Circulating tumour-derived predictive biomarkers in oncology

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Molecular characterization of tumour material will become increasingly important in selecting patients for clinical trials and offering appropriate treatment for patients in clinical practice. Recent advances in the field have indicated that the molecular characteristics of a tumour can be determined from circulating tumour cells and circulating tumour DNA; thus, a simple blood sample could provide these data in a simple, convenient and efficient manner. This article discusses progress towards guiding treatment decisions through measuring tumour-derived factors in the circulation.

A molecular approach to disease

Recent decades have seen significant steps forward in basic and translational research into cancer. It is beholden upon the oncology community to transform this improved understanding into tangible benefits for society at large and patients in particular. At the heart of the matter is a change of perspective from viewing cancer as a single disease defined by its anatomic site of origin to a disease defined by underlying molecular pathology. Historically, surgical treatment (and subsequent chemotherapy) was based upon the site of the primary tumour under the assumption that all tumours in one organ were homogenous in origin. Subsequently, tumours within a single organ were recognized as being histopathologically different (e.g. non-small-cell lung cancer, or NSCLC, and small-cell lung cancer). More recently, tumours with the same gross histopathological appearance have been recognized as molecularly heterogeneous, and it has been recognized that this heterogeneity impacts on the aggressiveness of the tumour and the likelihood of response to therapy (see e.g. [1]). Thus, an understanding of the molecular characteristics of a tumour will be of paramount importance for driving targeted therapies in the future. Access to tumour material will be essential, but this is where the challenges arise; access to such samples can prove problematic. However, recent advances in the field have indicated that the molecular characteristics of the tumour could be determined from circulating tumour cells (CTCs) and circulating tumour DNA. Thus, a simple blood sample could provide these data in a simple, convenient and efficient manner. This article discusses progress towards guiding treatment decisions through measuring tumour-derived factors in the circulation.

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Predictive tumour-derived biomarkers

The oncology community is committed to discovering new therapies by targeting the underlying molecular pathology of tumours. Most new therapies are aimed at one or a small number of targets hypothesized to be responsible for driving disease. *A priori*, this suggests that the maximum therapeutic index is likely to be achieved where the particular target is differentially important for tumour and normal cell growth or survival. Although this is akin to targeting differential proliferation and replication, as is done with traditional therapies, it is qualitatively different because it clearly signals the approach of measuring a molecular biomarker of 'pathway addiction' within the tumour. 'Biomarker' is a general term for a measurement and is generally defined as in Table 1. 'Predictive biomarkers' are a subgroup of useful biomarkers that inform treatment decisions for individual patients. It is important to distinguish between predictive markers and those that are solely prognostic. Prognostic markers inform of the likelihood or imminence of certain outcomes for a patient, but they might not help choose the best course of action for them. Predictive biomarkers are relevant to treatment decisions because the benefit/risk ratio

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TABLE 1

Definitions of biomarkers (from [4])	
Biological marker (biomarker)	A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention.
Clinical endpoint	A characteristic or variable that reflects how a patient feels or functions or how long a patient survives.
Surrogate endpoint	A biomarker intended to substitute for a clinical endpoint. A clinical investigator uses epidemiologic, therapeutic, pathophysiologic or other scientific evidence to select a surrogate endpoint that is expected to predict clinical benefit, harm, or lack of benefit or harm.
Useful biomarker	Informs risk/benefit ratio when there is a decision to be made. Does so in a better, faster, earlier and/or cheaper way than existing approaches. Generally applicable: sample and technology must be available and accessible. Has a known identity or known identities.

differs for competing courses of action according to marker status. For a marker to be considered predictive, clinical studies must demonstrate that there is a treatment by biomarker interaction such that the benefit/risk profile between two treatment options differs significantly. Note that a marker can be both prognostic and predictive and that logic tells us that specific measurements from within the tumour itself are the probable source for efficacy-driven predictive biomarkers.

Following this logic, notable successes in identifying the patients most likely to benefit from a particular therapy have included the presence of target (*BCR-ABL* and Gleevec in chronic myelogenous leukaemia); levels of target (oestrogen receptor, or ER, and HER2 for endocrine and Herceptin therapy in breast cancer); functionality of target (epidermal growth factor receptor, or EGFR, mutations and Iressa in NSCLC [2]); and target 'bypass' (*KRAS* mutations in anti-EGFR monoclonal antibody treatment of CRC [3]). Hence, although circulating tumour markers such as CA-125 and PSA are used routinely for disease monitoring purposes, we rely largely on tumour biopsies for the measurement of predictive markers.

Biology and practicalities

Predictive biomarkers seem to have the greatest chances of discovery or validation when the time interval between tumour biomarker measurement and treatment is short, minimizing the opportunity for the genetically unstable tumour to evolve further in the intervening period. It might be important that in some of the examples listed above, the predictive marker was not an integral part of the initial drug development plans and convincing data were only obtained much later in the clinical development programme (i.e. in large trials in first-line settings). New drugs are often not tested in a 'first-line' setting until late in their development, once the safety profile has been accurately documented, raising the prospect that measurements made on original diagnostic biopsies in the earlier later-stage clinical trials are 'fossils' that are many years old and not relevant to drug sensitivity of the current tumour. In addition, in later disease the original tumour might not be amenable to biopsy because it has been surgically excised, and most tumours are not amenable to multiple fresh biopsies at every occasion when you need to decide on the next therapeutic intervention. The latter point is extremely important within clinical trials in which it is necessary to validate a predictive marker where the proportion of patients with an evaluable sample can be so low as to endanger the validity of the study [4]. Furthermore, subjecting already very ill patients to invasive procedures for the sole purpose of exploratory research might be deemed unethical in many cases.

In several of the examples mentioned earlier, the predictive biomarker involves measuring tumour DNA related to a key biological difference between tumour and the patient's normal cells. It is important to distinguish this potentially dynamic tumour sample level measurement from a patient's heritable genotype. Such static, patient-level measurements might also be important indicators to forming tumours of a particular molecular type [5], but the required information can be gained from virtually any cell in the body and at any point during their lifetime. Interestingly, there are reports of drug resistance apparently arising as a result of alterations in DNA evolving under the selective pressure of treatment. In lung cancer, acquired resistance to EGFR inhibition has been associated with further mutations in the EGF receptor, and in ovarian cancer, resistance to platinum-based therapy has been associated with tumour-specific reversion of inherited mutations in the *BRCA2* gene [6,7]. Such observations reinforce the value of being able to characterize a tumour at the time of treatment choice. Thus, there is a real need to be able to monitor the molecular status of the tumour (i) without invasive tumour biopsy, (ii) at the time of treatment choice, (iii) rapidly and cheaply, and (iv) for all patients.

BOX 1

Circulating foetal cells and nucleic acids

There is a scientific parallel between the detection of circulating nucleic acid material from a tumour and from a foetus. Circulating foetal cells are rare (~2–20/20 ml of maternal blood). Cell-free foetal DNA, however, can be detected in maternal blood from the seventh week of gestation, and levels increase with gestational time, medical complications such as pre-eclampsia and after interventions such as chorionic villus biopsy and amniocentesis. Cell-free foetal DNA is generally less than 300 bp in length, comprises 3–6% of total circulating DNA (most is maternal in origin) and is cleared rapidly from maternal plasma with $t_{1/2}$ of approximately 16 min [8]. Foetal DNA, therefore, is significantly enriched compared with foetal cells. Foetal DNA can be used for the determination of foetal rhesus D status, sex and chromosomal aneuploidies. Work on foetal RNA is at an early stage, but it might be present in particles that protect it from degradation and could be useful for detecting foetal abnormalities, especially because there is a suggestion that no maternal RNA is detectable.

Circulating tumour cells

CTCs are found in some cancers and have been found to be prognostic in some indications (e.g. metastatic breast cancer [9], metastatic prostate cancer [10] and NSCLC [11]). Number of CTCs is also expected to be higher in advanced disease in which the tumour burden is higher than in primary disease. There exists an interesting scientific parallel between the detection of circulating material from a foetus with that derived from a tumour (Box 1).

There is a growing body of opinion that molecular profiling of CTCs should further enhance our understanding of cancer biology and enable us to personalize patient therapies [12]. Recently developed methodologies are reported to allow the detection of CTCs with sufficient purity to enable their characterization by means of several techniques: potentially, reverse transcription PCR, FISH and the detection of mutations [13].

Recent examples of the molecular characterization of CTCs include the identification of HER2 and EGFR on CTCs in the breast cancer setting, potentially opening the way for personalized treatment without the need for a tissue biopsy [14,15]. In the NSCLC setting, *EGFR* mutations have been detected on CTCs [11].

Circulating free tumour DNA

Small amounts of free DNA (~1 ng/ml) can be found circulating in the plasma of healthy individuals [16]. Increased levels have been reported in the serum and plasma of patients with a variety of illnesses, including autoimmune disease, chronic inflammation and cancer [16–18]. In addition, elevated levels have also been noted after exhaustive exercise or trauma [19,20]. The serum of cancer patients is enriched in DNA and contains, on average, approximately four times the amount of DNA of normal controls [16], although this is variable and overlap exists between the two groups. The mechanism and source of this DNA release remains largely uncertain, although several potential explanations have been explored. It is probable that several mechanisms contribute to its release, although it is generally thought that it originates from either malignant or haematopoietic apoptotic and necrotic cells.

In the 1990s, studies began to be published that looked at tumour-derived DNA, and there are reports of the identification of chromosomal and microsatellite aberrations, abnormal promoter hypermethylation and point mutations [21]. In many instances, these alterations are identical to those seen in the original primary tumour tissue. Indeed, recent studies have shown that there is good correlation between DNA mutations detected in serum and those of the diagnostic tumour [22]. RNA from tumours has also been detected in the circulation and might be useful for detecting and monitoring tumour-derived epithelial markers. A useful review of this field can be found in Refs. [23,24].

Circulating free DNA in lung cancer

Lung cancer is one of the most common cancers and the leading cause of cancer-related death. Access to high-quality tumour material is particularly challenging in this disease setting, making it an ideal target for approaches that utilize molecular characterization using a blood-based sample. Recent years have seen a large number of publications in this field, and this is extensively reviewed in Ref. [23]. Of particular note is the detection of mutations in the *EGFR* gene in circulating free DNA of patients with NSCLC. Recent studies have indicated that mutations, detected in serum or plasma, can be correlated with response to treatments such as Iressa and Erlotinib [25–27].

Circulating free DNA in colorectal cancer

Circulating free tumour DNA has reflected the total systemic colorectal cancer tumour burden, in that the DNA levels decreased upon complete surgery and generally increased as new lesions became apparent upon radiological examination [28].

Recent data have indicated that analysis of *KRAS* mutation status in metastatic colorectal cancer might be an important determinant for treatment choices with drugs such as panitumumab and cetuximab. Tumours harbouring mutations are more likely to be resistant to treatment with these agents and, as such, treatment choices will depend upon accurate and rapid determination of mutation status. Work in the field of analysis of circulating free DNA in colorectal cancer has also demonstrated its utility. *KRAS* mutations have been reported in 30–50% of colorectal cancers, and a review of studies reported that 24% of patients with colorectal cancer have detectable *KRAS* mutations in plasma or serum [29]. With the advent of more sensitive screening approaches (e.g. Beaming), detection rates will increase markedly.

Concluding remarks

Biomarker measurements from CTCs and nucleic acids have the potential to monitor the molecular status of tumours without invasive tumour biopsy, at the time of treatment selection, and rapidly and relatively cheaply, and they could do so for all patients that can provide a blood sample. A massive advantage for any predictive marker is the ability to be developed into a point-of-care approach. The circulating free tumour DNA methodology can currently run into problems where disease burden is low; however, technologies are improving at an exponential rate and might be able to identify tumour-derived DNA, where present, at progressively lower concentrations in the near future.

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