Standard Operating Procedure

SOP_All_B735_en_AA04

Clinical Evaluation of CTR-Test®

Purpose:

Goal of assessing the literature is the examination of the safety and performance of the CTR-Test.

Scope of SOPs / Responsibilities:

Product CTR-Test / Head of Development

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1 Result

1.1 Summary

Due to the comprehensive clinical evaluation, we came to the following results:

- The product has a benefit to the end user.
- All known risks are consistent with the benefits (see also risk management).
- All aspects of safety and performance were considered.
- The product complies with the current status of medical science and the state of art.
- The underlying literature is comprehensive and suitable.

1.2 General

Clinical evaluation of the **CTR-Test** was written on the basis of knowledge from publications, clinical studies (user information), validation studies and many years of experience.

Definitions:

- **Clinical examination / clinical studies:** A planned, systematical study on human trial subjects, which is conducted to determine safety and/or performance of a specific medical product.
- **Clinical investigation protocol:** Document, which defines reason, objective, design and intended analysis', methods, monitoring, implementation and reporting of a clinical investigation.
- **Validation studies:** Studies, in which the CTR-Test was validated on the basis of patient specimen. No further patient details were collected and correlated to the test.

This clinical evaluation is part of the technical documentation of the product. We declare the clinical evaluation to be complete.

2 Method

The clinical evaluation was planned as follows:

- Determining of the necessary standards
- Determining of publications
- Evaluation of the information obtained

2.1 Objective of the Clinical Evaluation

The aim of the literature review is to examine the safety and performance of the CTR-Test.

2.2 Types of Studies

The CTR-Test underlying studies are divided into two types:

1. Clinical studies
2. Validation studies

It should be noted that the test is formerly known as EDR assay (“Extreme Drug Resistance” assay) in the literature. The name EDR-Test is protected under trademark laws in the USA. Therefore, all studies in the USA used this term. After transfer of the technology to TherapySelect necessary adjustments to the European market were made. The change of name is part of these adjustments. The test was renamed to CTR-Test® (“Chemotherapy-Resistance-Test”) and is a registered trademark in Europe.

2.3 Determining Data

Collected data originate from recognized scientific publication. Data, which were collected by us (private or user studies), are included in the evaluation.

2.4 Requirements which were Considered During the Clinical Evaluation

All data sources are cited. For data collection, we analyzed the following sources:

- Data bases (especially PubMed)
- Further information from the market

2.5 Relevance of the Literature

Literature was evaluated by the management (Head of Development). It was either categorized as relevant or dismissed.

2.6 Evaluation of Suitability of the Literature

The literature was categorized as suitable because it contains all aspects, which are supposed to be considered, as well as positive and negative statements. Standards were also examined in regard to their suitability (see: relevance).

2.7 Relevance of Data

All data refer to general requirements and to the special area of our medical product (CTR-Test). All collected data can be regarded as relevant.

The studies directly refer to the product. Aspects, which do not directly refer to the product, are only partially considered. The amount of data is appropriate because all aspects of safety were looked upon.

2.8 Evaluation of Clinical Data

- Criteria of clinical data
- Direct assessment of the medical product
- The authors of data are known scientists.
- Conclusions are based on facts.
- Collected literature meets the state of the art medical practice.
- Sources are current publications, current literature or norms and decrees.
- The development of the state of the art was considered during compilation.

3 Critical Analysis of the Literature

3.1 General

- Analysis of literature was carried out by the Head of Development. He is adequately qualified for this because of his education and experience.
- The description of the medical product is found in the technical documentation “product description”. In order to avoid redundancy, description of the product is left out in this study
  .
- Available data were analyzed to assess critical as well as unfavorable aspects.
- The literature comprises all defined aspects.
- All aspects considered were gathered, assessed and minimized as far as possible as part of a risk analysis, which fulfills the requirements of DIN EN 14971. The technical documentation confirms that the name of the product serves its purpose.
- Data were sorted and analyzed according to their importance. In chapter they were listed chronologically for a better retrieval.
- The list of clinical data is regarded as a “list of publications”. No further lists are made in order to ensure timeliness of data.

3.2 Performance Evaluation – Correlation of the Test Result and Clinical Response

The most important clinical study on the CTR-Test was published by Kern and Weisenthal (1990). The test result was correlated to the clinical response and their way of analyzing was explained.

The goal of Kern and Weisenthal was to test the hypothesis whether resistance to chemotherapy can be predicted with high accuracy using the *in vitro* examination method CTR-Test prior to treatment. Tumor material of the patients was removed surgically and a CTR-Test was carried out in the laboratory. The following requirements had to be fulfilled for patients to be included in the study: A distinct tumor disease had to remain in the patients and they had to be treated with chemotherapy to be able to compare *in vivo* response to the *in vitro* test result. A total of 450 patient complied with those clinical and test entailed requirements.

The collection of clinical data and data of the CTR-Test were mutually blinded. Overall, 29 % of patients included in the study responded to chemotherapy *in vivo*.

Two separating lines were drawn in order to divide the CTR-Test results in three groups. The mean value of tumor cell inhibition was defined as the upper line and one standard deviation below the mean value was defined as the lower line.

1. **Extreme Resistance (ER) - group**
   Test results which rank below the lower separating line. Tumor cells hardly respond to extremely high exposure of chemotherapeutic agents *in vitro*. 127 patient specimen belonged to this group. It is remarkable how well extreme resistance is in accordance with clinical therapy failure. Only one patient from this group (less than one percent) responded to chemotherapy *in vivo*. Therefore, the test is 99% accurate in identifying clinical (*in vivo*) therapy failure while sensitivity is 42 %.

2. **Medium Resistance (MR) - group**
   Test results which rank in between the 2 separating lines were defined as medium resistance (MR). Tumor cells from this group respond a little to extreme high exposure of chemotherapeutic agents *in vitro*. 101 patient specimen were part of this group, of which 16 (16 %) responded to therapy in the clinic (*in vivo*).

3. **Slight Resistance (SR) - group**
   Test results above the upper separating line were defined as Slight Resistance (SR). 222 patient specimen were sorted in this group. 115 (52 %) of them responded to therapy in the clinic. This is considerably more than the 29 % response rate of the overall patient population.

Statistical Significance
For statistical significance calculations, clinical response rate above the upper separating line (SR group) was compared to the clinical response below the upper separating line (MR and ER group combined).
The resulting p-value for the total population of 450 patients is 10^{-9}, which is highly significant.

Subgroup Analysis
Different types of tumors as well as different chemotherapeutic agents were analyzed.
The following types of tumors were examined: breast cancer (48 patients), colorectal cancer (113 patients), non small cell lung cancer (35 patients), melanoma (68 patients), ovarian cancer (46 patients), sarcoma (38 patients), gastric cancer (38 patients) and tumors of unknown origin (34 patients). All types of cancer except sarcoma (p-value of only $p = 0.14$) have high statistical significance regarding the prediction of resistance when they are examined as a subgroup.
The same is valid if patients were divided into subgroups regarding the chemotherapeutic agents used. Subgroups of all individually examined substances showed high statistical significance regarding the prediction of resistance.

Conclusion
The hypothesis that high accuracy (99 %) of predicting resistance can be achieved by using extreme exposure of substances during the Chemotherapy-Resistance Test (CTR-Test) was confirmed by this clinical study. Substances, which show extreme resistance (ER) *in vitro*, should be avoided *in vivo* because there is a 99 % probability they will be ineffective. Furthermore, slightly resistant (SR) substances should be preferred over medium resistant (MR) substances.

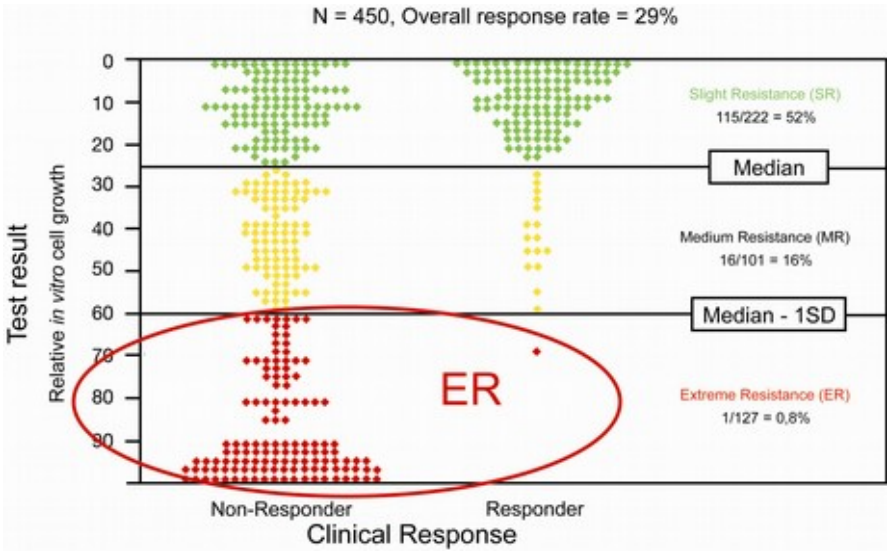

Figure 1: Correlation of the Test Result and Clinical Response. Data of study from Kern und Weisenthal (1990).

3.3 Cost / Benefit Study

To evaluate the cost / benefit of the CTR-Tests, a clinical study of Orr *et al.* (1999) was published.

Objective
Epithelial ovarian cancer is the fourth most common cancer-related death in women.
The 5 -year survival is 25 % and new approaches to the treatment of this disease are clearly justified. This study was designed to determine the feasibility of using an in vitro assay for drug resistance to guide treatment after cytoreductive surgery. The results were published after a median follow-up visits for 24 months.

Materials and Methods
In the study 66 patients with advanced ovarian cancer were treated by use of a combination of cytoreductive surgery and chemotherapy. Patient inclusion criteria included histological confirmation of epithelial ovarian cancer, International Federation of Gynecology and Obstetrics (FIGO) stage III, no prior chemotherapy or radiation therapy, no coexisting neoplasm, and optimal residual disease (< 2 cm). Malignant tissue from the involved ovary of each patient was tested *in vitro* for drug resistance, and chemotherapy was directed individually by assay results. On the basis of the assay 19 patients were treated with platinum/paclitaxel (TP) and 47 with platinum/cyclophosphamide (CP).

Results
The three- year survival (Kaplan- Meier estimate) was 69%, 95% confidence interval was 58% to 80%. There was no difference in 3-year survival between the 19 patients treated with TP (66%) and 47 with CP (74%). The cost-effectiveness of each treatment option was determined. The cost to achieve a 3-year survival for patients receiving CP was 4,615 US$ and 17,988 US$, to obtain a similar survival with TP. The cost-effectiveness of test-based therapy was 9,768 US$.

Discussion

Due to the high recurrence rate and poor long-term survival of women with advanced ovarian cancer, improved therapies for this disease are needed. After surgical debulking, the results of an *in vitro* assay for drug resistance were used to individually select chemotherapy for the patients in this study. Although the 3 year survival of 69% obtained in the present study appears good compared to previously published studies of optimally debulked patients, the results must be viewed with caution.
Patients were not randomized and differences in the prognostic factors, such as tumor grade, patient age and performance status, could account in part of the high survival found in the current study compared with previously published studies. Treatments with either CP or TP resulted in equivalent 3 -year survival. The costs to achieve 3 -year survival with this protocol, including the cost of CTR-Tests were 9,768 US$.

Conclusion
The consideration of the costs avoided by the elimination of ineffective treatments, unnecessary toxicity and loss of quality of life, would likely increase the cost effectiveness of the test CTR-based therapy as compared to conventional therapy. This study demonstrates that it is feasible to use an *in vitro* assay in the routine clinical practice to eliminate ineffective chemotherapeutic agents.

3.4 Correlation of Test Results with Progression-Free Survival (PFS) and Overall Survival (OS)

One important issue that has been addressed in other clinical studies, is whether the treatment with ER substances or SR substances have effects on the survival?

This question was addressed in the following studies:

Publication	Study Type	Tumor Entity
Mehta *et al.* (2001)	retrospective	Breast
Holloway *et al.* (2002)	retrospective	Ovarian (primary)
Loizzi *et al.* (2003)	retrospective	Ovarian (recurrent)
Parker *et al.* (2004)	prospective	Glioma (recurrent)
Verleye et al (2008) nur Abstract publiziert	prospective	Ovarian (primary)
Karam *et al.* (2009)	retrospective	Ovarian (primary and recurrent)
Kim *et al.* (2009)	prospective	Ovarian (primary)
Matsuo et al (2009)	retrospective	Ovarian (primary), Fallopian (primary), Peritoneal (primary)
d'Amato et al (2009)	retrospective	Lung (NSCL, Pt-resistant)
Pant *et al.* (2010)	retrospective	Ovarian (primary and recurrent) I.P.

Table 1: List of Publications Showing Correlation of Test Results with Progression-Free Survival (PFS) and Overall Survival (OS).

Below the studies are discussed in the context of individual tumor types.

3.4.1 Studies for Breast Cancer (Breast CA)

The study by Mehta et al (2001) deals with this tumor entity and comes to the conclusion that both results of the PFS and OS are significantly correlated with CTR-Test.

Objective

To determine whether *in vitro* extreme drug resistance (CTR-Test) assay results for patients with breast carcinoma were associated with clinical outcome after chemotherapy.

Patients and methods

CTR-Tests were performed on tumor tissue obtained from 103 newly diagnosed breast cancer cases. CTR-Test results scores of 2 for slight (SR), 1 for medium (MR), or 0 for extreme drug resistance (ER) were determined for each agent tested. *In vitro* ER scores for 4-hydroxycyclophosphamide (4HC) and doxorubicin were summed for patients treated with AC, or for 4HC and 5-FU for patients treated with CMF. Treatment selection was blinded to assay results.

Results

Median time to progression was significantly shorter for patients with extreme (ER) or medium i*n vitro* resistance (MR) (n=55, 48 months), compared to patients with slight *in vitro* resistance (SR), (n=41, 100 months, p =0.022). Patients demonstrating extreme (ER) to medium drug resistance (MR) also showed poorer survival than the slight resistance (SR) group (49.5 months vs. not reached, median follow-up 48 months, p =0.011). Summed CTR-Test scores, stage, and number of lymph nodes were significantly associated with survival in univariate and multivariate analysis. Compared to CTR-Test scores of 4, summed CTR-Test scores of 0–1 and summed CTR-Test scores of 2–3 were associated with a relative risk of death of 3.09 (95%, CI 1.05–9.06, Cox proportional hazards model, p=0.040) and 2.35 (95%, CI 1.07–5.15, Cox proportional hazards model, p=0.033), respectively.

Conclusion

CTR-Testing identified patients with individual patterns of drug resistance prior to therapy. In this cohort of breast cancer patients treated with chemotherapy, summed CTR scores were significantly associated with time to tumor progression and overall survival. CTR-Test results may offer a method for optimizing treatment selection.

3.4.2 Studies for Ovarian Cancer (Ovarian CA)

Till now it is by far the largest available data which are even homogenous with study (Karam *et al.*(2009)). Slight differences are probably due to the different designs of the studies discussed in the below summary.

Data for ovarian CA primary disease:

Holloway *et al.* (2002), Verleye *et al.* (2008), Kim *et al.* (2009), Matsuo *et al.* (2009) and Pant (2010) came all with the finding that the test results for both the PFS and OS are

correlated. However, there were slight differences, which might be caused by the study design and partly by the small number of cases found.

The data for Holloway *et al.* (2002) from 79 cases, whose tumors showed an extreme resistivity (ER) against platinum compounds, showed a significant shorter time to recurrence of the tumor (progression-free survival, PFS) and a lower survival (overall survival, OS), when treated with a platinum-based regimens (p = 0.0003).

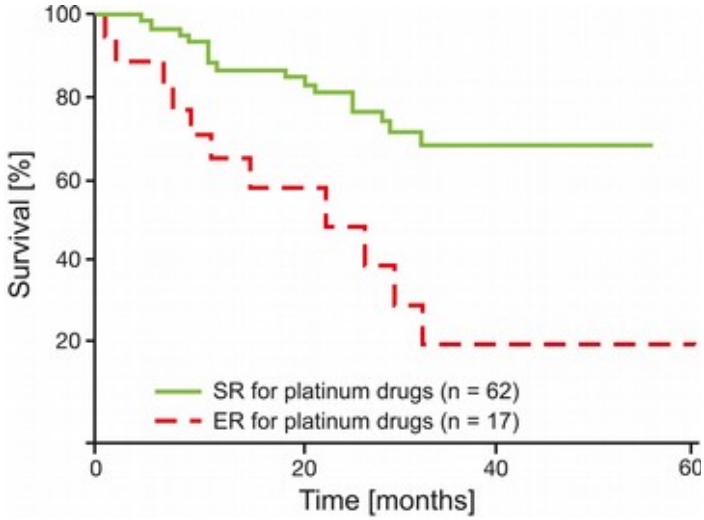

Figure 2: Correlation of Test Results with Overall Survival (OS). Data from the publication Holloway *et al.* (2002).

Verleye *et al.* (2008) repeated this study concept with 246 patients prospectively.
The PFS and OS data are not yet published, but the data shows that ER against Platinum compounds (*in vitro* platinum resistance test) correlates well with clinical failure with platinum therapy (p = 0.008). It has been shown that extreme drug resistance (ER) in the assay is an independent statistically significant parameter that indicates treatment failure before starting treatment in patients with ovarian cancer.

Kim *et al.* (2009) also repeated this concept in a prospective study. They also came to the conclusion that ER results in the CTR-Test against platinum compounds correlates with a poorer OS and a worse response. The PFS data revealed no difference however; this could be explained by the smaller number of cases from 43 patients.

Matsuo *et al.* (2009) made a follow-up of 173 patients who were treated with both platinum substances and with Taxanes and correlated this with the CTR-Test results.
In addition, subgroups of patients were formed, whether these were optimal or suboptimal operated. It was found that treatment with substances from the slight resistance (SR) group show a survival advantage, in particular, even when the patients were optimally operated (p <0.001).

Pant *et al.* (2010) examined the PFS of 56 patients who were given a treatment with platinum substances and paclitaxel intra-peritoneally (IP) (29 patients) or intravenously (IV) (27 patients). It was found that the patients, who were treated with ER tested substances, each had worse PFS: 15 months with ER substances compared to 23 months of total cohort of IP. For the I.V. patients there wasn't seen PFS difference in the subgroups. This is likely due to the small numbers of cases or to the fact that combination therapy were given here. Please refer to the chapter "Review of Combination Therapies".

The only study that concludes that there is no correlation of test results and PFS or OS, is study by Karam *et al.* (2009). In this study, 377 patients with both primary as well as recurrent were examined. This study was very much criticized because of its methodological errors and two publications from Holloway (2009) and Einenkel (2009) demonstrated these errors. The essential critics of the publication by Karam *et al.* (2009) were that the test results were indeed correlated with the survival of patients, but the following points were not checked and considered in the analysis:

1. How were the patients treated in individual cases?
2. Had the test results impact on the treatment at all or wasn't it use at all?
3. Was a direct comparison of SR and ER done for different substances?
4. Was it considered whether combination chemotherapy was applied and if yes, how?
5. How were the quality (optimal or suboptimal) of the surgeries performed?
6. Where did the tissues for analysis came from?

Since this study has methodological deficiencies, was heavily criticized among scientists and physicians and disproved by other prospective studies, it is not further included in the clinical evaluation.

Data for recurrent ovarian CA:
There are three publications that deal with the recurrent ovarian CA. These are Loizzi *et al.* (2003), Karam *et al.* (2009) and Pant *et al.* (2010). The criticism of Karam *et al.* (2009) publication was also applied here, and it won't be further discussed.

Pant *et al.* (2010) does not distinguish between recurrence and onset of disease, but shares patients in I.P. and I.V. Groups (see above). Thus, the statements for the ovarian CA were valid in general.

The Loizzi *et al.* (2003) study cannot be ideally used for a correlation with survival because the treatment was guided by the test result. However, one can see that a consequent treatment only with SR tested substances for the platinum-sensitive patients brings a survival advantage. Here, the control group should be untested patients, who are in principle a mixture of SR, MR and ER patients.

3.4.3 Study for Glioma

The study by Parker et al (2004) is the only study which deals with this tumor entity. It is a part of a prospective blind study phase II with 48 patients having recurrence of malignant glioma, who were treated with irinotecan. The CTR-Test was used for this study. This was done with the use of fresh tumor biopsies from patients, which was taken before the first cycle of chemotherapy.

The *in vitro* responsiveness to SN38 (bioactive derivative of Irinotecan used in the CTR-Test) was correlated with the actual response to irinotecan therapy, time to tumor progression (TTP) and survival. The SN38 activity was tested in 19 of 29 tumors, in which 15 of 18 test results could be evaluated for a correlation with the clinical outcome. The *in vitro* drug resistance was classified as extreme (ER), medium (MR) or slight (SR). TTP and survival were estimated by the Kaplan-Meier method and using the Coat-Haenszel version of the log-rank test and the Fisher exact test. The *in vitro* tumor response was

divided for comparison with the clinical output in an ER (n = 4) and MR/SR-category (n = 11).

The results significantly correlate with both the TTP and survival. The median TTP for the MR/SR cases was three months versus six weeks in the ER cases (Log-rank test, p=0.0288; hazard ratio=3.06). A median survival of 13 weeks was significantly shorter with the ER-cases, compared to 38 weeks for the MR/SR cases, (p=0.029). Furthermore, the 100-day survival favored the MR/SR cases (Fisher's test, p = 0.008). At the last follow-up were two of three survivors patients with tumors with an MR/SR for SN38. This prospective data supports the view that patients should avoid drugs, where their tumor shows an ER.

3.4.4 Studies for Lung Cancer

Also for the lung cancer, there is currently only one study. This was published by d'Amato et al (2009). This study demonstrates the utility of the CTR-Test to predict poor clinical outcome when platinum-based therapy is used to treat patients with biological evidence of tumor resistance to platinum.

It was investigated whether in non-small cell lung carcinoma exists a subpopulation that is platinum resistant and whether these patients have poorer survival. There were 189 patients included in this study and it was found that patients who had MR or ER test results against platinum compounds had a statistically significant shorter survival time (15.6 months compared with 29.8 Months for patients with SR test result to platinum compounds) (p=0.047). If the second substance, which was used in chemotherapy, was included, and the groups were divided into SR and MR/ER, the survival period for the SR group was longer as well. If the patients showed MR/ER to platinum-substances and to the second drug, out of this population only 58% survived over 3 years (Median 16.6 months). In the patients who showed SR to platinum compounds and to the second drug 78% survived more than 5 years and the median was not reached (p=0.0268).

3.5 Prolongation of Survival Time by CTR-Test Result-Directed Chemotherapy

The most important question, whether the consistent application of CTR-Test can prolong the lives of patients, was examined only in one clinical trial until now. This retrospective study was conducted and published by Loizzi *et al.*(2003). Subsequently, the design and results was as follows:

Objective
This study intended to compare CTR-Test-directed therapy with standard therapy.

Study design
Fifty women, who were treated with chemotherapy based on CTR-Test guidance were compared with 50 well-balanced control subjects, who were treated empirically.

Results
In the platinum-sensitive group, patients with CTR-Test-directed therapy had an overall response rate of 65% compared with 35% in the patients who were treated empirically

(P=0.02). The overall and progression-free median survival were 38 and 15 months in the CTR-Test-directed treatment group compared with 21 and 7 months in the control group, respectively (P=0.005, overall; P=0.0002, progression free).

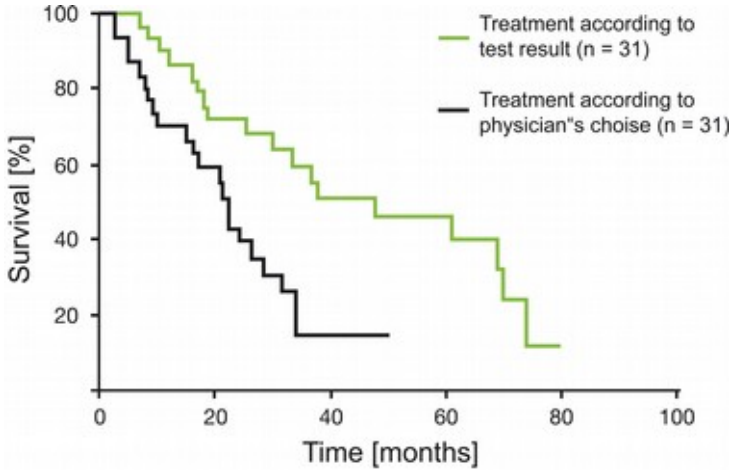

Figure 3: Prolongation of Survival Time by CTR-Test Result-Directed Chemotherapy. Data from the publication Loizzi *et al.* (2003).

In the platinum-resistant subgroup, there was no improved outcome in the patients who underwent assay-guided therapy. In multivariate analysis, platinum-sensitive disease, CTR-Test-guided therapy and early stage of disease were independent predictors for improved survival.

Conclusion
In this retrospective analysis, the results indicate an improved outcome in patients with recurrent ovarian carcinoma who have platinum sensitive disease and who underwent CTR-Test-directed chemotherapy.

3.6 Evaluation of Combination Therapies

The performance evaluation of Kern and Weisenthal was referring in the first place to the measurement and correlation of individual drug substances with clinical response. The combination of cytotoxic drugs in chemotherapy is more often used today, and the important question is whether the CTR-Test also can give answers and how this can be best done?

The answers are given by the following publications:

Publication	Combination Therapies	Tumor Entity
Orr *et al.* (1999)	Cisplatin+Cyclophosphamide Carboplatin+ Cyclophospha. Cisplatin+Paclitaxel Carboplatin+Paclitaxel	Ovarian (primary)
Mehta *et al.* (2001)	Doxorubicin+Cyclophospha. 5FU+Cycloph-+Methotrexate	Breast
Holloway *et al.* (2002)	Carboplatin+Cyclophospha. Cisplatin+Paclitaxel	Ovarian (primary)

	Carboplatin+Paclitaxel Cisplatin+Cyclo+Doxorubicin Carbopl.+Cyclo+Doxorubicin	
Loizzi *et al.* (2003)	Carboplatin+Paclitaxel Cisplatin+Paclitaxel Cisplatin+Gemcitabine Cisplatin+Cyclophosphamide	Ovarian (recurrent)
Geisler *et al.* (2007)	Carboplatin+Paclitaxel Cisplatin+Paclitaxel Carboplatin+Docetaxel	Ovarian
Verleye et al (2008) nur Abstract publiziert	Carboplatin+Paclitaxel Cisplatin+Paclitaxel Carboplatin+Docetaxel Cisplatin+Docetaxel	Ovarian (primary)
Kim *et al.* (2009)	Carboplatin+Paclitaxel Cisplatin+Paclitaxel Carboplatin+Docetaxel Cisplatin+Docetaxel	Ovarian (primary)
Matsuo et al (2009)	Carboplatin+Paclitaxel Cisplatin+Paclitaxel Carboplatin+Docetaxel Cisplatin+Docetaxel	Ovarian (primary), Fallopian (primary), Peritoneal (primary)
d'Amato et al (2009)	Carboplatin+Paclitaxel Carboplatin+Docetaxel Cisplatin+Docetaxel Carboplatin+Etoposide Cisplatin+Etoposide Carboplatin+Gemcitabine Carbo+Irinotecan+Celecoxib	Lung (NSCL, Pt-resistent)
Joo *et al.* (2009)	Carboplatin+Paclitaxel Cisplatin+Paclitaxel Carboplatin+Docetaxel Cisplatin+Docetaxel Platin+Taxan+Gemcitabine	Ovarian (primary)
Pant *et al.* (2010)	Carboplatin+Paclitaxel Cisplatin+Paclitaxel Carboplatin+Docetaxel Cisplatin+Docetaxel	Ovarian (primary and recurrent) I.P.

Table 2: List of Publications which Investigate CTR-Test and Combination Therapies.

All the above studies evaluated the effect of chemotherapy in which drugs (chemotherapy) were combined. All studies have measured individually the effect of the individual substances. The hypothesis behind it is: "The most effective combination therapy consists of single effective substances". In principle, the measurement of combinations is possible, with which one can determine synergistic effects. It is note to mention that there is a clinical trial (“Predictor-Study”, see chapter 4 Latest Publications) to address this question the first time with the combination therapy carboplatin plus paclitaxel. In this trial in

addition to the effect of the individual substances, the common effect is investigated also. The results of this trial are expected to be published in 2015.

However, the measurement of individual substances already leads to the desired result and seems to confirm the hypothesis "The most effective combination therapy consists of single effective substances". Whether this is a universal principle or only limited to certain combinations, still can't be assessed conclusively. Synergistic effects would not be determined anyway with the measurement of individual substances.

Based on the above publications, it can be concluded that it is possible to use the CTR-Test for combination therapy. The current state of the technique determines, for this purpose, the activity of the individual substances. This leads to the use of at least not ER but best SR-tested substances to obtain better PFS and OS (see also chapter 3.4 Correlation of Test Results with Progression-Free Survival (PFS) and Overall Survival (OS)).

3.7 Prognosis with the CTR-Test

The question is whether it is possible to use the CTR-Test not only to predict drug resistance but also to use the test for prognosis of tumor disease. The question cannot be answered conclusively.

It seems plausible that a patient whose tested medications all seem to be slight resistance (SR) will benefit maximally from chemotherapy. On the other hand, one would assume that a patient whose test for all drugs has extreme drug resistant (ER) will not benefit from chemotherapy. Since the chemotherapy is one of the three major treatment options in oncology (in addition to radiation treatment and surgery), the elimination of an option will reduce the treatment options and this would inevitably lead to a poorer prognosis. Unfortunately these scenarios had not been explicitly examined till now.

An indication that a prognosis is possible was partly shown in the publication of Holloway et al (2002). It showed that if the patient has a platinum resistance (ER) in the CTR-Test and still gets treated according to the standard (i.e. platinum), this will affect the survival time dramatically. For this it must be added that the patients with ovarian cancer are generally divided into two classes. The patients with platinum resistance show a recurrence within 6 months after chemotherapy. The platinum sensitive patients have a recurrence at a later time point. These two groups have different overall survival times (see also Loizzi *et al.* (2003)). In other words, a platinum resistance in the CTR-Test would means that this patient would have a poorer prognosis.

There is a publication that worked intensively on this topic (Matsuo et al (2010)) and below is the abstract listed:

Objective:
The objective of this study was to evaluate the clinical significance of the extent of extreme drug resistance (EDR) in in vitro drug resistance assays in advanced epithelial ovarian, fallopian, and primary peritoneal cancers.

Methods:

A retrospective study was conducted using the database for in vitro drug resistance assay (EDR Assay, Oncotech, Inc.) results for advanced stage ovarian cancer samples obtained at primary surgery between 1995 and 2009. In vitro drug resistance assay results were evaluated for thirteen drugs according to the following two groups: platinum and taxane (primary treatment group) vs remaining agents (secondary treatment group). Dual-resistance was then defined as at least one EDR in the primary and secondary treatment groups. Chemotherapy response and survival outcome were correlated with assay results.

Results:
There were 253 cases identified. Dual-resistance (n=53, 20.9%) was not associated with chemotherapy response (p=0.62) or survival outcomes (PFS, p=0.52; OS, p=0.11). Only one (0.4%) case exhibited complete EDR to all tested drugs, and 74 (29.4%) cases showed no EDR. There was no statistical correlation between total number of drugs in the EDR range and chemotherapy response (p=0.55), progression-free survival (PFS) (p=0.18), and overall survival (OS) (p=0.87). Proportion of EDR, defined as the ratio of the number of EDR drugs divided by all drugs for an individual patient, was also not related to chemotherapy response (p=0.37), PFS (p=0.13), or OS (p=0.13).

Conclusions:
Presence of extreme drug resistance to multiple agents in the in vitro drug resistance assays was not associated with survival outcomes in advanced stage epithelial ovarian, fallopian, and primary peritoneal cancers.

In this study an attempt was made to allow a general prognosis of the disease with the aid of a dual resistance detection. However, this used category did not enable progosis. This means that patients who have a dual resistance according to this study, has no worse prognosis than the other patients. This means that the patients, despite the ER to certain substances, are still able to respond to other sensitive substances and thus a better therapy is still possible.

Further studies or meta-analyses are needed in order to better answer this question.

3.8 Influence of Tumor Heterogeneity on the CTR-Test Results

The topic, whether and how the resistance pattern in patients varies over time or in different tumor sampling points, was examined in three validation studies: Tewari *et al.* (2005), Core (1998) and McAlpine *et al.* (2008).

In the study by Tewari et al (2005) ovarian cases were examined. This study recruited patients with different tumor sampling points as well as patients with tumor material examined before and after chemotherapy. The result is that the resistance patterns do not differ much and the conclusion was that the CTR-Test results can be used for future therapy selections.

In the study by Kern (1998) patients were examined with breast and ovarian cancer.
Ovarian CA patients with different sampling sites showed for cisplatin 4% and for Paclitaxel 13% extreme difference (a sample ER and a sample SR). For Ovarian CA patients with tumor samples before and after treatment showed 19% extreme difference for cisplatin.

Breast CA patients with different sampling sites showed 13% extreme difference for doxorubicin. For patients with breast tumor samples before and after treatment showed 24% extreme difference for doxorubicin. The conclusion in the study is that both the sensitization and the development of resistance against drugs can occur. To this end, the two models are identified and described in the paper. It seems that tumor heterogeneity within the body at a certain point in time is not as pronounced as tumor heterogeneity occuring over time.

In the study by McAlpine *et al.* (2008) ovarian ca samples were also examined. 2 to 3 samples per patient were collected: for primary disease (18 cases) and for recurrences (20 cases). These were tested with 9 drugs in the CTR-Test. It was found that an extreme difference (a sample ER and a sample SR) occurred in 4.1% of the primary disease and in 11.3% of recurrences. There were general differences in resistance classes at 18.6 % of the primary disease and 26.1% of recurrences. The conclusion was that there are differences in the measurements and this can be explained by tumor heterogeneity.

In summary it can be stated that the tumor heterogeneity seems to have an impact on the test results. This appears to be greater over time, so that ideally, the CTR-Test should be always performed shortly before the begin of a chemotherapy. It might be possible to increase the predictive precision of the CTR-Test, if different tumor samples per patient are tested simultaneously.

3.9 Feasibility Studies and Subgroup Analysis with the CTR-Test

There are a number of publications that deal with the feasibility of performing the CTR-Test in different tumor entities or in their different subgroups. The main findings are that the feasibility was shown and in addition the different resistance patterns for different chemotherapeutic agents were analyzed. These works allow the more targeted selection of new drugs for the tumor entities and thus enable conducting further studies. The relevant publications listed below:

Publication	**Study Type**	**Tumor Entity**
Kern und Weisenthal (1990)	Subgroup analysis	Breast, colon, NSCL, melanoma, ovarian, sarcoma, stomach, unknown primary site
Ellis *et al.* (2002)	Feasibility study	Breast (primary)
Haroun *et al.* (2002)	Feasibility study	Brain (primary)
Cloven *et al.* (2004)	Subgroup analysis	Ovarian (subtypes)
d'Amato *et al.* (2006)	Feasibility study	Lung (NSCLC)
Santillan *et al.* (2007)	Subgroup analysis	Ovarian (subtypes)
Lyons *et al.* (2009)	Feasibility study	Carcinoid
Mujoomdar *et al.* (2010)	Feasibility study	Malignant pleural mesothelioma (primary)
Mechetner et al (2011)	Feasibility study	Colorectal (primary and

		metastatic)

Table 3: List of Feasibility Studies and Subgroup Analyses.

3.10 Critical Evaluation / Conclusions of the Literature

The analysis of the clinical data shows that the CTR-Test is capable to identify the ineffective drugs prior to the start of chemotherapy. The confidence level is over 95% (with Kern and Weisenthal, 1990 by 99.2%).

It was likewise possible that the test result correlates with the progression-free survival and overall survival.

In another study it was shown that treatment in accordance with the test result, prolong the survival time. This test can actually extend the lives of patients according to the study of Loizzi *et al.* (2003). However, this was a retrospective study, so TherapySelect limits herself in advertising only the avoidance of ineffective drugs.

The only relevant risk, which is measured by the CTR-Test results from the fact that each diagnostic has a confidence level, i.e., the determined resistance categories may vary in each individual patient. However, here the benefit outweighs the risk.

Due to the comprehensive clinical evaluation we reach the following conclusions:

- The product has a benefit to the end user.
- All risks identified are consistent with the benefit (see also risk management).
- All aspects of the safety and performance of the product were considered.
- The product complies with the current status of medical science and the state of the art.
- The underlying literature is comprehensive and useful.

4 Latest Publications

The latest publications were published in 2017.

The first one is a validation study that explains the concept of testing efficacy of drug combinations. Patent based on this paper was filed and is pending.

> The title of the publication is “New in vitro system to predict chemotherapeutic efficacy of drug combinations in fresh tumor samples” and was published in PEERJ (Link: peerj.com/articles/3030.pdf).

The second publication is an observational study in ovarian cancer and evaluated the correlation between CTR-Test result and clinical response.

> The title of the publication is “Prediction of clinical response to drugs in ovarian cancer using the chemotherapy resistance test (CTR-test)” and was published in Journal of Ovarian Research (Link: rdcu.be/xNyM).

5 List of Publications

The CTR-Test underlying performance evaluation studies in the form of clinical studies are listed below that demonstrate the predictive power of the test and its benefit for the patient. It should be noted that the test is called in English literature as EDR test (extreme drug resistance assay).

5.1 Clinical Studies

The following studies verify the predictive power of the test and the benefit for the patient.

1. Highly specific prediction of antineoplastic drug resistance with an in vitro assay using suprapharmacologic drug exposures. Kern DH, Weisenthal LM
J Natl Cancer Inst. 1990 Apr 4;82(7):582-8.

2. Cost-effective treatment of women with advanced ovarian cancer by cytoreductive surgery and chemotherapy directed by an in vitro assay for drug resistance. Orr JW Jr, Orr P, Kern DH
Cancer J Sci Am. 1999 May-Jun;5(3):174-8.

3. Breast cancer survival and in vitro tumor response in the extreme drug resistance assay. Mehta RS, Bornstein R, Yu IR, Parker RJ, McLaren CE, Nguyen KP, Li KT, Fruehauf JP
Breast Cancer Res Treat. 2001 Apr;66(3):225-37.

4. Association between in vitro platinum resistance in the EDR assay and clinical outcomes for ovarian cancer patients. Holloway RW, Mehta RS, Finkler NJ, Li KT, McLaren CE, Parker RJ, Fruehauf JP
Gynecol Oncol. 2002 Oct;87(1):8-16.

5. Survival outcomes in patients with recurrent ovarian cancer who were treated with chemoresistance assay-guided chemotherapy. Loizzi V, Chan JK, Osann K, Cappuccini F, DiSaia PJ, Berman ML
Am J Obstet Gynecol. 2003 Nov;189(5):1301-7.

6. A prospective blinded study of the predictive value of an extreme drug resistance assay in patients receiving CPT-11 for recurrent glioma. Parker RJ, Fruehauf JP, Mehta R, Filka E, Cloughesy T
J Neurooncol. 2004 Feb;66(3):365-75.

7. Differences of chemoresistance assay between invasive micropapillary / low-grade serous ovarian carcinoma and high-grade serous ovarian carcinoma. Santillan A, Kim YW, Zahurak ML, Gardner GJ, Giuntoli RL, Shih IM, Bristow RE
Int J Genecol Cancer 2007. 2007 May-Jun;17(3):601-6.

8. Extreme drug resistance is common after prior exposure to paclitaxel. Geisler JP, Linnemeier GC, Thomas AJ, Manahan KJ
Gynecologic Oncology. 2007;106:538-540.

9. *In vitro* extreme drug resistance assay to taxanes and platinum compounds for the prediction of clinical outcomes in epithelial ovarian cancer: a prospective cohort study. Kim HS, Kim TJ, Chung HH, Kim JW, Kim BG, Park NH, Song YS, Bae DS, Kang SB
Journal of Cancer Research and Clinical Oncology. 2009 Nov;135(11):1513-20.

10. Low drug resistance to both platinum and taxane chemotherapy on an *in vitro* drug resistance assay predicts improved survival in patients with advanced epithelial ovarian, fallopian, and peritoneal cancer. Matsuo K, Bond VK, Eno ML, Im DD, Rosenshein NB
International Journal of Cancer. 2009 Dec 1;125(11):2721-7.

11. Chemotherapy time interval and development of platinum and taxane resistance in ovarian, fallopian, and peritoneal carcinoma. Matsuo K, Eno ML, Im DD, Rosenshein NB
Archives Gynecology and Obstetrics. 2010 Feb;281(2):325-8.

12. Survival among patients with platinum resistant, locally advanced non-small cell lung cancer treated with platinum-based therapy. d'Amato TA, Pettiford BL, Schuchert MJ, Parker RJ, Ricketts WA, Luketich JD, Landreneau RJ
Annals of Surgical Oncology. 2009 Oct;16(10):2848-55.

13. Efficacy of taxane and platinum-based chemotherapy guided by extreme drug resistance assay in patients with epithelial ovarian cancer. Joo WD, Lee JY, Kim JH, Yoo HJ, Roh HJ, Park J-Y, Kim D-Y, Kim Y-M, Kim Y-T, Nam J-H
J Gynecol Oncol**.** 2009 June;20(2):96-100.

14. Clinical relevance of extent of extreme drug resistance in epithelial ovarian carcinoma. Matsuo K, Eno ML, Im DD, Rosenshein NB, Sood AK.
Gynecol Oncol. 2010 Jan;116(1):61-5. Epub 2009 Oct 17.

15. Prediction of Chemotherapy Response With Platinum and Taxane in the Advanced Stage of Ovarian and Uterine Carcinosarcoma: A Clinical Implication of In vitro Drug Resistance Assay. Matsuo K, Bond VK, Im DD, Rosenshein NB.
Am J Clin Oncol. 2010 Aug;33(4):358-63.

16. Correlation of extreme drug resistant assay results and progression-free survival following intraperitoneal chemotherapy for advanced ovarian cancer. Pant AC, Diaz-Montes T, Tanner E, Ahmad S, Giuntoli RL, Holloway RW, Bristow RE.
J Chemother. 2010 Aug;22(4):270-4.

17. Extreme drug resistance for carboplatin predicts resistance to first line therapy in advanced stage ovarian cancer: results from the EORTC-GCG/NCIC-CTG neoadjuvant trial. Verleye L, Coens C, Amant F, van der Burg MEL, Johnson N, Verheijen R, Casado A, Reed NS, Parker RJ, Vergote I
Communication at the 12th Biennial meeting International Gynecologic Cancer Society IGCS, Bangkok, Thailand, October 25-28, 2008 (abs.).

18. Extreme drug resistance assay results do not influence survival in women with epithelial ovarian cancer.
Karam AK, Chiang JW, Fung E, Nossov V, Karlan BY.

Gynecol Oncol. 2009 Aug;114(2):246-52. Epub 2009 Jun 4.

19. Should the extreme drug resistance assay fade into oblivion?
Einenkel J, Wuttke P, Horn K.
Gynecol Oncol. 2010 Jan;116(1):148-9; author reply 149-50.
Commentary on Karam, A.K., Chiang, J.W., Fung, E., Nossov, V. and Karlan, B.Y. Extreme drug resistance assay results do not influence survival in women with epithelial ovarian cancer. Gynecol Oncol. 2009;114:246-252.

20. Extreme drug resistance assay does not influence survival in women with epithelial ovarian cancer.
Holloway RW.
Gynecol Oncol. 2010 Jan;116(1):147-8; author reply 149-50.

21. Prediction of clinical response to drugs in ovarian cancer using the chemotherapy resistance test (CTR-test). Kischkel FC, Meyer C, Eich J, Nassir M, Mentze M, Braicu I, Kopp-Schneider A, Sehouli J.
J Ovarian Res. 2017 Oct 27;10(1):72.

5.2 Validation Studies

The test was validated on patient specimens during the following studies.

1. Heterogeneity of drug resistance in human breast and ovarian cancers. Kern DH
Cancer J Sci Am. 1998 Jan-Feb;4(1):41-5.

2. Factors associated with success of the extreme drug resistance assay in primary breast cancer specimens. Ellis RJ, Fabian CJ, Kimler BF, Tawfik O, Mayo MS, Decelis CR, Jewell WR, Connor C, Modrell C, Praeger M, McGinness M, Mehta R, Fruehauf JP
Breast Cancer Res Treat. 2002 Jan;71(2):95-102.

3. Extreme drug resistance in primary brain tumors: in vitro analysis of 64 resection specimens. Haroun RI, Clatterbuck RE, Gibbons MC, Burger PC, Parker R, Fruehauf JP, Brem H
J Neurooncol. 2002 Jun;58(2):115-23.

4. In vitro chemoresistance and biomarker profiles are unique for histologic subtypes of epithelial ovarian cancer. Cloven NG, Kyshtoobayeva A, Burger RA, Yu IR, Fruehauf JP
Gynecol Oncol. 2004 Jan;92(1):160-6.

5. Conservation of in vitro drug resistance patterns in epithelial ovarian carcinoma. Tewari KS, Mehta RS, Burger RA, Yu IR, Kyshtoobayeva AS, Monk BJ, Manetta A, Berman ML, Disaia PJ, Fruehauf JP
Gynecol Oncol. 2005 Sep;98(3):360-8.

6. Prevalence of *in vitro* extreme chemotherapy resistance in resected non-small cell lung cancer. d'Amato TA, Landreneau RJ, McKenna RJ, Santos, RS, Parker RJ
Annals of Thoracic Surgery, 2006, 81: 440-447.

7. Tumor heterogeneity in ovarian cancer as demonstrated by in vitro chemoresistance assays. McAlpine JN, Eisenkop SM, Spirtos NM.
Gynecol Oncol. 2008 Sep;110(3):360-4.

8. *In vitro* chemoresistance testing in well-differentiated carcinoid tumors. Lyons JM 3rd, Abergel J, Thomson JL, Anthony CT, Wang YZ, Anthony LB, Boudreaux JP, Strauchen J, Idrees M, Warner RR, Woltering EA
Ann Surg Oncol. 2009 Mar;16(3):649-55.

9. Prevalence of in vitro chemotherapeutic drug resistance in primary malignant pleural mesothelioma: result in a cohort of 203 resection specimens. Mujoomdar AA, Tilleman TR, Richards WG, Bueno R, Sugarbaker DJ
J Thorac Cardiovasc Surg. 2010 Aug;140(2):352-5.

10. In vitro drug responses in primary and metastatic colorectal cancers. Mechetner E, Brünner N, Parker RJ.
Scand J Gastroenterol. 2011 Jan;46(1):70-8.

11. New in vitro system to predict chemotherapeutic efficacy of drug combinations in fresh tumor samples. Kischkel FC, Eich J, Meyer CI, Weidemüller P, Krapfl J, Yassin-Kelepir R, Job L, Fraefel M, Braicu I, Kopp-Schneider A, Sehouli J, De Wilde RL.
PeerJ. 2017 Mar 2;5:e3030.

6 Declaration of Author(s)

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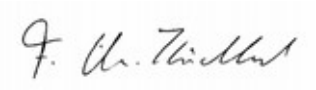

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