The transcription factor c-Myc (or "Myc") is a master regulator of pathways driving cell growth and proliferation. MYC is deregulated in many human cancers, making its downstream target genes attractive candidates for drug development. We report the unexpected finding that B-cell lymphomas from mice and patients exhibit a striking correlation between high levels of Myc and checkpoint kinase 1 (Chk1). By in vitro cell biology studies as well as preclinical studies using a genetically engineered mouse model, we evaluated the role of Chk1 in Myc-overexpressing cells. We show that Myc indirectly induces Chek1 transcript and protein expression, independently of DNA damage response proteins such as ATM and p53. Importantly, we show that inhibition of Chk1, by either RNA interference or a novel highly selective small molecule inhibitor, results in caspase-dependent apoptosis that affects Myc-overexpressing cells in both in vitro and in vivo mouse models of B-cell lymphoma. Our data suggest that Chk1 inhibitors should be further evaluated as potential drugs against Myc-driven malignancies such as certain B-cell lymphoma/leukemia, neuroblastoma, and some breast and lung cancers. Clin Cancer Res; 17(22); 7067-79. ©2011 AACR.