Functional drug response assay for cancer stem cells in the era of precision medicine

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Precision medicine/personalized medicine in oncology is centered on identifying which therapies are most effective for individual patients and the majority of the approaches have been based on the genetic characterization of their cancer.[1-3] Traditional chemotherapy has been largely established on cytotoxic drugs that destroy rapidly dividing cells, and this approach has been used for the past eight decades.

Precision medicine and personalized medicine have been popular words in the medical and health-care field worldwide since US President Barack Obama announced the Precision Medicine Initiative at his 2015 State of the Union address. Since the late 1990s, the basis of precision medicine has been to develop targeted therapies to inhibit specific molecules involved in tumor growth and dissemination of cancer cells.[2]

Several studies have been performed to discover targets that predict effectiveness in chemotherapy.[4] However, although over 100 chemotherapeutic agents are currently available for the treatment of cancer patients, the overall long-term clinical benefit is still unacceptable due to the lack of effectiveness or severe side effects from these agents. Additionally, the limited availability of effective medications and the high cost are still a major barrier for many cancer patients. Thus, alternative approaches to contain unnecessary cost still need to be developed.

There is a clear unmet demand for the development of diagnostic tools that may predict response in malignant tumors for an individualized treatment of these patients. Chemo-sensitivity and resistance assays (CSRA), which measure cell death or lack of cell death by drug-induced apoptosis, are one of such diagnostic tools. Several studies on CSRA focused on ovarian cancer,[5-7] gastric cancer,[8] colorectal adenocarcinoma,[9] breast cancer,[10,11] non-small cell lung cancer[12] and small-cell lung cancer,[12,13] following similar protocols with minor variations in their assay setup. The majority of these assays, which have been developed in the past 20-30 years, use culture of tumor cells that may also contain stromal cell contamination in the tested sample, which has been reported to preclude reliable chemosensitivity determination.[14] Testing cytotoxicity on bulk tumor cells containing a large presence of a contaminant of stromal cells may lead to the misinterpretation of test results due to an unselective determination of the overall response, because stromal and epithelial chemo-reactivity profiles may greatly differ. Also, the majority of these tests have been set up to assess chemotherapy cytotoxicity by exposing the bulk of tumor cells *in vitro* to drug concentrations that are lower than the plasma maximum concentration $[C]_{MAX}$ following a treatment, and therefore this procedure may not be clinically relevant. The majority of these chemotherapy sensitivity protocols treat the cancer cells for a period ranging between 24-72 h, which may also not be clinically relevant because it doesn't take into account that administered drugs undergo detoxification and body clearance leading to serum levels drop over time. Some of these tests have been improved over the years and are currently in use in clinical trials or in the clinics by progressive clinical oncologists for therapy of refractory malignant tumors especially in the OBGYN setting where no many options are available for platinum resistant tumors.[15-18] However, still none of these assays are in the routine standard-of-care clinical use due to their complex design and the lack of patient outcomes correlations.[19] In recent years, there has been a renewed trend towards personalized treatment approaches and in this context, CSRA-testing could be a further step in identifying the appropriate chemotherapeutics and molecular targeting agents.

A major breakthrough in the understanding of cancer progression has been the discovery of a cellular subpopulation with stem cell–like features, commonly referred to as cancer stem cells (CSCs), which is critical for tumorigenesis, treatment resistance and cancer recurrence.[20,21] Although the presence of somatic stem cells has been known since at least the 19th century, the demonstration that CSCs isolated from a patient reconstitute the full spectrum of malignant phenotypes in transplanted mice came from studies conducted between 1994 and 1997 on acute myeloid leukemia.[22,23] Existence of CSCs in solid tumor occurred in 2003 instead when these cells were first identified in breast cancer,[24] shortly followed by brain, lung, prostate, and colon cancer.[25]

The CSC fraction shares many properties with normal adult stem cells and is able to propagate the parental tumor in animal models.[20-23] Cancer lethality is mainly due to the onset of distant metastases and resistance to chemotherapy. Evidence has shown that CSCs are sheltered against widely used chemotherapeutic agents by means of different mechanisms, including increased expression of ATP-binding cassette drug transporters, augmented ability in DNA damage repair, and activation of PI3K/AKT and Wnt pathways.[26] Additionally, other indirect mechanisms involved in epithelial-mesenchymal transition and hypoxia may also contribute to chemo-resistance by inducing in cancer cells a stem-like phenotype.[27]

Selection of effective chemotherapy is extremely important not only when therapy is first initiated, but for recurrent disease as well. In fact, administration of ineffective anticancer therapy is often associated with unnecessary toxicity and the development of more aggressive cancer cell clones that are resistant to subsequent therapies (Figure 1).[28] The ability to initially choose the most effective chemotherapy may help to avoid the physical, emotional, and financial burden to patients of ineffective therapy, thereby improving their quality of life.[29] Because of the presence of therapy resistant CSCs, each time patients are treated they always have a chance of relapse, and their cancer will likely become more resistant to therapy.[30] Presently used anticancer drugs have a high rate of failure and cell culture

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chemotherapy testing has been used to identify which drugs are more likely to be effective against a particular tumor type. Measuring the response of the tumor cells to drug exposure is invaluable in any situation in which there is a choice between two or more treatments. Many attempts have been made over the years to develop an *ex-vivo* anti-cancer test that can provide clinically relevant treatment information. However, until now this approach has been limited to chemotherapy testing being performed only on bulk of tumor cells derived from cancer biopsies.[18,31-39]

Research on CSCs has failed thus far to discover universally clear and informative biomarkers, mutations, or gene-expression patterns.[40] The goal of individualized and targeted treatment and precision medicine requires the assessment of potential therapeutic targets (biomarkers) to direct treatment selection. Biomarkers, which are highly specific to a particular target or therapy are often called companion diagnostics and typically measure the therapeutic target itself or closely related partner molecules. Several clinical trials are under way to determine the role of biomarkers in stratifying patients who can benefit from a certain therapeutic molecule vs. another, but it will take probably another 10 years to assess the efficacy and reliability of these companion diagnostics.[41,42]

Recently, ChemoID® a new drug response assay has been developed that tests both CSCs and bulk of tumor cells directly derived from fresh tumor biopsies to predict the most effective chemotherapy agents' combination to treat individual cancers (Figure 1).[29,43-47] Targeting of CSCs alongside the bulk of other cancer cells is a new paradigm in cancer treatment. This constitutes an important advantage of ChemoID® approach over other cell culture testing methods. Understanding how CSCs overcome chemotherapy-induced death stimuli, and integrating such knowledge into clinical research methodology, has become a priority in the process of identifying innovative therapeutic strategies aimed at improving the outcome of cancer patients.

ChemoID® drug response assay is intended to assist the oncologist determine the optimum chemotherapy treatment options and the highest likelihood of efficacy for an individual cancer patient. ChemoID® assays ability to predictively test anticancer drugs efficacy for eradicating cancer stem cells (CSCs), personalized by the use of a patient's biopsy, measured in the lab and not in the patient, resolves the dangerous limitations of current cancer therapies. The test begins with a small tumor sample biopsy that is sent to the ChemoID® lab where bulk tumor cells and cancer stem cells are grown for testing. The process involves growing bulk tumor cells from individual patient biopsies in a medium that is unfavorable to normal stromal cells, followed by enrichment of the CSCs. Then those cellular fractions are treated with various standard-of-care chemotherapeutic agents selected by the patient's oncologist to determine how many tumor-derived cells and CSCs are killed using each drug or their combinations. A response curve is generated for each drug and drug combination evaluated, and the data are presented graphically as a cytotoxic index. This test enables faster reaction time to discover and administer the optimum selection of chemotherapy drug(s), and has been designed to increase patient survival, lower treatment costs by eliminating unnecessary chemotherapies, and decrease toxicity side effects. Any drug response assay – molecular or cellular – is only as good as the drugs that are available. A diagnostic test may be effective in predicting chemotherapy response; nevertheless, it will not improve the results of a poorly chosen therapeutic option. Unfortunately, cytotoxic chemotherapy is not yet good enough to provide a cure for most patients with malignant tumors; however, in a recent prospective study, we showed a statistically significant improved response rate (2.2-fold increase) in glioblastoma patients who were given assay-indicated chemotherapy.[48] Our results differ from other studies previously reported chemosensitivity assays based exclusively on bulk of tumor cells.[49,50] ChemoID® is the first and only CLIA compliant and CAP accredited drug response assay currently available that interrogates drug sensitivity of cancer stem cells from solid tumors. Results from our studies strongly suggests that a drug response assay that targets CSCs may be a very useful prognostic tool for optimizing treatment selection when first-line therapy fails, and when there are multiple clinically -acceptable and -equivalent treatments available.[29,44-48] Larger multi-institutional prospective clinical trials on the use of the ChemoID® drug response assay for guiding chemotherapy selection for glioblastoma, ovarian and breast cancers are being conducted to further demonstrate the clinical validity of this novel test. The ability to personalize therapy by providing the treating physician with drug response information on a panel of approved drugs should aid in the selection of most effective chemotherapy for individual patients, thus resulting in improved clinical outcomes.

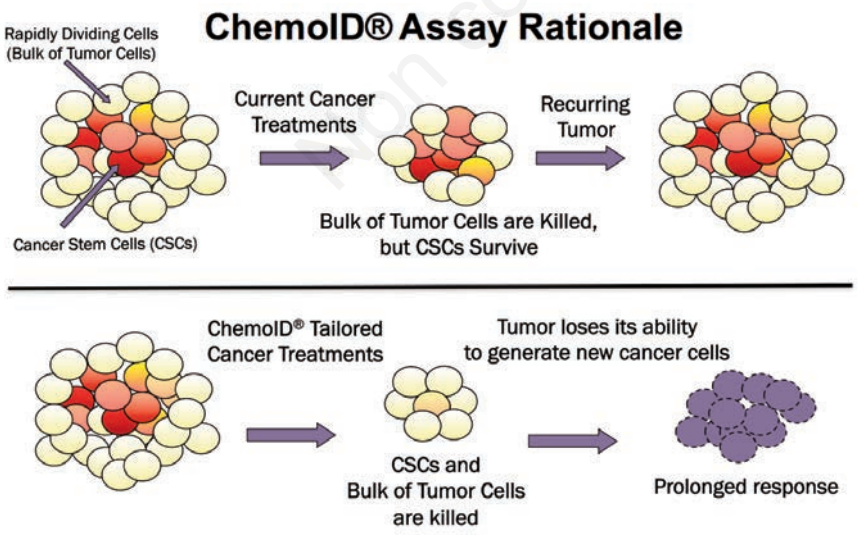

Figure 1. Cancer stem cell drug response assay rationale. Conventional chemotherapy initially kills most rapidly dividing cancer cells (bulk of tumor cells) causing shrinkage in tumor size, but the resistant cancer stem cells (CSCs) that are surviving eventually cause tumor recurrence. ChemoID® guided chemotherapy targets both CSCs and bulk of tumor cells leading to a prolonged clinical response due to the loss of self-renewal and proliferation capacity of the eradicated CSCs.

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