that...
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