ABSTRACT
Melanoma patients with B-Raf mutant tumors have been shown to respond to treatment with B-Raf inhibitors, e.g., dabrafenib. However, over time they become resistant to this treatment necessitating the need to discover additional treatments to target the resistant tumor. Tumors can be treated with a multitude of single drug or drug combinations, therefore, a platform is needed to help guide treatment decisions for patients. Patient-derived tumor xenograft (PDX) mouse models have been shown to have characteristics of the patient’s original tumor. The patient-derived tumor cells (PDC) obtained from the PDX models can be evaluated for response to anticancer agents. Transcriptic’s cloud laboratory offers a versatile high-throughput screening method, enabling scientists to quickly and precisely treat PDC lines against multiple drugs. In this poster, we describe our methods for PDC drug screening on Transcriptic’s Robotic Cloud Lab to identify a possible treatment option for a dabrafenib resistant melanoma.

INTRODUCTION
A 65 year old male with metastatic melanoma was enrolled in a clinical trial of dabrafenib and ipilimumab, and progressed following an initial partial response. Working with California Pacific Medical Center (CPMC)/Sutter cancer surgeons and oncologists, we created PDX and PDC models, and confirmed that the melanoma avatar MM-001 had a clinical and genetic profile similar to the original tumor. To predict tumor sensitivity to targeted therapies, we used the Transcriptic’s cloud laboratory to perform high throughput screening of PDC samples against multiple concentrations of 48 antitumor agents (currently being used in the clinic). The results were ranked to determine which treatments(s) were most effective at reducing the growth of MM-001 cells.

MATERIALS AND METHODS

PDX & PDC MODELS
Patient tissue was digested using a collagenase / hyaluronidase enzyme mix and 1x10⁵ cells resuspended in Matrigel were injected subcutaneously in a NSG mouse (Fig. 1). Once the PDX reached 1.5 cm in diameter, the tumor was harvested and a PDC was generated. Cultures were grown as tumorspheres in ultra low attachment dishes using DMEM and growth factors.

PHARMACOLOGY
Using the Transcriptic platform (see below), PDC were screened against a 6-point concentration-response curve of 48 drugs being used to target cancer in the clinic. After three days treatment of PDC, the CellTiter-Glo assay was used to quantify cell viability/proliferation. The resulting curves were fitted using non-linear regression analysis with the program GraphPad Prism.

TRANSCRIPTIC PLATFORM
A detailed protocol for dosing PDC was submitted to and evaluated by Transcriptic. The protocol was then translated into a python script to generate Autoprotocol, a data structure used to instruct Transcriptic’s workcells (Fig. 2) to execute experiments. Compatible plates and tubes are supplied to the workcell and a robotic arm moves the containers to and from the appropriate instruments without human intervention. Drugs were supplied to Transcriptic at 2X their effective concentrations in standard micro-1.5 tubes. These were transferred to a 384-acoustic liquid handler source plate which was then moved to a multi-channel liquid handler to serially dilute the drugs. This source plate was used to dose 384-well microplates containing PDC samples supplied by CPMC. Using a simple csv well map, the acoustic liquid handler automatically transferred each drug at each concentration from the source plate to the appropriate location on each of the cell-containing 384-well microplates. The drugged cell lines were then placed in an incubator for 72 hours, after which the CellTiter-Glo assay was used to quantify cell viability/proliferation. Data from luminescence reads were subsequently rendered graphically in Transcriptic’s web interface (Fig. 3) and made available for download in .csv format for further data analysis.

RESULTS

Figure 4. PCR sequencing confirmed that PDC MM-001 has a BRAF V600E mutation similar to the patient’s tumor.

Figure 5. Similar to the patient’s original tumor, melanoma avatar MM-001 is resistant to BRAF inhibitors.

Figure 6. Melanoma avatar MM-001 is sensitive to MEK inhibitors.

CONCLUSIONS
- HTS of PDC derived from a BRAF-resistant patient tumor identified sensitivity to MEK inhibitors
- Transcriptic’s Cloud Based Laboratory provided multiple advantages to development of a HTS platform including:
  1) Eliminating the need to develop an expensive in-house HTS platform
  2) Flexibility and control of protocol development and implementation, with the ability to control experiments from a web browser
  3) Reduce data cycle times by leveraging Transcriptic’s in-house expertise
- The Cancer Avatar Project at CPMCRI is using a multimodal approach that includes mouse avatars, genomics, high-throughput pharmacologic screening, and informatics to reach the goal of fully individualized cancer care through rapid prediction of likely response before treatment decisions must be made.

ACKNOWLEDGEMENTS
This work was supported by funding for the CPMCRI Precision Medicine Program.