Development of a Human Cell-Based BCRP Inhibition Assay
Using CellPort CPT-P1 Cell Monolayers and Cladribine as the Probe Substrate

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Objectives
The objective of this study was to validate the use of cladribine as a probe substrate of the breast cancer resistance protein (BCRP) when used with CellPort Technologies’ CPT-P1 cell monolayers, in which P-gp function has been substantially and stably knocked down compared to parental Caco-2 cells.

Methods
Bidirectional transport of cladribine (10 µM) was examined in confluent MDRI-MDCK, MDCK, Caco-2 and CPT-P1 cell monolayers in the absence and presence of a series of known transporter inhibitors. Reference compounds to qualify the cell monolayer batches were run in parallel and all analytes were quantified using LC-MS/MS.

Results
CellPort CPT-P1 is a cell line derived from Caco-2, which shows substantial and stable knock-down of P-gp expression (Figure 1), with little or no effect on other functional parameters of the parental cell line (Table 1). These cells have the potential to be a more relevant and accurate test system for determining drug transporter interactions vs. other traditional in vitro cell based models.1,2

The permeability of cladribine was initially tested in MDRI-MDCK and MDCK cell monolayers with and without the P-gp inhibitor PSC833 (valspodar). The efflux ratio in MDRI-MDCK was 3.1, which collapsed to approximately 1 in the presence of this P-gp inhibitor. The relative efflux ratio (efflux ratio in MDRI-MDCK divided by efflux ratio in MDCK) was 2.7, also indicating some involvement of P-gp (Figure 2).

Permeability was then assessed using Caco-2 and CPT-P1 cell monolayers. In both cell lines the efflux ratio of cladribine was robust (approximately 20), with the difference being the change in this value when challenged with inhibitors of P-gp and BCRP. In Caco-2 cells, both sets of inhibitors (each dosed individually) showed a significant (up to 50%) decrease in efflux ratios, whereas in CPT-P1 cells, significant inhibition was observed only with the BCRP inhibitors (Figures 3 and 4). While most of the efflux of cladribine appears to be mediated by BCRP, these results further confirm some involvement of P-gp and establish the need for a more specific test system such as CPT-P1 for using this BCRP probe substrate to assess BCRP inhibition potential.

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References
1. Wang Y et al. (2011), Applications of CellPort® CPT-P1 cells in identifying P-gp substrates in vitro (poster # W3023 at this workshop)
2. Li J et al. (2011), Use of Transporter Knockdown Caco-2 Cells to Investigate in vitro Effect of Stath Drug (submitted to Drug Metab Dispos)