Proposal for *Ex Vivo* Chemoadjuvant Testing

Mark F. McCarty, Catalytic Longevity, markfmccarty@gmail.com

Abstract

Ex vivo chemosensitivity testing, targeting markers of cell death, has proven utility for defining chemotherapy regimens most likely to be effective in a given cancer patient. In light of the fact that a number of chemosensitizing adjuvants have been characterized with potential for boosting the responsiveness of specific cancers to specific cytotoxins, it is proposed that *ex vivo* chemoadjuvant testing be employed to define those adjuvants most likely to boost response to the cytotoxic agents chosen for use against a given cancer. Use of *ex vivo* testing to identity the best cytotoxic agents *and* the best chemoadjuvants for use with these agents, in each patient, may enable a notable advance in cancer management.

Ex Vivo Chemosensitivity Testing Enables Effective Personalized Cancer Chemotherapy

Ex vivo cancer chemosensitivity testing, focusing on cell death endpoints, has repeatedly demonstrated its ability to predict clinical response to chemotherapy, both with respect to immediate response and progression-free survival.[1-6] This technology evidently has the potential to pinpoint those cytotoxic agents which are most likely to achieve control of an individual cancer, and also to avoid the use of agents to which the cancer is resistant, thereby preventing needless side effects. The current skepticism of many clinical oncologists toward this technology reflects a putatively definitive assessment[7] focusing inappropriately on obsolete chemosensitivity assays which examined the impact of cytoxic drugs on cancer cell proliferation.[2, 6]

Owing to the virtually limitless mutability of the cancer genome, both with respect to genetic and epigenetic changes, most disseminated cancers which cannot be outright eradicated will eventually evolve a measure of resistance to cytotoxic agents that they formerly responded to. Chemosensitivity testing may be able to identify new agents to which the cancer is responsive, but over the course of time resistance to these agents is also likely to evolve.

A Wide Range of Chemosensitizing Ajuvants are Currently Available

Fortunately, a number of available drugs and nutraceuticals have been identified which have the potential to intervene in a variety of the mechanisms whereby advanced cancers diminish their sensitivity to cytotoxic agents. Some examples include salicylic acid, which often suppresses NF-kappaB activation by inhibition of IKK-beta;[8-10] diindolylmethane and tocotrienols, which oppose Stat3 activation in some cancers;[11-14] metformin, which appears to promote chemoresponsiveness by lessening the formation or viability of cancer stem cells;[15-19] hydroxychloroquine, an inhibitor of the autophagic response which often enables cancers to evade chemotherapy-induced apoptosis;[20-23] nelfinavir, a mild proteasome inhibitor which evokes a phosphatase activity targeting p-Akt;[24-27] itraconazole, inhibitor of the hedgehog signaling pathway;[28] designer drugs such as imatinib or erlotinib that suppress tyrosine kinase activities promoting chemoresistance;[29-31] ribavirin, which may suppress chemoresistance mechanisms reflecting

hyperactivation of eIF4E;[32, 33] epigenetic modulators – such as the combination of valproate and hydralazine – which have the potential to reverse epigenetic changes that can give rise to chemoresistance;[34-37] and millimolar concentrations of ascorbate (achievable via i.v. infusion), that may boost tumor responsiveness to some cytotoxic agents by selectively amplifying oxidative stress in cancer cells.[38, 39] (Moreover, it may be noted that *ex vivo* chemosensitivity testing of low millimolar concentrations of sodium ascorbate might be employed to predict the utility of high-dose i.v. ascorbate as a direct tumoricidal measure.[38, 40-44]) With respect to testing epigenetic modulators *ex vivo*, it may not be feasible to examine the impact of demethylating agents (such as hydralazine, an inhibitor of DNA methyltransferase 1[45]), as the cancer cells would need to progress through several cell cycles to achieve optimal demethylation of CpG-rich DNA – whereas agents which inhibit histone deacetylases (such as valproate) would be expected to have a more immediate impact on cell function and presumably *could* be evaluated in this way.

***Ex Vivo* Chemoadjuvant Testing**

The chemosensitizing efficacy of any of these agents is likely to vary as a function of the cancer targeted and the cytotoxic drugs employed. It is therefore reasonable to suggest that *ex vivo* chemosensitivity testing could be employed, not only to identify cytotoxic drugs to which a cancer may be relatively sensitive, but also to pinpoint adjuvant agents capable of boosting cancer responsiveness to the most appropriate cytotoxic drugs. This novel strategy might be dubbed "*ex vivo* chemoadjuvant testing".

If the oncologist has already decided what cytotoxic agents he intends to employ in the upcoming course of therapy, he could then test the cell-killing efficacy of these agents alone and in the presence of each of the adjuvants he wishes to evaluate. If however he is unclear as to which cytotoxic agents should be used, he could test *each* of these alone and in conjunction with adjuvants whose use was contemplated. Evidently, this could entail a large number of simultaneous chemosensitivity tests if several drugs and several adjuvants were under consideration; the amount of tissue available, and possibly the entailed cost, would presumably place an upper limit on the number of tests which could be conducted. Theoretically, the oncologist could first do a set of tests to define the best cytotoxins to employ, and then a set of follow up tests to determine the best adjuvants to use with these agents; this approach would be more parsimonious from the standpoint of the number of tests employed, but might not prove feasible owing to the limited number of days that a tumor biopsy sample will remain viable for testing. To economize on the number of assays required, some of the cited chemoadjuvants might be employed standardly in the absence of *ex vivo* testing, if they are inexpensive, reasonably well tolerated, and judged fairly likely to be of benefit (e.g metformin, diindolylmethane, tocotrienols, salsalate).

It will be important to define plasma levels of the tested adjuvant agents which can be achieved and maintained with optimal tolerated dose regimens, and to employ such levels in the testing; otherwise, chemoadjuvant testing might yield an overoptimistic assessment of the degree of sensitization likely to be achieved clinically.

It is envisioned that the insightful use of this strategy, in conjunction with additional feasible measures which slow tumor growth (directly anti-proliferative or anti-angiogenic) and support the immune system's cancer-scavenging potential, and coupled with the judicious use of locally targeted therapies (radiation, hyperthermia, intra-arterial chemo, surgery) that address critical metastases, may notably increase the chances for long-term survival in advanced malignancies. Furthermore, as an adjunct to adjuvant or neo-

adjuvant chemotherapy, the employment of appropriate chemoadjuvants guided by *ex vivo* testing might increase the chances that initial cancer surgery will achieve a curative outcome.

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