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Practical Guidelines for Diagnostic Use of *In Vitro* Chemosensitivity Tests

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Abstract. *To date, a multitude of chemotherapy sensitivity and resistance assays (CSRAs) have been developed to examine the efficiency of diverse cytostatic drugs on a patient's tumour material in vitro, and therefore provide the opportunity of customizing the particular treatment strategy in vivo. In an attempt to assess the impact of the hitherto developed CSRAs on routine clinical practice, in 2004, the American Society of Clinical Oncology (ASCO) performed a comparative analysis of previously published studies (1966 until January 2004) referring to the diverse assays and came to the conclusion that none of these assays was suitable for routine clinical practice. As the CSRAs aim to support the clinician in selecting the most efficient regimen to be applied to the individual patient, they must comply with several technical requirements. In this report, we examined the indispensable criteria for a CSRA to be broadly applicable to clinical routine use and to be meaningful for treatment recommendations not only at present but also with respect to newer cytostatic drugs in the future.*

Cytostatic drugs are an inherent part of today's cancer therapy, beside surgery and radiotherapy. The introduction of the first cytostatic drugs in the 1940s represented a major breakthrough in cancer therapy. Since then, many successful regimens were developed for adjuvant therapy and some even for curative therapy.

The experience with chemotherapy shows that its success always depends on individual factors. The patient's individual genetic background may influence the concentration of an active drug or may result in a modulated expression level of the molecular target. For the same reasons, a patient may suffer intolerably from side-effects with the consequence that a drug cannot be applied at effective concentrations.

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All current chemotherapy recommendations are based upon empirical data from well-designed clinical studies that have been performed to a representative population of patients. From these studies, the probabilities of therapeutic response are known. It is a binding standard that the clinicians provide the patient with an individualized prognosis and a therapy recommendation after considering a multitude of diagnostic parameters resulting from imaging methods, biochemistry, immunocytology, cytogenetics and molecular biology. In the end, despite all prognostic parameters, the clinician generally has the choice between a limited number of therapies.

An additional approach to individualize the prognosis and to assist the clinician in the process of therapy decision has its origin in the 1950s (1). The idea was to simulate the therapy *in vitro* with tumour material of the individual patient and to predict thereby the *in vivo* efficiency. The concept was simple and logically convincing. Fifty years later, in 2004, the American Society of Clinical Oncology (ASCO) concluded its comparative analysis of studies on chemosensitivity and resistance assays (CSRA) with the statement: "...there does not appear to be a single assay that is ready for routine integration into the clinical setting" (5). Nevertheless, ASCO is still convinced that "the increasing number of choices makes the rational basis for developing CSRAs even more compelling" and that better assays will be developed "as laboratory techniques become more automated and high throughput analyses become easier to obtain and interpret".

Here, we assess those features required for a CSRA to be broadly applicable to clinical routine use and to be meaningful for treatment recommendations. First of all, a routine assay must use standard laboratory techniques for the preparation of tumour samples and for cell cultivation. This means, for example, that liquid cell culture in standard plates must be favoured. Additionally, a CSRA assay must be performed with standardized material (drug preparations, culture media) and common equipment. This is a prerequisite to reproducibly obtain reliable results wherever the test is performed. A requirement that is also important for reproducibility and for future acceptance of a

CSRA is the potential to automate the process. This is directly associated with the criterion that test analysis should not require expert knowledge. The easier the test can be performed, the fewer will be the drop-outs caused by loss of sample material or by false analysis. Moreover, an automation of the CSRA provides the possibility for high throughput analyses.

The quality of the chemotherapy prediction absolutely depends on the evaluated parameter. To provide valid data, a routine CSRA must be the cell survival assay. The cell survival should be displayed in a dose-response curve that spans an adequate concentration range. The analysis of this curve must result in a simple informative value that summarizes all information comprised in the dose-response curve. One approach for such an informative value is presented by Staib *et al.* in 2005, as the 'chemosensitivity index' (6).

An informative value should also include data on therapy success that was generated in clinical studies. This calibration with the clinical outcome, *i.e.* 'clinical training' of the CSRA, is a seldom-used approach to increase the yield of interpretable results (2, 6).

The ASCO has made the criticism that the CSRA results are at best in accordance with the evidence-based therapy recommendation. ASCO states that any additional assay can be dispensed if it has no corrective function. Nevertheless, the comparison of tumour response rates [Table II of the ASCO report (5)] showed a benefit of CSRA-based therapies. In about 70% of the studies, they resulted in higher tumour response rates than the empirical therapies. Therefore we claim that CSRAs should only lead to recommendations of standard therapies that are empirically validated. Any information that might be interpreted as a recommendation of an experimental therapy has to be strictly avoided. The CSRA should not irritate the clinician, but rather provide him with assistance in the difficult process of choosing the right standard therapy for rapid initiation of the regimen. In addition, the assay should be suitable for new cytostatic drugs that will be available in the future.

To date, no assay exists giving consideration to all these demands at once. The CSRA assays so far established do not conform to the certain requirements pointed out above.

CSRAs were originally oriented to the antibiotic susceptibility test. For this microbiological routine test, antibiotics are diluted in a nutrient solution to which bacteria are added. The lowest concentration that effectively inhibits bacterial proliferation is defined as the minimal inhibitory concentration (MIC). A proliferation assay seems to be a good model for a tumour CSRA as well. Cancer cells are characterized by a nearly unhindered cell proliferation since parts of the normal control mechanisms are out of order (*e.g.*, tumour suppressor genes, activation by growth factors, contact inhibition). The human tumour cloning assay (HTCA) and the capillary cloning system (CCS) assess proliferation by counting the number of colony forming units in a nutrient agar. Due to the comparatively long doubling time of tumour cells, these tests take at least 14 days (7, 8). Another proliferation test assesses proliferation by measuring the size of a human tumour 6 days after its implantation in the kidney of a mouse that was chemotherapeutically treated (4). These procedures are judged as labour intensive, lengthy and technically challenging (5). Cell proliferation is also measured in the extreme drug resistance assays (EDR). Incorporation of tritium-labelled thymidine in the DNA is used in an EDR to predict failure of a therapy (3).

Principally high tumour cell proliferation is an unmistakeable sign of the failure of a therapy. But a lack of cell proliferation does not necessarily indicate therapy success. Tumour cells that do not proliferate during the therapeutic cycle may nevertheless cause a bad prognosis if they stay alive. Therefore the differential staining cytotoxicity assay counts all living cells (DiSC). At day 4 of *in vitro* cultivation, cells are counted microscopically (6).

All the tests mentioned so far require manual analysis by skilled personnel. An automatic analysis can be performed if tests measure the metabolic activity of cells, as the ATP and MTT assays do (2, 9). Plate readers simplify the analysis of these luminescence or colorimetric assays. But the data of ATP and MTT assays have a constitutional interpretation problem. Metabolic activity values exactly represent neither cell proliferation nor cell survival. If all tumour cells are alive with down-regulated metabolic activity they cannot be detected by these tests.

A natural handicap of all CSRAs is that even the highest concentrations of cytostatic drugs must not induce total growth inhibition of tumour cells. This is a fundamental difference to the antibiotic susceptibility test of bacteria. The consequence is that a simple MIC-like concentration cannot be read out and that single cut-off concentrations like the concentration that inhibits 90% cell growth (IC_{90}) are not optimal either.

The aim of the ASCO study was to judge whether CSRAs have a positive impact on therapy success. Among all studies from 1966 until January 2004, those have been selected that directly compared the empirically selected and the CSRA-based therapies with groups of at least 20 patients. The major criticisms have been the low proportion of rateable assays, either caused by high drop outs or by non-interpretable data, and the therapy recommendations, because they have been mostly congruent with the empirically chosen treatment (5).

Conclusion

Applying the ASCO's requirements, each CSRA currently used has some severe limitations. We believe that a CSRA in clinical routine must use standardized materials, that

cytostatic drugs must be tested over an adequate concentration range to allow the creation of a dose-response curve, and that cell survival is necessary to be measured. The 'clinically-trained' interpretation model must provide the clinician with reliable advice on the best standard treatment.

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