Appendix 2

Small-Molecule Toxicity Curves in NK-92 Cells

Introduction

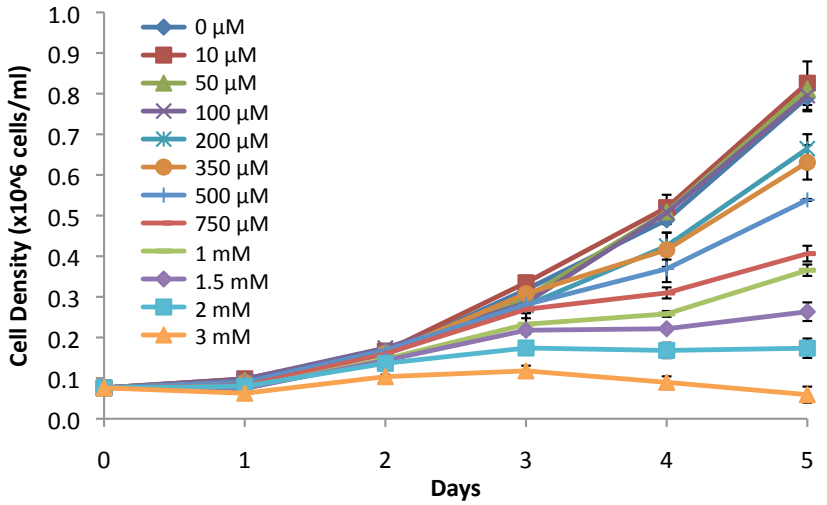
The toxicity of various small molecules to NK-92 cells was measured to determine the optimal ligand input concentration for regulatory device evaluations and to determine whether certain small molecules of interest are sufficiently nontoxic to be useful candidates for aptamer selection. Molecules were chosen for toxicity measurements for the reasons described in Appendix 1.

Results and Discussion

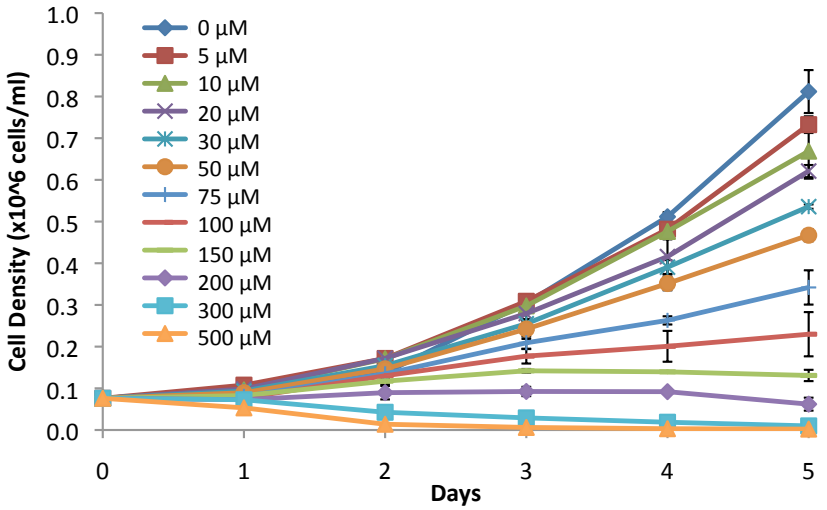
Compared to CTLL-2 cells, NK-92 cells are slightly more robust when cultured in the presence of theophylline (Appendix Figures 1.1, 2.1) and significantly more viable in the presence of tetracycline (Appendix Figures 1.2, 2.2). Therefore, the concentrations used for characterization studies in CTLL-2 cells were considered acceptable and adopted for use in NK-92 cells. Vitamin B_{12} is essentially non-toxic to NK-92 cells (Appendix Figure 2.3), and phenobarbital can be administered to 1 mM without significant toxicity (Appendix Figure 2.4), thus confirming these small molecules as desirable candidates for apatmer selection. Interestingly, folinic acid shows significant toxicity to NK-92 cells at 50 μM and above (Appendix Figure 2.5). Visual inspection of cultures containing 2 mM or more of folinic acid reveals fine precipitates, suggesting concentrated folinic acid could have non-specific interactions with the X-VIVO 20 media used to culture NK-92 cells but not with the RPMI-1640 media used to culture CTLL-2 cells.

Materials and Methods

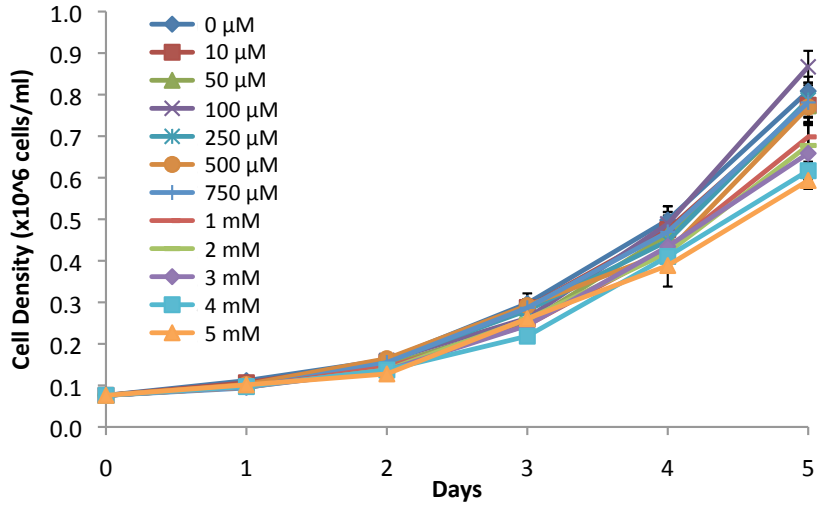
NK-92 cells stably expressing the *cd19-tk-t2a-il15* transgene were cultured in X-VIVO 20 media (Lonza) without exogenous cytokines or antibiotics. On Day 0, 12-well plates were seeded with cells at 0.08 x 10^6 cells/ml, 3 ml/well, 3 wells for each ligand concentration tested. The following small-molecule stocks were prepared by dissolving in X-VIVO 20 media and used without sterile filtration: 6 mM theophylline anhydrous (Sigma), 1 mM tetracycline hydrochloride (Sigma), 4 mM phenobarbital (Sigma), 10 mM folinic acid calcium salt (Sigma), and 10 mM vitamin B_{12} (cyanocobalamin, Sigma). Stocks were further diluted with media as necessary so that >3 μl of dissolved small molecule was added to each well to bring cultures to the appropriate final ligand concentrations. Culture plates were wrapped in foil to prevent excessive evaporation and stored in a 37°C incubator. All wells were split two-fold (phenobarbital and folinic acid samples) or three-fold (all remaining samples) using media containing the appropriate ligand concentration on Day 3. Starting on Day 1, 200 μl of each well was sampled every 24 hours for cell density measurements using a Quanta Cell Lab Flow Cytometer (Beckman Coulter). Viable populations were gated based on side scatter and electronic volume, and cell density was determined by viable cell count divided by total volume of sample analyzed on the flow cytometer. Reported values are the mean of triplicate samples ± s.d.



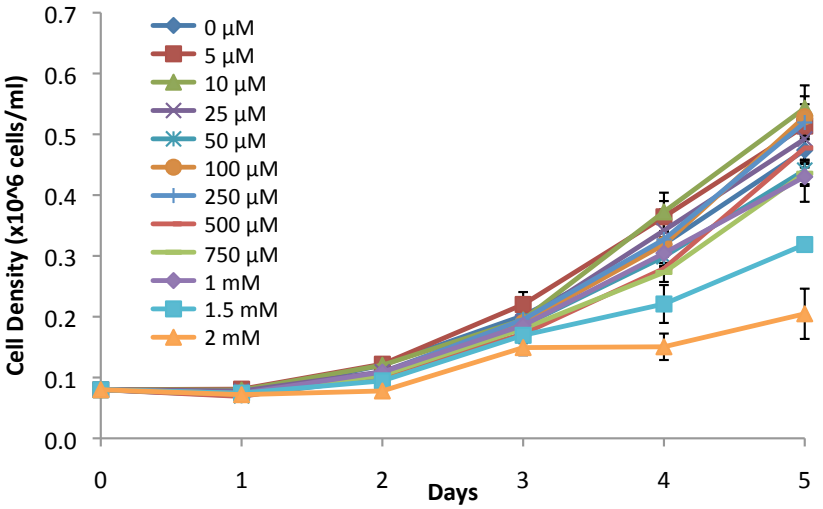
Appendix Figure 2.1. Theophylline toxicity curve in NK-92 cells.



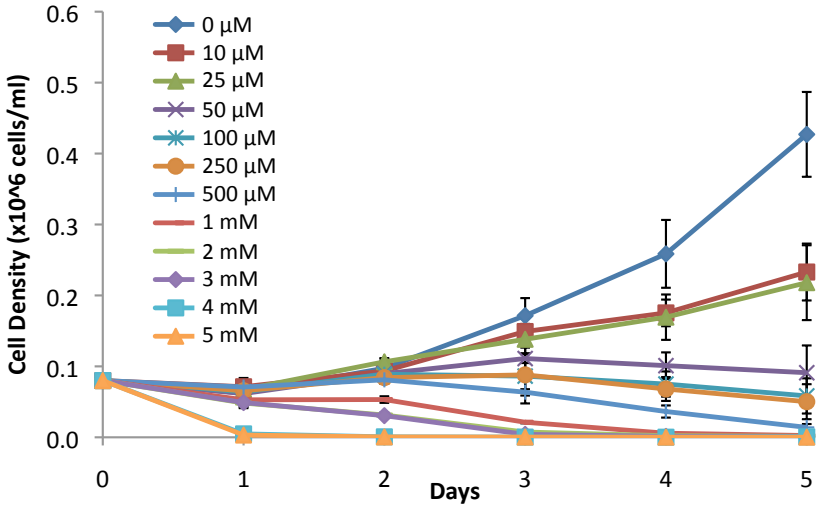
Appendix Figure 2.2. Tetracycline toxicity curve in NK-92 cells.



Appendix Figure 2.3. Vitamin B_{12} toxicity curve in NK-92 cells.



Appendix Figure 2.4. Phenobarbital toxicity curve in NK-92 cells.



Appendix Figure 2.5. Folinic Acid toxicity curve in NK-92 cells.