LETTER

Nonsense suppression activity of PTC124 (ataluren)

Auld et al. (1) suggest that PTC124's nonsense suppression activity may be an indirect consequence of the compound's effects on firefly luciferase (FLuc) enzymatic activity. However, our initial characterization of potential nonsense-suppressing compounds in FLuc assays also utilized independent assays of nonsense suppression in disease-relevant systems, including assays measuring synthesis of full-length protein in mdx myotubes, mdx mice, and Cftr$^{-/-}$ transgenic mice (2, 3). These tests eliminated inactive chemical scaffolds and identified PTC124. Subsequently, PTC124 demonstrated nonsense suppression activity in cystic fibrosis and Duchenne muscular dystrophy patients (2, 4). These independent confirmations in validated suppression assays are to be contrasted with Auld et al.'s (1) use of a Renilla luciferase reporter that was an unsuitable and misinterpreted gauge of nonsense suppression because it failed to respond appreciably to the established nonsense-suppressing aminoglycoside, G418.

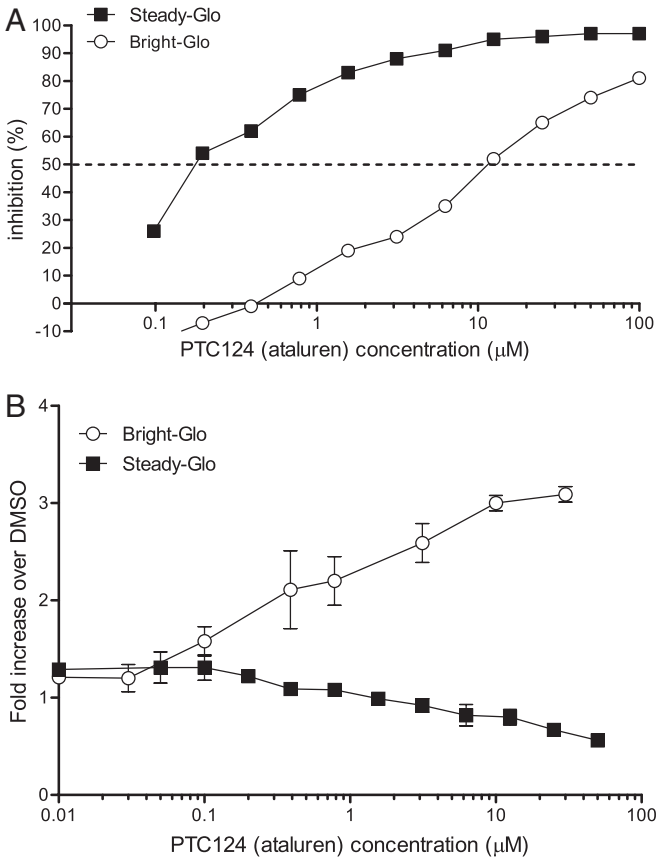

Fig. 1. PTC124 luciferase inhibition varies depending on the luciferase substrate used in the assay. (*A*) Inhibition of luciferase enzymatic activity is substrate-dependent. Recombinant luciferase protein (Sigma) was incubated with the indicated concentrations of PTC124 (synthesized by PTC Therapeutics). Luminescence was determined by using either the Bright-Glo or Steady-Glo luciferase substrate (Promega) as described by the manufacturer, and monitored with a Viewlux CCD imager (Perkin-Elmer). Luminescence data were normalized to reactions containing solvent alone (DMSO) and the percentage luciferase inhibition was calculated. Dotted line: 50% inhibition. (*B*) Inhibition of luciferase activity in the cell-based nonsense suppression assay is substrate-dependent. 293H cells were transiently transfected with a LUC nonsense suppression reporter gene containing a premature UGA terminator at codon 190 (2) and treated with the indicated concentrations of PTC124 for 20 h. After incubation, luminescence was determined by using either the Bright-Glo or Steady-Glo luciferase substrate as described for *A*. Luciferase activities were normalized to that produced with solvent alone (DMSO), and the fold suppression over background was calculated as $PTC124_{light\ units}/DMSO_{light\ units}$.

Although Auld and colleagues demonstrated elsewhere (5, 6) that luciferase IC_{50} activities vary widely with different luciferase substrates, in their recent PNAS article they presented only data from the Steady-Glo substrate (1). However, Steady-Glo has a 60-fold lower IC_{50} for PTC124 than Bright-Glo, the substrate we used (2) (Fig. 1*A*). Detection of nonsense suppression at 30 nM PTC124, well below the IC_{50} value of Bright-Go, indicates that the FLuc suppression activity we reported (2) cannot be due to enzyme inhibition. Auld et al. (1) also utilized transient transfections, whereas we used stable cell lines (2). Using transient transfections and both FLuc substrates, we found that PTC124 treatment recapitulated our previously reported Bright-Glo results (2) (Fig. 1*B*). However, consistent with the potent PTC124 IC_{50} with Steady-Glo, no luciferase activity was observed at any concentrations tested (Fig. 1*B*).

PTC124's activity in animal models and clinical trials, and the failure of Auld et al. (1) to replicate our assay conditions, indicates that their study is not relevant to the nonsense suppression efficacy of PTC124.

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