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Point of View

Selection of Markers to Predict Tumour Response Or Survival: Description of a Novel Approach

H.L. McLeod,[1] G.I. Murray,[2] J. Mollison,[3] J. McKay[1] and J. Cassidy[1]

[1]Department of Medicine and Therapeutics; [2]Department of Pathology; and [3]Department of Public Health, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, U.K.

THE PROBLEM

A MAJOR ISSUE with current cancer therapy is patient selection to ensure maximum benefit with minimum toxicity. Current methodologies for the prediction of patient outcome at diagnosis are almost exclusively based on the size of the primary tumour and the degree of local (lymph node) invasion or distant metastasis. Biomarkers have not made a major impact as yet for any solid tumour [1]. However, the Human Genome Mapping Project and other initiatives are producing a large amount of new data with potential relevance for predicting response or survival. In fact, the number of potential biomarkers is in near exponential increase, bringing further problems in selecting which marker will be most important for each tumour type. As we move into the post-genome era we need practical methods to assess rapidly multiple markers to evaluate their potential as prognostic predictors. Guidelines have been proposed for assessing the utility of putative markers of prognosis from existing literature [2], but no approaches appear to be identified for objectively selecting markers with the greatest potential to provide useful information in terms of predicting prognosis, predicting response to therapy, or in guiding therapy selection.

The currently used clinicopathological staging systems have the advantage of the availability of standardised criteria for assessing tumour stage for most tumour types (e.g. Dukes' or UICC stage for colorectal cancer, FIGO stage for ovarian cancer) and a relationship between advancing tumour stage and poor prognosis has been established for most cancers [3, 4]. Although these approaches provide gross information (i.e. patients with early stage disease generally have a better survival than patients with advanced stage disease), they have not led to clear criteria for the selection of individual patients who will benefit from radiotherapy, chemotherapy or alternative approaches. For example, in the Intergroup study of 929 patients with lymph node positive colorectal cancer (Dukes' stage C), 45% of patients treated with surgery alone were alive and disease free after 5 years compared with 65% of patients treated with 5-fluorouracil/levamisole [5]. This suggests that in retrospect, nearly half the patients with Dukes' C colorectal cancer did not require adjuvant chemotherapy and 35% died despite treatment with systemic chemotherapy. These data highlight the need for more informative prognostic markers which will identify the most appropriate therapy approach for individual patients.

The spiralling cost of healthcare has led to the need for rationing, or at least rationalisation, of the use of expensive chemotherapeutic agents. The therapeutic use of these agents are also often associated with great personal 'costs' in the form of systemic toxicity. Usually these toxicities are short lived and reversible, but even the most 'gentle' of chemotherapy can be fatal (e.g. toxicity from 5-fluorouracil therapy in patients with dihydropyrimidine dehydrogenase deficiency [6]). This has led to a greater emphasis on identifying prospectively predictive markers of response to chemotherapy or patient survival and highlights the need to identify new markers of clinical relevance in cancer. There are currently a large number of proteins which are of potential interest as markers of clinical outcome, including markers of proliferation, oncogenes, tumour suppressor genes, factors involved in tumour invasion, or cellular targets for chemotherapy [7–11]. The majority of published studies have assessed the utility of a protein as a prognostic marker by measuring its expression in 100–300 (usually primary) tumour samples from patients with a range of disease stages. Statistical associations are then made between the expression of the protein of interest and patient survival. However, this approach does not provide definitive information on how to correctly apply the results of such a marker study in the management of individual patients with cancer. The inclusion of patients with a variety of disease stages increases the generalisability of any findings, but usually does not provide sufficiently robust information regarding specific patient groups, because of a lack of statistical power within each subgroup. Most studies to date have also ignored the variable of patient therapy and analyse a combination of patients who have received a variety of different chemotherapy and/or radiation treatment regimens. These studies are thereby inadequate in their design and of little value to the practising oncologist in selecting individual patient therapy.

Correspondence to H.L. McLeod, e-mail: h.l.mcleod@abdn.ac.uk
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A more fundamental problem with the traditional approach for assessing putative tumour markers is the testing of such markers in isolation. Advances in tumour biology have made it clear that no single variable regulates tumour growth, invasion, and/or chemosensitivity. Analysis of a single or a small group of markers is akin to only checking the battery if your car will not start and ignoring the myriad of complex functions which make up the engine. The battery is a good place to begin to look but ignores potential contributing factors such as fuel and the components of the engine itself. However, there are many practical and financial limitations to the analysis of a large number of putative markers in definitive prospective studies. Time, expense and personnel required are a few of the reasons why these studies are rarely performed. Therefore, methodology must be developed for rapid identification of those putative prognostic markers worthy of more extensive assessment.

The 'enrichment approach'

We are proposing an approach for the rapid selection of markers of potential interest, on which larger definitive studies can then be performed. This method, which we have termed the *enrichment approach*, provides an initial selection of patients based on their clinical outcome (response to therapy or long-term survival) (Figure 1). The patients need to have the same disease stage, be at a similar time since diagnosis, and have received uniform surgical treatment, radiation, and/or chemotherapy. Two extreme patient groups are selected; those with a very *good* outcome (for example, complete tumour response or greater than 5 year survival) and those with a very *poor* clinical outcome (for example, progressive disease while receiving chemotherapy or rapid death from disease progression). Factors such as surgical mortality and inadequate radiological or pathological staging must be considered during the selection process. The enrichment approach operates on the assumption that factors important for tumorigenesis will be equally distributed between the two groups (both groups having already developed a tumour), but variables important for clinical outcome will be segregated to one group (or the other). This should give two relatively homogeneous groups of patients, in terms of clinicopathological demographics, on which to evaluate tumour markers. The patient numbers required to identify a large difference in the expression of a putative marker are smaller than that required to provide definitive evidence that a marker is associated with outcome in more heterogeneous patient groups.

The enrichment approach is designed to provide pilot information on which larger, more definitive randomised controlled studies of prognostic markers can be based. The design is similar to case–control studies, a common approach in epidemiology, where cases and controls are selected to differ only in the presence of disease and to be as similar as possible with respect to all other patient characteristics. The testing of a large number of putative markers in a relatively small number of patients using the enrichment approach is similar to that currently used by the U.S.A. National Cancer Institute, where the sensitivity profile of a new anticancer drug in a panel of 60 human cancer cell lines is evaluated in the context of the pattern of sensitivity of over 10 000 compounds previously analysed [12, 13]. The intention of the NCI new drug screen is to identify compounds on which further pre-clinical and clinical studies can be based. The enrichment approach has the same goal, in that it attempts to provide an environment which is more likely to identify clinically important markers, whether they be molecular, cellular or otherwise, associated with response to therapy or long-term survival. Markers which only have a small contribution to prognostic or therapeutic prediction will not likely be identified, due to sample size constraints. This reflects the lack of practical utility of such markers in patient clinical management.

Putative markers identified from the enrichment approach must then undergo prospective evaluation in a large definitive analysis programme (e.g., randomised control trials). It is no longer appropriate to analyse a single marker in 100–300 patients of mixed tumour stage, who received their treatment in the pre-chemotherapy era, and then attempt to make statements on the application of the marker to patient management today. Rather, an approach such as that proposed, could be used to 'weed-out' low yield markers and allow focus on those which are more likely to have a high impact on outcome.

We propose using a practical approach, such as the difference in the presence of markers which is thought to be clinically significant. We have defined this a priori in our ongoing studies as an absolute difference of 25% for the presence of a marker between the 'good' and 'bad' clinical outcome groups. Variables which demonstrate at least 25% difference will be taken forward to the more classical definitive analysis. This 'threshold' for clinical significance raises questions about the sample size needed to suggest a difference. The observation of over expression of a protein in 60% of 'good' clinical responders versus 30% of the 'poor' group would identify the marker as a candidate for predicting favourable outcome (i.e. a 30% difference between groups). However, the confidence intervals for this difference will be -4 to 64% if $n = 15$ in each group and 6 to 54% if $n = 30$ for each group.

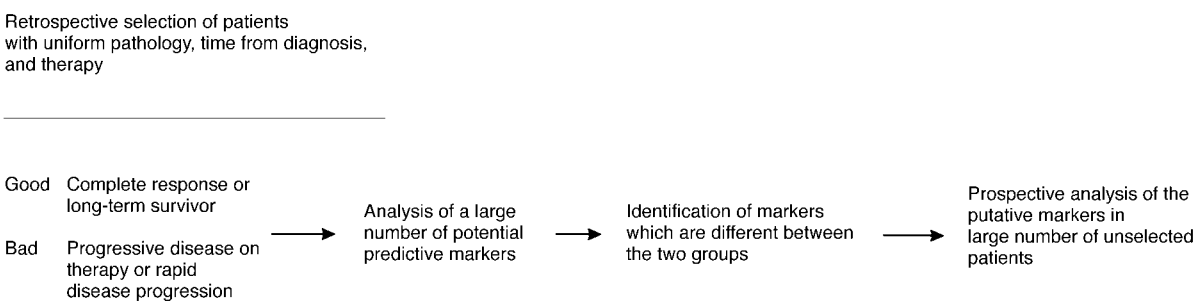

Figure 1. Proposed strategy for use of the enrichment approach to identify or exclude markers of outcome. 1-patient section based on outcome, 2-tumour studies, 3-identified markers carried through to prospective randomised controlled trials.

Although we advocate use of clinical significance, rather than statistical significance, factors such as sample size cannot be ignored. Again similarity with the epidemiology literature can be seen in the enrichment approach, where relatively small case–control studies are often followed by a larger prospective cohort study to establish relevance for risk-factors identified to be associated with disease.

Several new techniques, such as cDNA microarrays or tissue microarrays, have been recently described and are broadly applicable to the enrichment approach [14]. It would be feasible to construct tumour tissue micro-arrays containing samples from good and poor outcome patients, on which high-throughput molecular profiling could be performed [14].

The enrichment approach is now being applied in our laboratory for studies of colorectal, ovarian, and non-small cell lung cancer. As with all new methods, this approach will likely need to undergo further refinement to optimise the potential for generating clinically relevant markers of outcome. It is hoped that this commentary will stimulate further discussion on the best way forward for efficiently identifying putative markers of tumour response or survival which have a high likelihood for practical application in routine patient management.

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