New light on polyvalent agents for cancer treatment

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Abstract

Options for the definitive treatment of cancer are: facilitating immune responses, restoration of growth controls, removing the cancer cells, or killing them. All treatments fail more often than not because of the intrinsic malignant properties of spread and progression and because cancer cells are so like their normal neighbours. If we want improved performance in radio- and chemo therapy we need better agents than are at present available, of vastly greater power to select cancer cells. There is reason to suppose that multivalent agents can be made that will achieve the necessary standard of performance – capable of identifying and eliminating all cancerous cells in a human patient – by detecting the unique combination of (mostly "passenger") mutations that characterises each individual tumour. tom@boyde.com, www.trcboyde.net

Introduction

The word 'cancer' refers to tumours of a wide range of properties and aggressiveness, having in common that growth of the characteristic cells is not under normal restraint (true also to a lesser degree of benign tumours); progression; the propensity to invade and/or metastasise; and the presence of many somatic mutations, including passengers: so many and so varied that every individual cancer must be considered unique in respect of the constellation of mutations present (T.R.C. Boyde, "Singularity: The Achilles' heel of cancer?" *Medical Hypotheses* 73 (2009) 503-505, and references therein). Classification remains quite crude, based on tissue of origin, not reflecting the genomic site or nature of the driver mutations and of limited value in predicting the response to treatment. It appears that many would-be cancers are removed by the immune system (understood in the broadest possible sense) so that we see only the failures, though there is perhaps better evidence of cancers being aided or concealed by a subverted immune system (for example, M.L. Markiewski *et al*, *Nature Immunology* 9 (2008) 1225-1235).

Our options for definitive treatment are limited to: 1. Enhance immune activity (even if the immune system is often two-faced). 2. Restore cellular growth controls. 3. Remove the cancer cells (surgery). 4. Kill them (radio- and chemotherapy). The immune system may be helping us in ways that we know little about whilst option 2 is the new wave which has a number of remarkable successes to its credit over the past decade. Yet surely the gold standard is to get rid of all abnormal cells and the mainstays of treatment for more than one hundred years have been options 3 and/or 4. Their effectiveness is limited by properties intrinsic to malignancy, namely spread and progression, together with the great similarity between cancer cells and the normal cells of the body; so that option 4 has been directed to cells that are rapidly dividing rather than to cancer cells *per se*.

If radiotherapy and chemotherapy are to be improved, we will require much better-directed agents, more selective for the abnormal cells over their normal neighbours.

Improved Discrimination

How can that be done? There is something to be learned from analytical biochemistry.

In 'sandwich' immunoassay, a method now widely used in routine hospital laboratories, a capture antibody detains the desired analyte in contact with a solid phase so that it can be washed free of interfering contaminants; the measurement is then done by means of another antibody also specific for the analyte but directed to a distinct and different epitope. This detector antibody has been labelled with a signal-generating moiety of some kind; fluorescent or similar, radioactive or enzymatic. Specificity of such a method is much enhanced over the first-generation immunoassay methods because a positive signal requires the simultaneous presence on the analyte of two different features, each separately identifying it. Neither alone will suffice. Diagnostic precision may be increased by several orders of magnitude.

Even more nearly related in principle to what is described below are: [i] The proximity-ligation assay (S. Fredricksson *et al*, *Nature Biotechnology* 20 (2002) 473-477) where a positive result is only possible if two different epitopes are close enough together on the target that polymerase-chain-amplification occurs involving nucleic acid sequences attached to the detector entities. [ii] The long-known phenomenon of chelation (complexation) where remarkably large association constants and discrimination factors are achieved because several binding residues or orbitals are combined in one molecule and all can act at once.

What all these methods share is recognition of multiple, different, independent aspects of the target, which must all be present for identification to be accepted, somewhat like having several independent witnesses to the identity of a criminal suspect; more reliable than one alone. Agents based on this principle of heteropolyvalency should prove far better than existing agents both for the detection and destruction of cancer cells.

We emphasise that the approach here is quite unlike the known 'cancer antibodies' or immunotherapy of cancer. In immunotherapy, the aim is to enhance production of antibodies or T cells against an existing cancer and it is an example of treatment option [1], not [4]. Cancer antibodies are ordinary antibodies with binding sites of identical specificity, bivalent but monospecific, depending for any chance of success upon a unique antigen which is present on all the cancer cells of a particular patient and only on them – which may be true of some immortal, unchanging laboratory cell lines, but never occurs in the clinic.

How it might work in practice

To grasp how this kind of thinking may be applied in treatment requires clarification of some less-widely-appreciated properties of cancers.

Each patient's cancer is unique: those within one formal classification do not share the same somatic mutations; usually not even the same set of driver mutations. Further, cancers are clonal and sub-clones have mutations additional to those of their parent clone, because more and more are acquired during progression. Nevertheless we can expect to identify a ***constellation*** of mutations and derived from them a lesser number of mutant surface epitopes, common to all the cancer cells but not found on normal cells (T.R.C. Boyde, *op cit*).

Scenario. So, the projected scenario is that in any patient all the cancer cells possess several, accessible target epitopes that are not present on his or her normal cells, or not in the same combination, allowing specific recognition of those cells. Ligands to a sufficient number of these targets are required, and will be linked in pairs to yield diagnostic or therapeutic agents by way of adding suitable labelling, cytotoxic or radio-sensitising residues.

This model has several unconventional and challenging consequences, but the approach should not be discarded for that reason alone since all have been carefully considered.

There is no implication that Fab fragments or antibodies will be the only raw materials. Ligands may be in principle of any chemical nature and nucleic acid aptamers are included as possibilities. In this paper, a 'ligand' means a monovalent entity. Throughout, higher multiples are included where pairs are mentioned or implied; bivalent includes multivalent, etc.

Reality check. It is not yet established that cancer cells have suitable sets of epitopes that are peculiar to themselves, either individually or in combination, but it ***IS*** established that typical, mature cancers have thousands of random somatic mutations (T.R.C. Boyde, *op cit*) and it is reasonable to expect that some of these will be detectable phenotypically in the form of altered structures in accessible locations. Experiments are required, not dogma.

Ligands, diagnostic reagents and therapeutic agents. Given the timescale of an individual patient, the ligands that will be used in a given case must be known very quickly after initial diagnosis, whereas development of an antibody may take months to years. Therefore, ligands must be available in advance (and stored) or be made easily and quickly for the individual patient. There is no need for very high affinity or specificity of the monovalent ligand. Nucleic acid aptamers may well serve, or protein fragments that are easier to derive or engineer than antibodies. It may be positively advantageous to start with ligands having monovalent affinities in the region of 10^6 to 10^7 l/mol (rather than Fab's whose affinities may be typically 10^8 to 10^9 l/mol) and not absolutely specific but able to react with structurally similar targets over a modest range, since the properties of selectivity and high affinity will be obtained by linking ligands in pairs. Broad specificity of the monovalent ligands will make it much easier to select a suitable set for an individual patient and will allow the majority of patients to be served by means of a battery of perhaps only a few hundred ligands – still a major logistic exercise but manageable.

Chemistry. Making the bivalent diagnostic and therapeutic agents for a particular patient is much easier than appears at first sight (given a supply of monovalent ligands). An appropriate nucleic acid 'tail' is added to each ligand and the agent assembled simply by allowing hybridisation between the tails of a pair, as has been described (GB Patent 2 229 1877, US Patent imminent). These 'tailed' ligands will perhaps be the form that is kept in storage awaiting patient use.

Flexible, extensible agents. Target epitopes may be so far apart or so disposed that a bivalent agent of the old kind cannot bind to both at the same time. To overcome this objection, the nucleic acid link between ligands is flexible and extensible, being single-stranded in the segments not involved in hybridisation. The only targets that are of use therapeutically will be close enough to each other for such an agent to bind to both epitopes.

Detailed diagnosis. When preparing for treatment of a patient it will be necessary to identify what combination of epitopes to attack. Tailed ligands are allowed to react with samples

obtained by biopsy or from peripheral blood (S. Nagrath *et al, Nature* 450 (2007) 1235-1239). Only if a pair of target epitopes are close enough will hybridisation occur between the tails of the bound ligands and, for example, a fluorescent signal be emitted to indicate that fact. That is to say, the final diagnostic agent assembles itself *in situ*.

Therapy. The tailed ligands used in diagnosis and therapeutic agents differ in whether the residues to be added (see 'Scenario', above) are signalling, cytotoxic or radio-sensitising residues, but the original ligands are the same and show the same binding properties to target epitopes (and to each other - by hybridisation). Thus a positive diagnostic test will show directly which combination of tailed ligands should be used as the therapeutic agent**:** better still, it is expected that the final, functional therapeutic agent will assemble itself *in situ, in vivo*, after parenteral administration of the appropriate tailed ligands, separately.

Regulatory Barriers. A bivalent therapeutic agent may be unique to one patient or common to a limited group; but in neither case can we imagine trials of the kind now demanded for small-molecule drugs, even if there were time. So there is an ethical dilemma, enhanced by the current regulatory process, to be tackled as such rather than allowing it to become an obstacle. Possible solutions include the 'orphan drug' exemptions, approval only of the less numerous tailed ligands rather than the therapeutic agents proper, and recognising that patients facing death should be free to choose unusual treatments if that is what they want.

Costs. Costs will be astronomical at first, though some very wealthy individuals will be willing to pay for themselves and it is this feature which makes the venture practicable. If established as described, there is no reason why this approach should not reduce in cost later on, to become comparable with present-day advanced treatments.

Discusssion

Research centred on cancer continues to enlarge our understanding of the immensely complicated nature of life in multicellular organisms. Treatment of cancer has lagged somewhat behind. There is every reason for the community to feel proud of recent successes based on understanding the controls of cellular growth and division – and yet it remains true that most cancers are refractory to treatment of any kind and crude death rates are increasing if only because the population is ageing. Very likely, cancers will be treated in future by a combination of options 1 to 4. The approach described in this paper is the only one known that may allow the absolute identification of every cancer cell in the patient's body, and thus has unique potential. Progress has been slow, but nothing has happened to diminish confidence in its eventual success, given appropriate financial and logistic support.

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