CANscript™
SEE What Works.

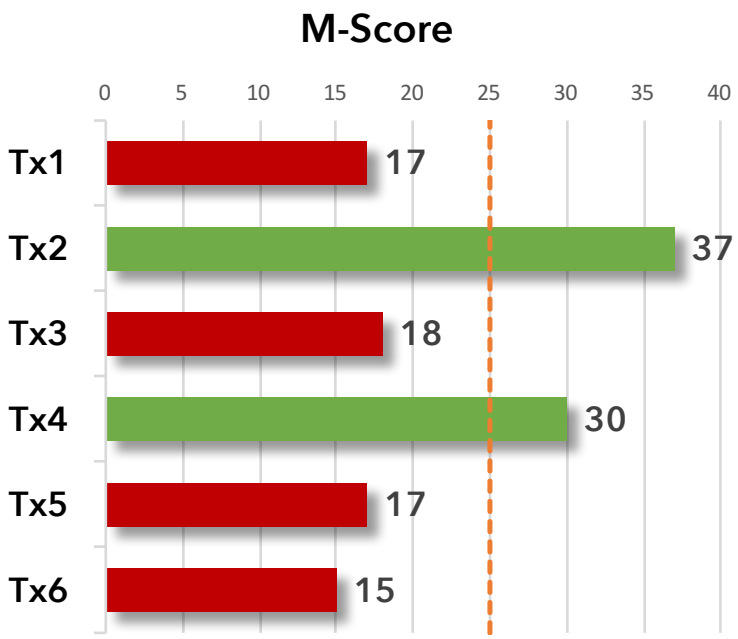
CANscript™ Report

REPORT INFORMATION			Requisition ID: XXXXXX
Sample Received	12/5/20XX	**Ordering Physician**	Dr. Fred Example
Report Delivered	12/12/20XX	**Hospital**	Example Hospital

PATIENT DETAILS			Mitra Biotech ID: 1234567
Patient Name	Jane P. Sample	**Cancer Type**	TNBC
Age/Sex	55/F	**Cancer Stage**	IV
Date-of-Birth	12/1/19XX	**Specimen Site**	Lung

TEST RESULTS

TREATMENT RESPONSE	M-Score
Predicted Responders (M-Score > 25)	
Tx2: paclitaxel, albumin-bound	37
Tx4: doxorubicin + cyclophosphamide	30
Predicted Non-Responders (M-Score ≤ 25)	
Tx1: gemcitabine + carboplatin	17
Tx3: gemcitabine	18
Tx5: capecitabine + docetaxel	17
Tx6: Paclitaxel + bevacizumab	15
Not Tested	
Tx7: *additional treatment*	Not Tested
Tx7: *additional treatment*	Not Tested

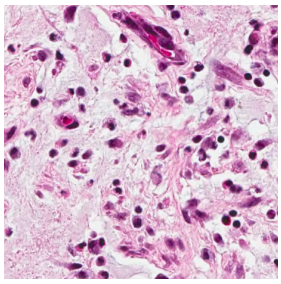
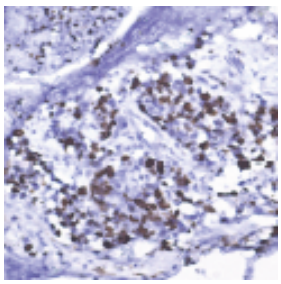


Dana Robin Semmel, MD, Lab Director: _____ Date:_____
CLIA ID: 5418

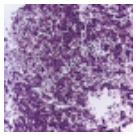
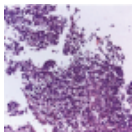
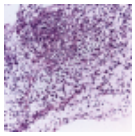
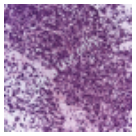
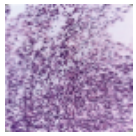
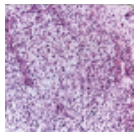
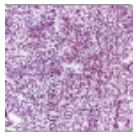
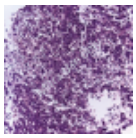
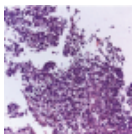
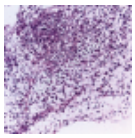
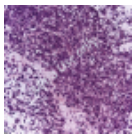
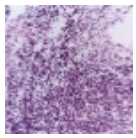
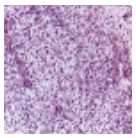
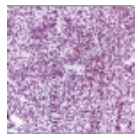
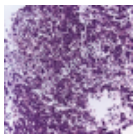
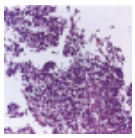
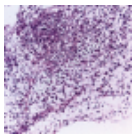
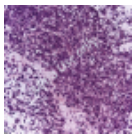
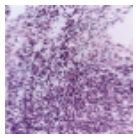
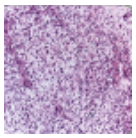
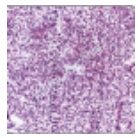
@2017 Mitra Biotech, Inc.
12 Gill St. Suite 3150 Woburn, MA 01801 +1.339.999.2300 MitraBiotech.com

CANscript™
SEE What Works.

BASELINE TUMOR

H&E Staining Score: 30%	Ki67 Tumor Index: 72%
	

HISTOPATHOLOGY EVALUATION

CTRL	Tx1	Tx2	Tx3	Tx4	Tx5	Tx6	
							H&E
							Ki67
							Ac-Cas3

Results of Histopathological evaluation along with multiple kinetic assays determined M-Score calculation for each drug/combination tested. The description of assays follows in the next page.

@2017 Mitra Biotech, Inc.
12 Gill St. Suite 3150 Woburn, MA 01801 +1.339.999.2300 **Mitra**Biotech.com

CANscript™
SEE What Works.

DRUG INFORMATION

1. **gemcitabine + carboplatine**	Gemcitabine (Item code: 1759) inhibits DNA replication as a nucleoside analog. The drug concentration used in the test was 22.3µg/ml (100µM). Carboplatin affects DNA replication by cross-linking. It is used at the concentration of 37.1 µg/ml.
2. **paclitaxel, albumin-bound**	Paclitaxel (Item code M2011) inhibits mitosis and activates apoptosis. The drug concentration used in the test was 3.19µM.
3. **gemcitabine**	Gemcitabine (Item code: 1759) inhibits DNA replication as a nucleoside analog. The drug concentration used in the test was 22.3µg/ml (100µM).
4. **doxorubicin + cyclophosphamide**	Doxorubicin intercalates between base pairs in the DNA helix, thereby preventing DNA replication and ultimately inhibiting protein synthesis. The drug concentration used in the test was 0.93µg/ml. Cyclophosphamide (Item code: NA) acts by DNA crosslinking (alkylating agent) which leads to apoptosis and induces IFNs. The drug concentration used in the test was 37.2 µg/ml.
5. capecitabine + docetaxel	Capecitabine is selectively converted by tumor cells into its active moiety 5-FU which is catabolized into two active metabolites FdUMP and FUTP. FdUMP inhibits DNA synthesis and cell division by reducing normal thymidine production, while FUTP inhibits RNA and protein synthesis by competing with uridine triphosphate for incorporation into the RNA strand. The drug concentration used in the test was 3.9 µg/ml. Docetaxel (Item code: 2232-10) affects proliferation by inhibiting mitotic spindle assembly and thereby induces cell apoptosis. The drug concentration used in the test was 2µg/ml (3.4µM).
6. paclitaxel + bevacizumab	Paclitaxel (Item code M2011) inhibits mitosis and activates apoptosis. The drug concentration used in the test was 3.19µM. Bevacizuman is an angiogenesis inhibitor that slows down the growth of new blood vessels. The drug concentration used in the test was 450ng/ml.

CANscript Assays

Cell Viability: Tumor slices were cultured in tmultiples. Prestoblue viability reagent was added to the culture wells containing tissue specimens. Outcome of the viability assay was calculated from the raw data inputs to derive percent inhibition to all the treatment arms. Reduction in viability compared to control was observed.

LDH Release: Cell death was assessed in multiples by measuring released Lactate dehydrogenase (LDH) enzyme into the culture supernatant from damaged cells as an indicator of cytolysis and cellular cytotoxicity. Culture supernatant was collected and assayed for LDH levels. Change in LDH levels with respect to control was compared.

Estimation of ATP Levels: Tumor tissue specimens post-culture from vehicle or drug treatment arms were processed for measuring intracellular ATP levels by luminometry. Assessment of ATP is one of the gold standard techniques in estimating the tumor(s) drug sensitivity in *ex-vivo*.

Histopathology Evaluation:
Assessment of tumor morphology, cell proliferation & cell death: Tumor slices were tested against the drugs indicated above. Drug treated and control tumor slices at the end of culture were fixed in formalin and embedded in paraffin. Four micron sections were prepared, stained with H&E and immunostained with Ki-67 and Caspase3 (active/cleaved) specific antibodies following heat induced antigen retrieval. Number of parameters including change in cell numbers, morphology, mitotic index, tumor proliferation are observed.

@2017 Mitra Biotech, Inc.
12 Gill St. Suite 3150 Woburn, MA 01801 +1.339.999.2300 **Mitra**Biotech.com

CANscript™
SEE What Works.

Publications

Majumder B, Baraneedharan U, Thiyagarajan S, Radhakrishnan P, Narasimhan H, Dhandapani M, Brijwani N, Pinto DD, Prasath A, Shanthappa BU, Thayakumar A, Surendran R, Babu GK, Shenoy AM, Kuriakose MA, Bergthold G, Horowitz P, Loda M, Beroukhim R, Agarwal S, Sengupta S, Sundaram M, Majumder PK.
Predicting clinical response to anticancer drugs using an ex vivo platform that captures tumour heterogeneity. Nature Communs. 2015 Feb 27; 6:6169.

Goldman A, Majumder B, Dhawan A, Ravi S, Goldman D, Kohandel M, Majumder P K , Sengupta S.
Temporally sequenced anticancer drugs overcome adaptive resistance by targeting a vulnerable chemotherapy-induced phenotypic transition. Nature Communs. 2015 Feb 11; 6:6139.

GovindBabu K, Majumder B, Thiyagarajan S, Baraneedharan U, Surendran R, Shenoy AM, Kuriakose MA, Loda M, RameenBeroukhim, Peleg Horowitz, Shivani Agarwal, Radhakrishnan P, Sundaram M, Sengupta S, Majumder PK.
Application of a robust and novel ex vivo platform mimicking patient heterogenous tumor microenvironment for personalized cancer treatment. J ClinOncol 33, 2015 (Suppl; Abstract# 6029).

Majumder B, Baraneedharan U, Thayakumar A, Thiyagarajan S,Brijwani N, Tewari B, Shanthappa BU,Radhakrishnan P,Majumder PK. **Identification of responders for Anti- CTLA4 in refractory colorectal cancers using CANscript™ platform.** Proceedings of the 106th Annual Meeting of the American Association for Cancer Research (AACR). 2015 Apr 18-22, Philadelphia, PA: Abstract# 1304.

DISCLAIMER: CANscript should not be used as the sole input for the purposes of diagnosis, prognosis, or as the primary basis for making therapeutic decisions. CANscript should be used by an experienced physician in conjunction with other recognized laboratory and diagnostic procedures, in accordance with current literature and practice guidelines. An individual patient's condition and medical history should always be considered when making any treatment decisions, and the experience and clinical judgment of the treating physician should ultimately determine which treatment is selected. The Mitra Biotech laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and as such is qualified to perform high-complexity clinical testing.

@2017 Mitra Biotech, Inc.

12 Gill St. Suite 3150 Woburn, MA 01801 +1.339.999.2300 **Mitra**Biotech.com