**Inhibitor Assay Version 1 Design Summary:**

**Drug List:**

Drugs for version 1 of the HNSCC specific inhibitor assay were determined from analysis of five different measurements.

1. **INHIBITOR ASSAY’S**

The top 15 drugs from previous inhibitor assays with and without an EGFR inhibitor from 11 patient derived HNSCC tumor cell lines were added. One of which was an HPV+ tumor cell line (10159). Effective drugs were also added based on inhibitor assay analysis of 3 cutaneous SCC patient derived tumor cell lines.

1. **TCGA HNSCC LIGHT PATHWAY ANALYSIS**

Shannon’s group analysis of TCGA HNSCC data set. Aberrational pathways were ranked based on number of a mutations in a certain pathway and frequency of mutations in a given gene. Using Rory’s analysis of FDA approved cancer drugs, Rory came up with a list of drugs that best targeted the aberrational pathways of the TCGA HNSCC dataset. For more details see Rory’s summary in box.

1. **RAPID ASSAY’S**

Rapid assay targets below the mean – 2 SD for 11 patient cell derived tumor cell lines were cross referenced to the targets from the drugs curated from the above two analyses. If a target came up from the rapid assay analysis and the TCGA analysis that did not have a drug already listed that hit that target, we used literature searches to determine which drug(s) should be added to the panel for that target.

1. **TCGA HNSCC DARK PATHWAY ANALYSIS**

Using Gabby’s ranking of TCGA dark pathways, Steve created a list of top natural products that would target the dark pathways. In addition to TCGA dark pathways, Steve also looked for natural products that would target p53 or RAS, as they were two targets from the rapid assay that did not have any drugs on the panel targeting them. See Steve’s summary in box for more details.

1. **COMBINATIONS**

Lastly, a few combinations were added to the panel based on data of effective drugs or targets from the inhibitor assay’s and rapid assays of 11 patient derived tumor cell lines in combination with an EGFR inhibitor. Most of these were combinations of drugs with the EGFR inhibitor, Gefitinib, and a few were other drugs in combination with a PI3K/mTOR inhibitor, BEZ235.

After curation using the four methods above, drugs for a few other targets of interest were added. This included BRD4 inhibitors and their combination with Gefitinib and BEZ235.

**Plate Layout:**

After all analyses above were completed, the position of the drug on each plate was randomized by Rory and Shannon. The randomization took into account x, x, x.

Plate layout design was based on design of Tyner panel where starting concentration of drug’s were added (1 to each well, going down the column) of column 1, 9, and 17. Columns 8, 16, and 24 were blanks and just included DMSO. Then 7, 3-fold serial dilutions were plated across the plate from columns 1-7, 9-15, and 17-23. Total volume in each well was 60uL. Starting concentrations of drugs were determined from previous use on Tyner panel. For new drugs not tested on Tyner panel, the starting concentrations were all 10mM (for master plate but final concentration will be 10nM on daughter plates after addition of cells) to test for initial version but can be adjusted for future versions.

**Drug Dilutions:**

All drugs were diluted to 20mM unless otherwise stated on vial due to solubility difficulties. All drugs were diluted in DMSO, aside from Pemetrexed which was dissolved in DMEM/F12 media due to insolubility in DMSO. Drugs were organized in alphabetical order and placed in boxes in the -80 closest to the BSL2 back room of the equipment room.

**Plate Making:**

Plates were made 1 day at a time for three consecutive days from 4/19-4/21 2017. All drugs were thawed in 37degreeC metal bead bath and vortexed prior to adding to the plate. For drugs at 20mM concentration, 30uL of DMSO was added to the well first, followed by 30uL of the drug. For drugs at other various concentrations, this was adjusted accordingly. All drugs were added to columns 1, 9, and 17. Then 40uL of DMSO was added to all other empty wells. Next, 7 three-fold serial dilutions were plated across the columns, picking up 20uL from first column and mixing up and down 5 times before transferring to next well. Lastly, DMSO was removed from wells 8H and 16H, and positive controls (flavopiridol + staurosporine + velcade) were added instead. Plates were covered with plate cover sticker/microseal Biorad type B) and spun down at 2,000RPM for 30 seconds before placing directly in -80 freezer. Plates were transferred to Dylan Nelson (OTRADI, OSU) on dry ice on 4/21/2017 for making the daughter plates.

Dylan creates three master plates from the original master plate (for purposes of avoiding repetitive freeze thaw cycles based on the number of plates we order at one time) and each of these is used to create 20 daughter plates by aliquoting 46nL from each of the master wells into 23uL of base media. These plates are then delivered on dry ice back to our lab and stored in the -20 freezer until use. The plates are thawed and 25uL of base media + cells is added to each well using the automated plating equipment in the Tyner laboratory.