ASC ONCOLOGY
REVERSE CLINICAL ENGINEERING®

Final report

Reverse Clinical Engineering® test procedure

PATIENT	PHYSICIAN	SPECIMEN
Jane Doe 1 Example Street London EC1A 1AA UNITED KINGDOM 18 May 1972 Female	Dr. John Doe University Hospital 118 Example Road WC2R 2LS London UNITED KINGDOM	Breast Cancer **COLLECTION** 30 Sep 2022 **RECEIVED** 4 Oct 2022 **REPORT** 25 Oct 2022 **OB** 0000XXX

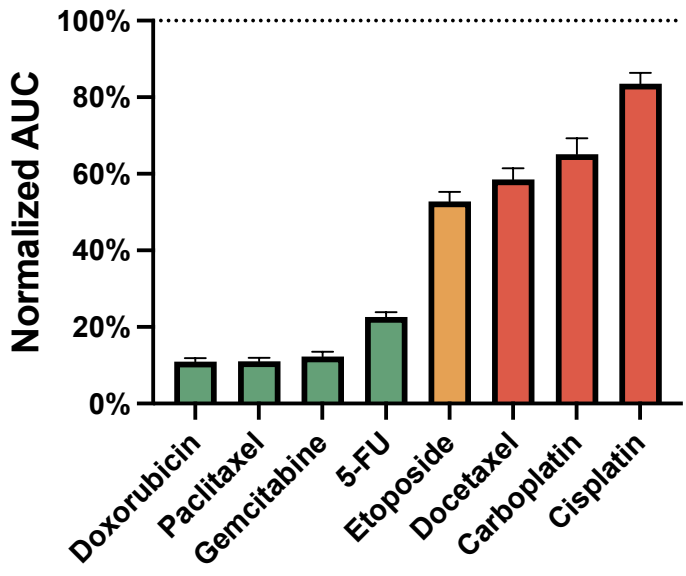
DRUGS FOR TESTING

Doxorubicin	Etoposide
Paclitaxel	Docetaxel
Gemcitabine	Carboplatin
5-FU	Cisplatin

A patient-derived 3D (PD3D®) tumour model was generated in the laboratory from a tissue fragment of your breast cancer to test the efficacy of the drugs above with the Reverse Clinical Engineering® (RCE) test procedure.

Results

Using your individual tumour model to test drug efficacy with the RCE® test procedure showed that Doxorubicin, Paclitaxel, Gemcitabine and 5-FUreduced tumour cell growth by at least 50% in a clinically relevant range of concentrations. These drugs are therefore the most promising therapeutic options.



Use this link to download pictures of your individual PD3D® model: asc-oncology.com/downloads/...

Definitions and explanations

$IC_{50} < c_{max}$	$IC_{50} \sim c_{max}$	$IC_{50} > c_{max}$
Tumour cell growth decreased over 50% at relevant concentration	Tumour cell growth remained constant at relevant concentration	Tumour cell growth decreased less than 50% or uninhibited at relevant concentration
Chance of effective treatment likely	**Chance of effective treatment possible**	**Chance of effective treatment unlikely**

AUC (Area under the curve) The area under the dose-response curve. The smaller the value, the stronger the effect of the active substance on the tumour models.

C_{max} This value indicates the maximum concentration of an active pharmaceutical ingredient in the blood plasma after its administration. The blood plasma is the liquid fraction of the blood, excluding the blood cells.

IC_{50} This value indicates the concentration of an active pharmaceutical ingredient at which tumour cell growth is half-maximally inhibited, i.e., at which half (50%) of the maximum possible growth inhibition is achieved.

PD3D® A 3D cell culture model created from the patient's own tumour tissue. Grown in a cell culture dish (*in vitro*), the tumour model functions as a copy of the original tumour and largely preserves the tissue's original architecture as well as the expression patterns of relevant tumour marks.

RCE Abbreviation for Reverse Clinical Engineering®, a diagnostic procedure offered by ASC Oncology GmbH. In this process, PD3D® tumour models are used to test anticancer drugs to predict their efficacy in treating a patient's individual tumour.

Further information and support

German Cancer Society
With 124 counselling centres throughout Germany, these regional cancer society's offer onsite support and guidance for patients, their families, and friends.
www.krebsgesellschaft.de

National Cancer Institute
The National Cancer Institute (U.S.A.) provides comprehensive information about cancer and explains technical terms in a dictionary.
https://www.cancer.gov

Cancer Rebels
This charitable association financially supports cancer patients and their relatives in their fight against cancer, whenever other support systems are not effective.
www.cancer-rebels.club

War on Cancer
This social cancer app connects cancer patients with each other and enables them to share their experiences and approaches to living with the disease.
https://waroncancer.com

Information for healthcare professionals

Detailed overview of Reverse Clinical Engineering® test results

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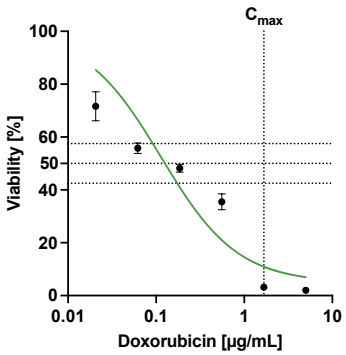
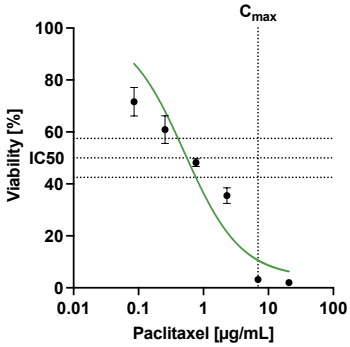
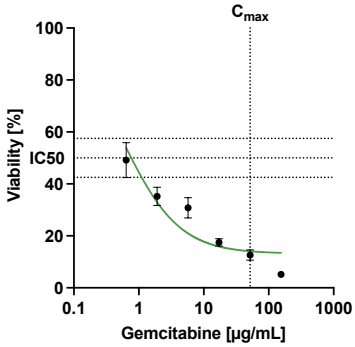
Using the patient-derived tumour models, the following IC_{50} values have been calculated for the tested drugs with the Reverse Clinical Engineering® test procedure.

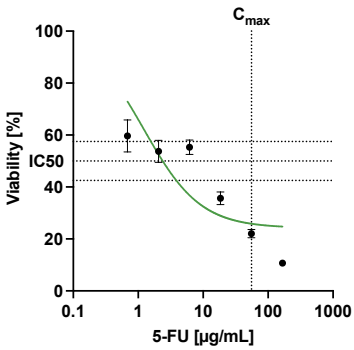
	Drug class	c_{max} value (µg/ml)	Calculated IC_{50} value PD3D® cell culture (µg/ml)	*In vitro* response
Doxorubicin	**Anthracycline**	**1.67**	**0.13**	⊕
Paclitaxel	**Taxan**	**2.47**	**0.56**	⊕
Gemcitabine	**Pyrimidine analogue**	**13.58**	**0.77**	⊕
5-FU	**Pyrimidine analogue**	**55.44**	**1.02**	⊕
Etoposide	Topoisomerase inhibitor	33.89	Not reached	~
Docetaxel	Taxan	2.10	Not reached	−
Carboplatin	Alkylating agent	14.09	Not reached	−
Cisplatin	Alkylating agent	4.30	Not reached	−

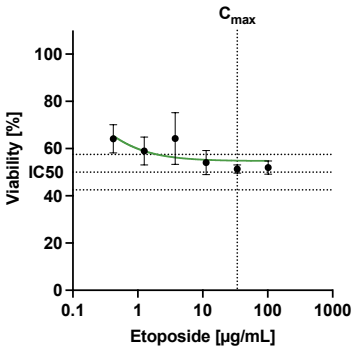
The individual tumour model's comprehensive chemosensitivity profile is shown for each tested drug in the following dose-response curves. Values in the lower left area of the diagram (cell viability < 50% at a drug concentration < c_{max}) correspond to an in vitro response in which cell viability has been at least halved by a clinically relevant drug concentration.

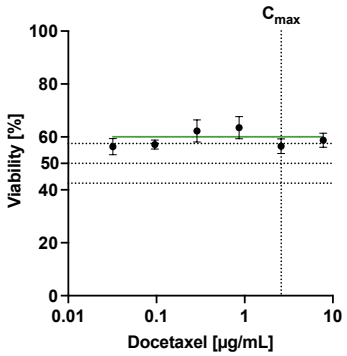


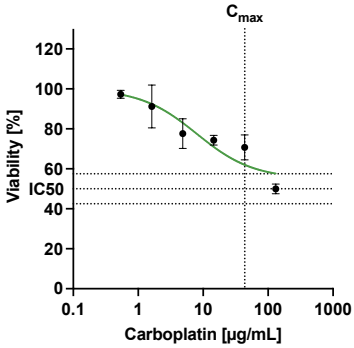


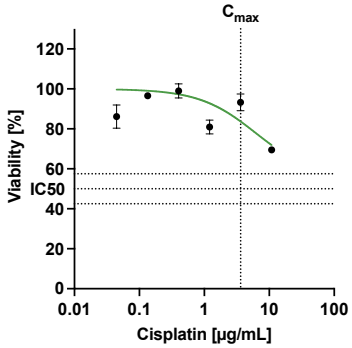












Method

The Reverse Clinical Engineering® test procedure uses the patient's own tumour tissue, which is mechano-chemically dissociated and embedded in a supporting matrix. Under optimized culture conditions, the tumour cells are selectively expanded, and patient-derived three-dimensional (PD3D®) organoids are generated (Boehnke et al., 2016). The organoids are incubated for chemosensitivity testing with clinically relevant concentrations (dilution series with six serial 1:3 dilutions) of the drugs to be analysed; in daily or single administration, depending on the clinical protocol. After four days, cell viability is determined in triplicates using an ATP-dependent luminescence assay (CellTiter-Glo®). The half maximal inhibitory concentration (IC_{50}) is then calculated. An IC_{50} value less than the maximum achievable plasma concentration ($IC_{50} < c_{max}$) indicates a response to treatment in the patient, potentially leading to tumour regression. An IC_{50} value greater than the maximum achievable plasma concentration ($IC_{50} > c_{max}$) is unlikely to result in a meaningful effect on tumour cell viability in the patient. If IC_{50} and c_{max} are approximately equal, there is a chance of stable disease.

Tests performed by CELLphenomics GmbH on behalf of ASC Oncology GmbH
Laboratory director: Dr. Lena Wedeken

Diagnostic significance

The test procedure is based on three-dimensional tumour models, called tumour organoids, which differ markedly from traditional cell culture models. Since 3D organoids largely retain their cell-cell and cell-matrix interactions, they are more similar to the original tumour than cells grown two-dimensionally on a rigid plastic substrate (Kapałczyńska et al., 2018). In co-clinical studies that compared patient drug response with PD3D® tumour model prediction, a positive predictive value of up to 88% and a negative predictive value of up to 100% was found (Vlachogiannis et al., 2018). In addition, a meta-analysis revealed a sensitivity of 0.81 (95% CI: 0.69-0.89) and a specificity of 0.74 (95% CI: 0.64-0.82) for PD3D® testing procedures (Wensink et al., 2021).

Clinical benefit

This report makes no promises or guarantees that a particular drug will be effective in the treatment of disease in any patient. This report also makes no promises or guarantees that a drug with potential lack of clinical benefit will in fact provide no clinical benefit.

Reimbursement

ASC Oncology makes no promises or guarantees that a healthcare provider, insurer or other third party payor, whether private or governmental, will reimburse a patient for the cost of Reverse Clinical Engineering®.

Therapeutic decision

Drugs referenced in this report may not be suitable for a particular patient or approved by the European Medicines Agency (EMA) or the United States Food and Drug Administration (FDA) for the particular dosage, administration, or indication (off-label use, compassionate use). The selection of any, all or none of the drugs associated with potential clinical benefit (or potential lack of clinical benefit) resides entirely within the discretion of the treating physician. Indeed, the information in this report must be considered in conjunction with all other relevant information regarding a particular patient, before the patient's treating physician recommends a course of treatment. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the current standard of care. A treating physician's decisions should not be based on a single test, such as this test, or the information contained in this report.

References

Boehnke K, *et al.* Assay Establishment and Validation of a High-Throughput Screening Platform for the Three-Dimensional Patient-Derived Colon Cancer Organoid Cultures. *J Biomol Screen*. 2016; 21(9):931-41.

Kapałczyńska M, *et al.* 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. *Arch Med Sci* 2018; 14(4):910-919.

Vlachogiannis G, *et al.* Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 2018; 359(6378):920-926.

Wensink G, *et al.* Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. *NPJ Precis Oncol*. 2021; 5(1):30.

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