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Designing personalised cancer treatments

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ABSTRACT

The concept of personalised medicine for cancer is not new. It arguably began with the attempts by Salmon and Hamburger to produce a viable cellular chemosensitivity assay in the 1970s, and continues to this day. While clonogenic assays soon fell out of favour due to their high failure rate, other cellular assays fared better and although they have not entered widespread clinical practice, they have proved to be very useful research tools. For instance, the ATP-based chemosensitivity assay was developed in the early 1990s and is highly standardised. It has proved useful for evaluating new drugs and combinations, and in recent years has been used to understand the molecular basis of drug resistance and sensitivity to anti-cancer drugs.

Recent developments allow unparalleled genotyping and phenotyping of tumours, providing a plethora of targets for the development of new cancer treatments. However, validation of such targets and new agents to permit translation to the clinic remains difficult. There has been one major disappointment in that cell lines, though useful, do not often reflect the behaviour of their parent cancers with sufficient fidelity to be useful. Low passage cell lines — either in culture or xenografts are being used to overcome some of these issues, but have several problems of their own. Primary cell culture remains useful, but large tumours are likely to receive neo-adjuvant treatment before removal and that limits the tumour types that can be studied. The development of new treatments remains difficult and prediction of the clinical efficacy of new treatments from pre-clinical data is as hard as ever. One lesson has certainly been that one cannot buck the biology — and that understanding the genome alone is not sufficient to guarantee success. Nowhere has this been more evident than in the development of EGFR inhibitors. Despite overexpression of EGFR by many tumour types, only those with activating EGFR mutations and an inability to circumvent EGFR blockade have proved susceptible to treatment.

The challenge is how to use advanced molecular understanding with limited cellular assay information to improve both drug development and the design of companion diagnostics to guide their use. This has the capacity to remove much of the guesswork from the process and should improve success rates.

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1. Introduction

The goal of truly personalised cancer treatment is within our grasp. It is no longer acceptable to suggest one size fits all treatment to patients who understand that their cancer is unique to them, as indeed it is. Treatment based on randomised clinical trials has improved cancer outcomes, but has done this incrementally. Standard therapy inevitably fails many patients as it ignores the heterogeneity of tumour response to drugs (Fig. 1). Richard Klausner described the situation at the turn of the century very well in 1997 when he said, "Right now we lump patients together and treat them with the same drugs and then deal with their variable response to treatment. We're essentially treating different diseases with the same medicine." That cannot be good medicine, and recent gains made suggest that individualised therapy based on companion diagnostics can do better. The problem of course is to know who has which disease, or at least who will benefit from which drug. Oncologists need pathologists to do a test and tell them which drugs are likely to work. They can then decide on the appropriate treatment and exercise the real skill of getting the best from the drugs in patients despite variable side effect profiles.

When considering the sort of test to use, it is important to distinguish between prediction and prognosis. A test that defines prognosis simply implies that two (or more) groups are different and that you they can be identified by the test. However, the test does nothing to improve the prognosis this contrast to a predictive test which alters the outcome in one group resulting in improved prognosis or for some of the patients (Fig. 2). Some tests do both — for instance Herceptest (Dako) which defines a group of patients who do worse if not treated, and predicts that they will do better if treated with an anti-HER2 antibody, traztuzumab (Herceptin).

2. Cellular cytotoxicity assays

There have been many attempts to individualise cancer therapy using tests based on exposure of cells to drugs. None of these have entered clinical practice, but many have provided very useful research tools for the development of new drugs and have increased the

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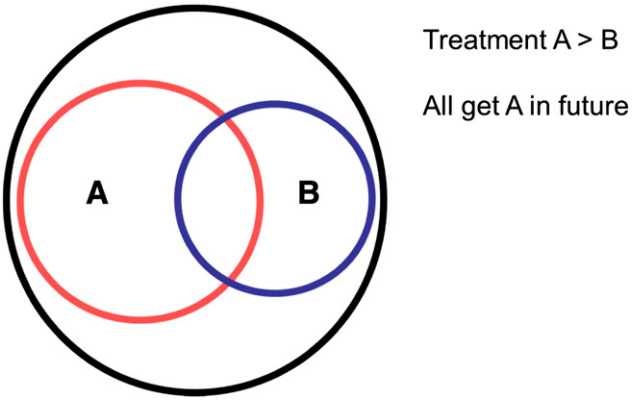

Fig. 1. This Venn diagram shows the hypothetical result of a standard clinical trial of two treatments — A and B. Treatment A does better than B, and becomes the new standard of care. However, those outside the circles representing patients responsive to either treatment get the toxicity and no benefit, while those within B but not A, would have responded to an older treatment which they will not even know they could have benefitted them.

understanding of anti-cancer mechanisms. The field started in the 1970s with clonogenic assays pioneered by Salmon and Hamburger [1]. These proved relatively difficult to handle with a high failure rate as many patients' tumours could not be grown *in vitro*. Many other assays have been produced since [2]. The enzyme release assays look for cell death by release of enzymes into the medium surrounding the cells [2–5]. Viability assays such as the trypan blue assay are in regular use by labs growing cells as they are simplicity itself. They show that the cells have intact membranes and recent automation of these methods has extended their utility. The NCI60 panel has made extensive use the Sulforhodamine B (SRB) assay which determines the total protein content of the culture compared with controls, and correlated the results with gene expression [6]. There are a number of cell death assays on the market that are particularly useful for looking at apoptosis and other cell death mechanisms. They include the caspase assay, the Annexin V assay [7], and various methods to assess DNA fragmentation. Cell survival assays are probably the most widely used. The Neutral Red uptake assay was the first such test, but was rapidly supplanted by the widely used MTT assay, which relies on succinate dehydrogenase activity and hence intact mitochondrial function [8,9]. The ATP Tumour Chemosensitivty Assay (ATP-TCA) is an example of a cell survival assay and is covered

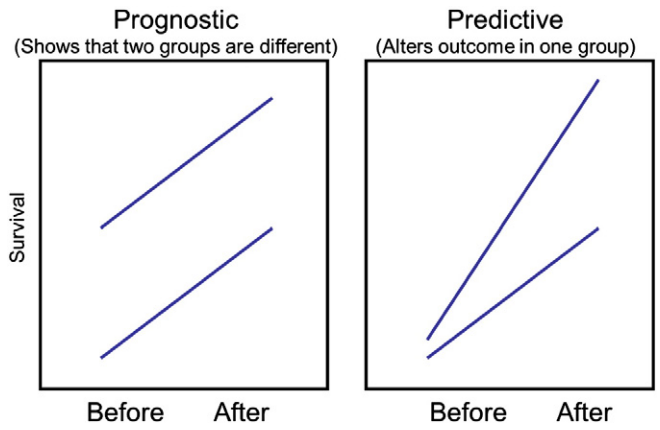

Fig. 2. The difference between a prognostic and a predictive test. Prognostic assays distinguish groups according to their risk of an adverse outcome, while predictive tests define a group in which treatment will improve outcome.

in greater detail below. All of these assays [2] have their advantages and disadvantages and it is a case of choosing the correct test for the job.

3. The ATP-TCA

The ATP-based Tumour Chemosensitivity Assay (ATP-TCAP) was developed by Peter Andreotti and the author in the early 1990s as a standardised way of assessing the effect of anticancer drugs in primary cell cultures [10,11]. It uses fragments of tumour from fresh tumour resection specimens or biopsies obtained directly from the operating theatre. The fragments are minced, and incubated overnight with a relatively gentle collagenase based medium resulting in a suspension of single cells and small clumps which can be washed, counted and then plated easily at 20,000 cells per well in a 96 well polypropylene plate. Drugs are added and the plates of incubated for six days at 37 °C in a CO_2 incubator with high humidity. At the end of that time, the cells are extracted and ATP content measured by luciferin-luciferase. In each 96 well plate it is possible to look at four different drugs or combinations at six dilutions, using triplicate wells to ensure accuracy. Quality assurance is an important aspect of any assay: most cellular sensitivity assays relate their results to a medium only (MO) control and the best also check a complete inhibitor which should kill all the eukaryotic cells present, allowing subtraction of background [11,12]. In common with most such assays, the results are then expressed as the percentage inhibition against a test drug concentration.

The test drug concentration used in such tests can be hard to determine, as the concentration of drug to which the tumour is exposed may differ from the free drug concentration in blood and there is usually reduced protein binding in tissue culture media. Data from manufacturers and phase I trial reports contain most of the information needed. For the ATP-TCA, we were able to come up with a test drug concentration for most cytotoxic drugs, allowing simple comparison of the effect of individual drugs against tumours. The other issue is drug metabolism: drugs such cyclofosfamide require activation by the liver, but in this and some other cases, it was possible to obtain metabolites for use *in vitro*.

The ATP-TCA proved to be a very useful test for drug development, allowing early testing of compounds against different cancer types and giving some indication of likely activity. This included cytotoxic agents, anti-tumour antibodies and targeted small molecules [13–21]. The assay is particularly helpful for the *in vitro* design of new combinations, particularly where there was a molecular hypothesis to test. For instance, we were able to show that the effect of mevalonate pathway inhibitors was limited, but that they enhanced the effect of N-bisphosphonates, probably via the production of metabolites not normally present in the cell [17]. We have most recently used the assay to show that EGFR and PI3K blockade is synergistic in ovarian cancer cells (Glaysher et al., unpublished data). While it is certainly true that primary cell culture can mislead, this is less likely than cell line alternatives, particularly when serum is present in the medium [22,23]. As an example, the sensitivity of uveal melanoma to treosulfan + gemcitabine, a combination suggested by the ATP-TCA, proved disappointing, though this may well have been due to previous treatment of most patients with dacarbazine and ineffective dosage [24–26].

One important use of the assay was to show the level of heterogeneity present between patients in terms of their response to new and old drugs. We were able to do this for a variety of tumour types [27–31], and this suggested that the results might be useful to guide treatment. We undertook the first randomised clinical trial of individualised chemotherapy and were able to show impressive progression free survival and response rates in the assay-directed arm of the study in recurrent platinum-resistant ovarian cancer, though the size of the trial was limited and the effect just fell short of statistical significance [32]. There was, however, a statistically significant

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