Cancer cells can induce an immunological response resulting in the production of tumor-associated (TA) autoantibodies. These serum immunobiomarkers have been detected for a range of cancers at an early stage before the development of clinical symptoms. Their measurement is minimally invasive and cost effective using established technologies. TA autoantibodies are present in a clinically significant number of individuals and could supplement current screening modalities to aid early diagnosis of high-risk populations and assist the clinical management of patients. Here we review their production, discovery, and validation as biomarkers for cancer and their current and future potential as clinical tools.

**TA Autoantibodies: Early Biomarkers**

Cancer is a growing burden and, despite considerable therapeutic advances, identifying tumors at early stages to enable curative treatment represents the most promising route for reduction of mortality rates [1–4]. It has been over 100 years since it was first proposed that the immune system could play a role in preventing tumors [5–7] (Box 1). Tumorigenesis leads to the production of many abnormal substances that can be antigenic. As antigens (see Glossary) are no longer recognized as ‘self’, the immune system triggers a reaction by producing autoantibodies [8], also referred to as TA autoantibodies.

Hundreds of TA autoantibodies have been identified since their discovery [9,10] (Figure 1). While not all TA antigens will elicit an immune response in all individuals, and not all immune responses may be tumor specific, the measurement of TA autoantibodies appears to hold hope for an alternative method for cancer detection, provided that levels are higher in individuals with tumors than in healthy individuals and those with benign conditions.

**Autoantigens** are well established biomarkers for the clinical diagnosis of some autoimmune disorders [11]. TA antigens are currently the most commonly measured biomarkers during management of cancer patients [12]. However they are often of little utility in early disease due to serum levels increasing with the size of the tumor, limiting their clinical use to monitoring of treatment and disease recurrence. miRNAs and cell-free circulating tumor DNA are gaining popularity as tumor biomarkers [13], although the technical issues with sample collection and reproducible measurement are not insignificant.

TA autoantibodies are currently emerging as strong candidates for clinically useful cancer biomarkers identified for a range of cancers. They are produced early in tumorigenesis, being measurable up to 5 years before the development of clinical symptoms [14–17]. As antibodies they represent biologically amplified markers, increasing the detectable signal for the corresponding antigen. Antibodies persist in the circulation with half-lives of typically up to 30 days [18] and are more stable outside the body than other biomarkers. TA autoantibodies are...
detectable in archive material and do not require expensive or complex technologies for detection, and quantification platforms are already in common clinical use.

This review describes the immunological response of tumor cells thought to lead to the production of TA autoantibodies in vivo and current technologies for their detection and identification in vitro. Recent progress in this field, including growing evidence that TA autoantibody assays can potentially aid cancer diagnosis and treatment, is discussed. Finally, we summarize the requirements for translation of these biomarkers to clinical practice and predict their future utilization.

**In vivo Production of TA Autoantibodies**

**B Cells**

All immature B cells that bind 'self' antigens during maturation in the bone marrow undergo negative selection, including clonal deletion, and are removed from the cellular repertoire. This results in an immunological tolerance to self where mature B cells should bind only negative selection, including clonal deletion, and are removed from the cellular repertoire. This promotes the generation of cell variants with increasing capacity to survive an immune attack. It is the longest of the three phases and may occur over a period of many years.

(iii) Escape results when the host immune defenses are breached and the tumor expands and proliferates in an uncontrolled manner [137].

**Tumors** represent outgrowths of a single abnormal cell. It is now generally accepted that the immune system recognizes these cell abnormalities early in their development and initiates a response [133,134] that has three phases.

(i) Immunosurveillance/elimination is where the first few transformed cells are detected and bound by natural killer (NK) cells that secrete cytokines to recruit other immune cells [139]. The ensuing destruction of some transformed cells and the uptake and processing of their fragments by the recruited cells initiates the adaptive immune response. A cascade of events further promotes the activation of innate immunity and supports the expansion and production of T and B cells, the latter of which secrete antibodies [136].

(ii) Equilibrium results when tumor cells with a non-immunogenic phenotype escape elimination and are allowed to grow. This promotes the generation of cell variants with increasing capacity to survive an immune attack. It is the longest of the three phases and may occur over a period of many years.

(iii) Escape results when the host immune defenses are breached and the tumor expands and proliferates in an uncontrolled manner [137].

**Box 1. Cancer Immunooediting**

Tumors represent outgrowths of a single abnormal cell. It is now generally accepted that the immune system recognizes these cell abnormalities early in their development and initiates a response [133,134] that has three phases.

(i) Immunosurveillance/elimination is where the first few transformed cells are detected and bound by natural killer (NK) cells that secrete cytokines to recruit other immune cells [139]. The ensuing destruction of some transformed cells and the uptake and processing of their fragments by the recruited cells initiates the adaptive immune response. A cascade of events further promotes the activation of innate immunity and supports the expansion and production of T and B cells, the latter of which secrete antibodies [136].

(ii) Equilibrium results when tumor cells with a non-immunogenic phenotype escape elimination and are allowed to grow. This promotes the generation of cell variants with increasing capacity to survive an immune attack. It is the longest of the three phases and may occur over a period of many years.

(iii) Escape results when the host immune defenses are breached and the tumor expands and proliferates in an uncontrolled manner [137].

**Clinical validation:** how accurately a test detects the analyte.

**Antigen:** substances recognized by the immune system triggering a host immune response including antibody generation.

**Autoantibodies:** when the immune surveillance system no longer recognizes native molecules as self, an immune response is initiated including the production of antibodies against host substances, termed autoantibodies. This pathological disturbance of self-tolerance can be indicative of autoimmune disease and has been identified in other diseases, including cancer.

**Autoantigen:** host proteins or substances recognized as non-self, initiating a host immune response.

**Autoimmune disorder:** a disease in which the body produces antibodies that attack its own healthy tissues leading to the deterioration and in some cases the destruction of such tissue.

**Clinical utility:** whether a test improves current patient management (diagnostic, treatment).

**Epitope:** the part of an antigen molecule to which an antibody binds.

**False-positive rate:** the proportion of negatives falsely identified as positives (i.e., 1-specificity).

**Immunogenic:** relating to or denoting substances able to produce an immune response.

**Indolent:** causing little or no pain, slow growing, and unlikely to shorten life expectancy.

**Major histocompatibility complex (MHC):** a large family of proteins divided into two subgroups: class I and class II. Host and non-host proteins are constantly degraded in the cell and the resultant peptides are bound by these proteins and transported to the cell surface for T cell detection. The diversity of peptides bound is achieved as MHC genes are polygenic, their expression is codominant (using both alleles), and gene variants are highly polymorphic, predominantly in the peptide- and T cell receptor-binding regions.
mutations, frame shift, or coding sequence extension or truncation [37]. This can also lead to protein misfolding [38], potentially altering stability and location and therefore detection by the immune system [39]. Most genetic mutations related to cancer are somatic rather than hereditary and can be oncogenic as well as immunogenic. (iii) Cancer-testis antigens are expressed in many tumors of various histological types, having no expression in normal tissues with the exception of male germine cells and trophoblastic cells (both of which do not produce MHC and therefore cannot present antigens to T cells [40,41]). Over 60 genes with similar patterns of expression have been identified and, despite progress, the functions of most of these remain unclear although they are expressed in a substantial fraction of tumors [42].

Some TA autoantigens have lower tumoral specificity than tumor-specific antigens as they are also present, albeit at lower levels, in normal tissues (Table 1). They include differentiation antigens and proteins that are over expressed in tumors. Differentiation antigens are expressed in the tumor and in the normal tissue of origin only; for example, prostate specific antigen (PSA) in prostate cancer and alpha-fetoprotein (AFP) in hepatocellular cancer [43]. A threshold level of antigen is required for T cell recognition and if tumor cells are above this threshold, while normal cells are not, a specific antitumoral response could occur [44]. Nucleic acids or nucleic acid-like structures often form targets for the immune system in autoimmune diseases and paraneoplastic disorders, which are often associated with cancer and so also belong to this antigen class [45].

**TA Autoantibodies**

TA autoantibodies have been described in most common human malignancies in both early- and late-stage disease and can have both diagnostic and prognostic relevance [43,46]. The mechanisms of their secretion have not been clearly determined but their production is thought to occur due to increased immunogenicity of the corresponding antigen. This is thought to occur through mechanisms such as mutation, resulting in, for example, the generation of new or altered epitopes, abnormally high expression levels (leading to loss of tolerance), or exposure of antigens usually expressed in immune-privileged sites (e.g., fetal proteins). These mechanisms could enable extracellular and intracellular host proteins to be detected by B cells and antibodies. A recent study estimated that most TA autoantigens are overexpressed or mutated proteins (42% cytoplasmic, 26.1% nucleus derived, 21.4% membrane bound, and 10.3% extracellular) [46]. The specificity of TA autoantibodies for intracellular molecules rather than their more usual cell surface targets is not fully understood. However, there is evidence that a proinflammatory environment, as in cancer, can enhance vascular permeability for autoantibodies and cytoplasmic proteins [47,48].

TA autoantibody epitopes can be highly conserved. Many sequentially discontinuous B cell epitopes have also been identified, indicating that the epitopes are frequently dependent on conformation [49,50]. This could be characteristic of epitopes situated at functional sites of the antigen and so TA autoantibodies would inhibit antigen function on binding to the antigen, as observed in *in vitro* studies [51–58]. Interestingly, TA autoantibodies can often bind both the unmodified and the modified form of the protein that elicited their production, probably due to epitope spreading [59]. Mainly TA autoantibodies of Immunoglobulin G class are detected in patient sera [46], indicating a strong secondary immune response. There is also evidence that isotypes can switch during cancer progression from inflammatory to anti-inflammatory effector functions [60].

The role of TA autoantibodies is largely undefined but they are likely to contribute to the immune response to cancer in several ways, including the typical antibody effector
functions (Box 2). Individuals with autoimmune disorders can be at higher risk for development of cancer [61,62] and many TA autoantigens are oncogenic with associated titers of TA autoantibodies. These findings further corroborate the hypothesis that TA autoantibodies are immunological biomarkers of aberrant cellular mechanisms associated with tumorigenesis [18,31,63,64].

**Box 2. Humoral Immunity**

The humoral response provided by B cells works in combination with the cellular response of T cells to form the antigen-specific adaptive immune response (Figure 3). Cytotoxic T cells directly destroy antigen-expressing target cells, helper T cells regulate the strength, duration, and efficacy of the immune response, and B cells produce antigen-specific antibodies [138].

Antibodies are immunoglobulins produced exclusively by B cells and divided into five classes in mammals. All contribute to humoral immunity, directed principally at extracellular antigens. Each B cell produces an antibody with a single antigen specificity. The B cell receptor (BCR) binds the antigen either free floating in the lymph or cell-surface bound. Antigens can include proteins, polysaccharides, and nucleic acids. The BCR and the resultant antibodies bind whole proteins/molecules and do not require prior antigen processing, unlike T cells, which will only bind peptides processed and bound on the surface of antigen-presenting cells. Proteins bound by the BCR are ingested and processed, resulting in extracellular presentation of peptides (antigen presentation) by the B cell. Helper T cells activated by the same antigen will bind to the extracellular B cell peptide complex. The T cell in turn will activate the B cell via excretion of various cytokines. This activation promotes B cell proliferation as well as sustaining T cell growth and differentiation. Once activated B cells proliferate, leading to the differentiation of plasma and memory B cells.

Plasma cells secrete different isotypes of immunoglobulins with distinct functions; G, M, and A constitute the majority. Hypermutation in combination with isotype switching (mainly from M to G isotype) results in a stronger and more sustained immune response. B cell activation without T cell help can occur for antigens including polysaccharides and nucleic acids, generating class M antibodies, which tend to have lower affinity and less versatility than antibodies of the Immunoglobulin G class. Class A antibodies are found in mucosal areas only.
In addition, circulating antibodies bind antigen or antigen-bound cells allowing a variety of effector mechanisms (isotype dependent). These include neutralization (antigen binding prevents detrimental activity of the antigen or the cell it is bound to), opsonization (recruitment of phagocytes to destroy antigen-bound cells), complement-dependent cytotoxicity (resulting in cell lysis and phagocyte recruitment), antibody-dependent cell-mediated toxicity (NK cell recruitment destroys antigen-bound cells), and antigen presentation (enhanced antigen presentation to T cells, inducing cytotoxic T cell activity).

**Biomarker Development**

**Discovery**

Discovery techniques include serological analysis of tumor antigens by recombinant cDNA expression cloning (SEREX) [65], phage display, serological proteome analysis (SERPA) [66], multiple affinity protein profiling (MAPPING) [67], and protein microarrays [68–70]. It is crucial, however, that potential biomarkers be validated by independent immune techniques and their performance be established with independent and well-matched case-control cohorts for clinical use.

It has been observed in systemic autoimmunity that some autoantibody profiles are unique and others are shared between disorders, acting as immune signatures and proven diagnostic tools [71,72]. Many assays to measure autoantibodies have been developed with ELISA being the gold standard, although more economical, higher-throughput methods are emerging [73–75].
However, in contrast to what occurs in autoimmune diseases, assays that measure a single TA autoantibody appear to have little diagnostic use for cancer due to their low frequency, as they rarely exceed $10^{-30}\%$ in a patient population [49,76]. This is probably due to diverse patient immune responses resulting from the highly heterogeneous nature of cancer and inherent genotypic (and epigenetic) variations within a population. The heterogeneity provided by the measurement of panels of TA autoantibodies is therefore likely to lead to better diagnostic performance than single markers. However, identifying such a panel for a specified clinical utility will require balancing the increase in positivity achieved by increasing panel size with the reduction in clinical specificity that each biomarker may contribute to the test performance.

### Validation

Measurement of TA autoantibodies may be of clinical benefit in the early diagnosis of various solid tumors including breast cancer [50,77,78], lung cancer [79–82], hepatocellular carcinoma [83,84], esophageal cancer [85], gastric cancer [86], colorectal cancer [87], ovarian cancer [88], thyroid cancer [89], and prostate cancer [90]. These publications focus on the utility of TA autoantibody panels and while research in this area has accelerated, virtually all remain to be translated to the clinical setting.

A clinically useful serological assay should be reasonably simple to perform, highly robust, reproducible, and cost effective both in production and in the appropriate health economic

### Table 1. Summary of TA Autoantigens That Elicit a Humoral Immune Response

<table>
<thead>
<tr>
<th>Antigen class</th>
<th>Example</th>
<th>Protein role</th>
<th>Cancer incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific antigens</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral</td>
<td>Epstein-Barr</td>
<td>Virus</td>
<td>Lymphoma, nasopharyngeal, stomach</td>
</tr>
<tr>
<td></td>
<td>Human papilloma</td>
<td>Virus</td>
<td>Cervical, head and neck</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B and C</td>
<td>Virus</td>
<td>Hepatocellular</td>
</tr>
<tr>
<td>Mutated</td>
<td>p53</td>
<td>Tumor suppressor, oncogenic</td>
<td>Lung, breast, ovary, hepatocellular, colon</td>
</tr>
<tr>
<td></td>
<td>Ras</td>
<td>Cell signaling, oncogenic</td>
<td>Pancreatic, hepatocellular</td>
</tr>
<tr>
<td></td>
<td>c-myc</td>
<td>Transcription factor, oncogenic</td>
<td>Cervical, lymphoma, colon, lung, breast, stomach</td>
</tr>
<tr>
<td>Ectopically expressed</td>
<td>MAGE</td>
<td>Cancer-testis antigen</td>
<td>Lung, breast, ovary, hepatocellular, colon</td>
</tr>
<tr>
<td></td>
<td>NY-ESO-1</td>
<td>Cancer-testis antigen</td>
<td>Lung, breast, ovary, hepatocellular, colon</td>
</tr>
<tr>
<td></td>
<td>LAGE</td>
<td>Cancer-testis antigen</td>
<td>Lung, breast, ovary, hepatocellular, colon</td>
</tr>
<tr>
<td></td>
<td>SSX</td>
<td>Cancer-testis antigen</td>
<td>Lung, breast, ovary, hepatocellular, colon</td>
</tr>
<tr>
<td><strong>Associated antigens</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td>PSA</td>
<td>Peptidase enzyme</td>
<td>Prostate</td>
</tr>
<tr>
<td></td>
<td>AFP</td>
<td>Unknown</td>
<td>Hepatocellular</td>
</tr>
<tr>
<td>Overexpressed</td>
<td>SOX2</td>
<td>Transcription factor, stem cell</td>
<td>Lung</td>
</tr>
<tr>
<td></td>
<td>MUC1</td>
<td>Cell signaling, pathogen binding</td>
<td>Breast, ovary, colon, prostate</td>
</tr>
<tr>
<td></td>
<td>Cytokeratins</td>
<td>Scaffold proteins</td>
<td>Lung, hepatocellular, bladder, pancreas</td>
</tr>
<tr>
<td></td>
<td>HER2/neu</td>
<td>Cell proliferation, oncogenic</td>
<td>Breast</td>
</tr>
<tr>
<td>Nucleic acids</td>
<td>DNA, RNA</td>
<td>Autoimmune disorders</td>
<td>Lung, colon</td>
</tr>
</tbody>
</table>

However, in contrast to what occurs in autoimmune diseases, assays that measure a single TA autoantibody appear to have little diagnostic use for cancer due to their low frequency, as they rarely exceed 10–30% in a patient population [49,76]. This is probably due to diverse patient immune responses resulting from the highly heterogeneous nature of cancer and inherent genotypic (and epigenetic) variations within a population. The heterogeneity provided by the measurement of panels of TA autoantibodies is therefore likely to lead to better diagnostic performance than single markers. However, identifying such a panel for a specified clinical utility will require balancing the increase in positivity achieved by increasing panel size with the reduction in clinical specificity that each biomarker may contribute to the test performance.
setting. For high-volume testing such as screening, large amounts of reagents including capture ligands are required and the batch-to-batch reproducibility of these reagents is of paramount importance. The stability of all reagents over the shelf-life of the test has to be demonstrated and quality control measures need to be in place to monitor accuracy and reproducibility on an ongoing basis [91]. Only if this is possible can a patient's results be reliably compared from one measurement to the next.

Techniques for detecting TA autoantibodies "capture" them from sera utilizing the respective TA autoantigen usually bound to a solid support, which is easy to perform and minimally invasive. The TA autoantigen proteins are typically produced by a recombinant organism or cell culture. The choice of system should not be underestimated as it can greatly influence batch size, purity, and the processing of target proteins, which in turn can affect reproducibility and assay performance [92].

The general lack of clinical translation of TA autoantibody biomarkers could be due to research studies not accurately reflecting clinical need in terms of sample set size (the power of the studies) or population demographics. Consequently, performance is lost during confirmatory studies on larger and/or more suitable sample sets. TA autoantibody levels can also increase with age [93,94], a factor that is not always addressed (control cohorts need to be age matched).

The path to developing any biomarker assay from the research laboratory to a test for use in the clinic is long and fraught with technical and regulatory challenges [91]. A recent review suggested a set of guidelines for new biomarkers [95] that include analytical validation, clinical validation, clinical utility demonstrated from performance data preferably in a clinical trial setting, and regulatory approval. Despite a clear and unmet need, few biomarker assays have been incorporated into clinical practice for early cancer detection. A lack of scrutiny of biomarker publication by journals and a lack of evidence of analytical validity and clinical utility has exacerbated this problem [96], as well as insufficient investment [97]. Implementation of future diagnostic tests could be improved by reforming the regulatory review of tests, increasing reimbursement of tests with proven clinical utility, and adherence to evidence-based recommendations for test use [98].

In the case of lung cancer, health economic modeling for a TA autoantibody test has been conducted and is cost effective [91,99], with analytical and clinical validation studies also published. Therefore, TA autoantibody tests appear to be a long way along the biomarker development path and may prove to be of future clinical utility in a range of solid tumors.

**Biomarker Utility**

**Early Detection**

Early detection continues to represent one of the most promising approaches to decrease the rising worldwide burden of cancer. Despite increased focus in this area, a significant proportion of individuals are diagnosed late, presenting with advanced disease at which time the opportunities for successful treatment are drastically reduced and treatment costs significantly increased.

Population-wide screening can be an effective method for early detection of cancer but only where early diagnosis will lead to improved outcomes, where a clearly defined high-risk screening population exists, where no alternative efficient and cost-effective screening tests are available, and, finally, where tumors can be subsequently localized.

Currently, imaging techniques remain the gold standard for cancer detection and localization; however, they are generally unable to detect the early development of malignant cells.
Furthermore, many imaging techniques are unsuitable for population-wide screening due to their expense, required operator skill, and, for some, associated radiation exposure. This is especially true for countries where health budgets are constrained.

Cancer screening programs for defined risk groups do already exist for some cancers; for instance, mammography for breast, CT for lung, and occult fecal monitoring for colorectal cancer. However, a proportion of individuals remain unwilling to participate and some may find a blood test more acceptable. Also, within such programs a considerable proportion of cancers can be missed depending on the sensitivity of the screening method employed.

TA autoantibodies have been discovered in early- and late-stage disease for most common human malignancies, being measurable up to 5 years before clinical symptoms [14–17]. Recent work described their use as aids for early cancer identification with encouraging results [80,100]. These studies investigate their use in lung cancer, where there are clear and defined risk groups. This is also the case for several other cancers, including lung, breast, ovarian, colorectal, prostate, and hepatocellular, in which TA autoantibodies have been detected. This suggests that TA autoantibody measurement may provide an opportunity for early cancer detection for a wide range of malignancies. TA autoantibody biomarkers could complement existing screening modalities to improve performance and detect more cancers, especially for early diagnosis where the disease is at a curable stage. TA autoantibody detection does not require specialist equipment, personnel, or premises and therefore testing is cheap and easy, making its use especially attractive when health budgets are limited.

**Patient Stratification**

Imaging techniques can be highly sensitive and specific for the detection of abnormal masses but generally are unable to distinguish malignant from benign disease. This can result in poor specificity and high false-positive rates. The follow up to diagnose malignancy can be expensive and lengthy and, more importantly, can pose a significant risk to patients due to complications (biopsy procedures can result in complications for the patient and can have associated mortality rates as a result [101]). A highly specific tumor biomarker could help to supplement this poor specificity and be used in conjunction with imaging to improve screening accuracy. If a patient’s cancer risk could be more accurately estimated, higher-risk patients could receive more active follow up and those with a lower risk would not. Alternatively, a biomarker could be used as a prescreening tool to prioritize access to screening for those with a positive result and at higher risk. This approach could be extended to medium-risk populations. Either of these approaches would significantly aid stratification of patient management and reduce costs.

The presence of some TA autoantibodies has been correlated with indolent tumor growth and increased patient survival [102]. Imaging tests are generally unable to distinguish indolent from malignant tumors, which can result in high over-diagnosis rates and in turn lead to overtreatment. This not only results in unnecessary patient harm, both mentally and physically, but is also a huge financial burden. A marker that could aid the identification of indolent disease would hugely benefit cancer surveillance and screening programs, with these patients receiving less frequent surveillance.

**Therapeutic**

TA antigen levels are used to monitor disease progression and recurrence and can also be used as predictors of responses for some tumor types. TA autoantibody levels can fluctuate during disease progression [103,104] and could be utilized in a similar way. Raised levels of TA autoantibodies indicate not only that the corresponding antigen is involved in tumorigenesis but that it is inducing an immunogenic response. An increase in TA autoantibody levels correlates
with complete tumor remission for some anticancer therapies [102,105]. The detection of some TA autoantibodies also correlates with different disease subtypes. For example, in lung cancer some TA autoantibodies are more sensitive for small cell while others are more sensitive for non-small cell cancer [100,102]. Measuring TA autoantibody levels could therefore enable the identification of populations that may respond well to cancer therapies. Monitoring their levels could also help in monitoring therapy effectiveness during and after treatment.

Increasing evidence suggests that adaptive immunity contributes to the long-term clinical benefits of chemotherapy and radiotherapy. Various immunotherapies are in development that attempt to harness the adaptive immune system to reject tumors or to prevent recurrence, but work to date suggests that success is limited to patients without an immunosuppressive tumor environment [106]. Tumors can proliferate by utilizing checkpoint inhibitors to downregulate immune effector functions and allow evasion of immune attack and evasion of immune therapies. There is currently growing interest in therapies that target these checkpoint inhibitors, especially towards PD1 and CTLA-4 [107]. Using these targets in combination with other therapies is proving successful, sometimes providing complete eradication of disease in late-stage patients, although patient responses are typically on the order of 20–60%. The presence of some immune inhibitory molecules in tumor biopsies seems to be a predictor of response for several tumor types. However, recent studies suggest that this ability to predict the efficacy of combination therapies may be limited [108,109]. Predicting optimal immunotherapy combinations accurately and confirming clinical response will require the identification of reliable surrogate biomarkers [107]. It is increasingly recognized that the presence of a lymphocytic infiltrate in a tumor often correlates with improved survival, and the role of the B lymphocyte in this response is accepted, as discussed above. Autoantibody measurements have shown correlation with positive response to anti-CTLA4 therapy [108]. It is also well documented that PD1 is strongly involved in the regulation of B cells [110,111]. There is also evidence that TA autoantibodies may be useful for synthetic lethality treatment of cancer, with promising agents especially in combination with other therapeutics including checkpoint blockade agents [112,113].

Further studies of the interaction of B cells and autoantibodies with tumor and other immune cells should facilitate better understanding of the tumor–host interaction. This knowledge could help in the design of novel and more effective immunotherapies for cancer. TA autoantibody measurement is inexpensive, fast, and easy to perform compared with the biopsies and cell assay techniques that are currently used to assess patients’ immune profiles and response to immune therapies.

**TA Autoantibodies in Lung Cancer: Case Study**

Lung cancer is a prime example of where combining biomarkers and imaging techniques could maximize patient benefit in a cost-effective manner. In 2011 the National Lung Cancer Screening Trial (NLST) in the USA demonstrated a 20% reduction in lung cancer mortality (due to the identification of earlier-stage disease) with low-dose CT screening of high-risk populations [2,114]. These data resulted in a recommendation to introduce population screening in the USA of high-risk-group individuals; that is, those between 55 and 80 years of age with a smoking history of greater than 30 pack-years who currently smoke or who have quit within the past 15 years [115]. However, about 70% of all cases of lung cancer in the USA fall outside high-risk groups [116]; therefore, a test that could maximize the benefits of a CT screening program by improving pre-CT patient risk stratification would have utility.

The presence of a TA autoantibody response has been documented in lung cancer and demonstrated to be present irrespective of disease stage [117] (Figure 2). It may, therefore, be an ideal test to add to CT, but its utility in this area remains to be demonstrated in retrospective
clinical studies. To date there are two TA autoantibody assays with significant clinical and analytical validation data published (Table 2), both of which aid lung cancer detection. The first of these uses a panel of seven TA autoantibodies [100] (p53, NY-ESO-1, GBU4-5, MAGE A4, SOX2-B, and Hu-D [100]). Vertical dashed line represents sensitivity at 40% (all stages of lung cancer). NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; LD, limited disease; ED, extensive disease; early, stage I and II NSCLCs and LD SCLCs; late, stage III and IV NSCLCs and ED SCLCs. The number of samples in each group is represented in parentheses.

Table 2. Validated TA Autoantibody Tests in Current Clinical Use

<table>
<thead>
<tr>
<th>Product name</th>
<th>TA autoantibody marker</th>
<th>Use</th>
<th>Manufacturer</th>
<th>Platform</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Release date</th>
</tr>
</thead>
<tbody>
<tr>
<td>EarlyCDT-Lung</td>
<td>NY-ESO-1</td>
<td>Lung cancer diagnostic aid</td>
<td>Oncimmune Ltd</td>
<td>Semiautomated ELISA</td>
<td>41%</td>
<td>93%</td>
<td>2009 (limited)</td>
</tr>
<tr>
<td></td>
<td>p53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2012 (full)</td>
</tr>
<tr>
<td></td>
<td>CAGE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hu-D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAGE A4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SOX2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GBU4-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAULA’s Test</td>
<td>NY-ESO-1*</td>
<td>Non-small cell lung cancer diagnostic aid</td>
<td>20/20 Gene systems</td>
<td>Luminex xMAP</td>
<td>71–77%</td>
<td>80%</td>
<td>2012 (limited)</td>
</tr>
</tbody>
</table>

*CA-125, CEA, and CYFRA 21-1 antigen markers also.
This test has been clinically validated on three discrete and independent high-risk case-control populations, the third of which was blinded \[117, 118\], and four post-validation cohorts were also independent \[120\]. All cohorts were appropriately matched for age, sex, and smoking history. The test also identified lung cancer in a high-risk group of 1600 individuals whose physician ordered the test during clinical practice, at similar levels to those determined in case control studies \[121\] and with a failure rate (invalid test result) of <1%. The test consistently identifies lung cancer with 92% accuracy (compared with 50% for CT) with a sensitivity of ~40% for all stages and types (small cell and non-small cell) of lung tumors and a specificity of 93% for all cohorts, showing the robustness and reproducibility of this assay system (Figure 2) \[100\]. It was launched commercially in 2012 and had some clinical acceptance for the early detection of lung cancer \[121, 122\] and for follow up of patients who had a positive result on CT (Table 3) \[79\].

The second analytically validated test (Table 2) is for non-small cell lung cancer and detects a panel of three TA antigens (CEA, CA-125, and CYFRA 21-1) and one TA autoantibody marker (NY-ESO-1) \[80\]. This test is performed on the Luminex xMAP technology platform using beads coated in either commercially available capture antibodies (for the antigens) or commercially available capture antigen (NY-ESO-1) utilizing a biotin-streptavidin detection system. High and low controls on each plate were utilized to estimate interassay precision, which met precision industry standards. A multiple of medium (MoM) algorithm was used to predict lung cancer risk for samples by converting data for each biomarker to a multiple of a population median value by dividing by the median value of the control group \[80\]. This model was validated on a cancer cohort matched to a healthy cohort and using AUC ROC analysis to determine the sensitivity and cut-off point value at 80% specificity. Both the training and the validation cohort were independently sourced and approved by an independent review board. This panel was able to discriminate cases from controls with 72.7% and 82.8% sensitivity for early- and late-stage non-small cell lung cancer, respectively. However, it was also noted that the current form of this test may result in substantial positivity for benign conditions (11%) and so it may not be suitable for patient stratification of CT-screened patients, but it could be used instead to predict lung cancer risk and enrich the population that is offered CT screening. Failure rates are not reported and this test seems to be in an earlier stage of development than the previous one, as the authors discuss further optimization and the need to investigate more extensively the clinical validity and utility.

Another issue revealed by the NLST study is the very high rate of false-positive test results (96.4%) \[2\] for CT screening, predominantly due to benign pulmonary nodules. The Fleischner Society recommends patient follow up for pulmonary nodules identified by CT \[123\] and balancing early detection and treatment against overuse of invasive procedures. The potential for malignancy increases with nodule size, which tends to result in later-stage detection of lung cancer as the nodule grows \[124\]. It is also worth noting that in most countries the follow up of so many false positives is beyond available budgets and that lung biopsy diagnosis is not without significant complications for the patient, recently estimated to be as high as 20% \[123\]. A biomarker test that discriminates benign nodules from malignant ones, especially for smaller

<table>
<thead>
<tr>
<th>Screen</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EarlyCDT-Lung</td>
<td>41</td>
<td>93</td>
<td>13(c)</td>
<td>92</td>
</tr>
<tr>
<td>Low-dose CT</td>
<td>93</td>
<td>73</td>
<td>3.8(c)</td>
<td>50</td>
</tr>
</tbody>
</table>

\(a\) CT cohort: 55–74 years old with 30-pack-year smoking history; EarlyCDT-Lung cohort: lung cancer prevalence of 2.4%.
\(b\) Stage independent.
\(c\) Proportional to nodule size.
nODULES, could be powerful in maximizing limited diagnostic resources. It was recently reported that the seven-panel test discussed above may also be clinically useful in patient stratification by nodules when combined with CT [79]. The high specificity and positive predictive value (PPV) make the test a potentially complementary tool to CT for early lung cancer detection, where patients with a pulmonary nodule and a positive test have an increased probability of malignancy.

There are three ongoing prospective trials investigating the clinical utility of autoantibody biomarkers and their use in a clinical setting. All trials evaluate the first test. The ECLS study, the largest (12 000 high-risk subjects) randomized trial for early detection of lung cancer using biomarkers ever conducted, is currently being run by the NHS in Scotland [125] to evaluate this TA autoantibody panel as a pre-CT screening tool. The primary end point is the identification of more early-stage lung cancers in the test arm compared with the standard of care. Early results demonstrate a cancer detection rate of 81% with a specificity of 91% as expected [126], with the trial due to report in 2018. The same test is being evaluated in two further trials to assess clinical utility and health economic benefit as a lung cancer clinical tool in combination with CT [127] to aid pulmonary nodule management. These trials are expected to report at the end of 2017 and 2018.

Concluding Remarks

We stand at a relatively early point in the development of TA autoantibody use in cancer. Further investigation into their exact role and method of production by the immune system during tumorigenesis is vital for a more complete understanding of the immune response to cancer. It would also contribute to the realization of the full potential of TA autoantibodies as clinical aids (see Outstanding Questions).

Results of ongoing clinical trials for lung cancer are set to provide much-needed information and determine the true clinical utility of TA autoantibodies. Once this is achieved for lung cancer it would be envisaged that tests for all solid tumors would follow. Patients with cancers that present predominantly at a late stage with high mortality rates, and where difficulties in differentiating malignant and benign masses exist, are likely to have the most to gain from the application of TA autoantibodies to high-risk individuals; for example, ovarian cancer in women over 50 years of age. Access to appropriate sample cohorts is likely to be the limiting factor to progress due to the low incidence and late-stage presentation of some cancers. Access to large cohorts of early-stage samples is vital to prove a test’s utility for early-stage disease. Greater collaboration and access to independent cohorts would greatly accelerate this process.

Increasing TA autoantibody assay performance further may be possible if a patient’s baseline measurement in early life is recorded and any changes are monitored over time. This should mitigate problems created by ‘noise’ when using population-based cut offs and is being proposed across several other standard cancer markers [128,129]. Additionally, recent studies in ovarian, hepatocellular, and prostate cancers suggest that combining TA autoantibody panels with existing antigen biomarkers (CA125 [130], AFP [131], and PSA [132], respectively) could also improve performance. The non-small cell lung cancer test described earlier (PAU-LA’s Test; Table 2) detects a panel of three tumor antigens and one TA autoantibody marker, with the latter significantly improving detection of early-stage disease [80].

While debate remains regarding over-diagnosis and increased concern about the costs incurred following false-positive tests for current cancer screening and surveillance programs, any test that can detect cancer at an early and curable stage and distinguish malignant from benign disease is worth further investigation. TA autoantibodies have the potential to provide...
innovative tools not only for screening but for patient stratification, treatment monitoring, and recurrence of all solid tumors. It is predicted that their use will significantly contribute to the combinatorial and personalized approaches currently gaining momentum in cancer surveillance and treatment programs.

**Disclaimer Statement**
I.K.M. and C.B.P-K are employed by Oncimmune Ltd.

**References**

52. Shero, J.H. et al. (1986) High titers of autoantibodies to topoisomerase I (Scl-70) in sera from scleroderma patients. Science 231, 737–740
53. Guldener, H.H. et al. (1986) Scl 70 autoantibodies from scleroderma patients recognize a 95 kDa protein identified as DNA topoisomerase I. Chromosoma 94, 152–158
58. Shero, J.H. et al. (1986) High titers of autoantibodies to topoisomerase I (Scl-70) in sera from scleroderma patients. Science 231, 737–740
59. Guldener, H.H. et al. (1986) Scl 70 autoantibodies from scleroderma patients recognize a 95 kDa protein identified as DNA topoisomerase I. Chromosoma 94, 152–158
64. Shero, J.H. et al. (1986) High titers of autoantibodies to topoisomerase I (Scl-70) in sera from scleroderma patients. Science 231, 737–740
65. Guldener, H.H. et al. (1986) Scl 70 autoantibodies from scleroderma patients recognize a 95 kDa protein identified as DNA topoisomerase I. Chromosoma 94, 152–158