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REVIEW

Clinical utility of anti-*p53* auto-antibody: Systematic review and focus on colorectal cancer

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Abstract

Mutation of the *p53* gene is a key event in the carcinogenesis of many different types of tumours. These can occur throughout the length of the *p53* gene. Anti-*p53* auto-antibodies are commonly produced in response to these *p53* mutations. This review firstly describes the various mechanisms of *p53* dysfunction and their association with subsequent carcinogenesis. Following this, the mechanisms of induction of anti-*p53* auto-antibody production are shown, with various hypotheses for the discrepancies between the presence of *p53* mutation and the presence/absence of anti-*p53* auto-antibodies. A systematic review was performed with a descriptive summary of key findings of each anti-*p53* auto-antibody study in all cancers published in the last 30 years. Using this, the cumulative frequency of anti-*p53* auto-antibody in each cancer type is calculated and then compared with the incidence of *p53* mutation in each cancer to provide the largest sample calculation and correlation between mutation and anti-*p53* auto-antibody published to date. Finally, the review focuses on the data of anti-*p53* auto-antibody in colorectal cancer studies, and discusses future strategies including the potentially promising role using anti-*p53* auto-antibody presence in screening and surveillance.

Key words: *p53* gene; *p53* mutation; Anti-*p53* auto-antibody; Cancer; Colorectal cancer

Core tip: Anti-*p53* auto-antibodies are commonly produced in response to *p53* mutations. Anti-*p53* auto-antibody titres generally increase with tumour load, but not all patients who are initially sero-negative develop an auto-antibody response despite disease progression and metastases. Conversely, sero-positive patients do not lose their anti-*p53* auto-antibodies despite the cancer being completely excised. In general, cancers with the highest *p53* mutation rate, *e.g.*, oesophageal and ovarian, demonstrate the highest anti-*p53* auto-antibody rates; conversely, melanoma and testicular carcinoma with the lowest mutation rate have the lowest serum auto-antibody levels. Measurement of anti-*p53* auto-antibodies may be useful in screening or monitoring for tumour recurrence.

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INTRODUCTION

The *p53* gene is located on the distal band of the short arm of chromosome 17p13[1,2]. It consists of approximately 20000 base pairs spread over 11 exons[2-5]. *p53* was initially discovered in 1979 as a protein binding to a viral

oncogene, Simian Vacuolating 40 (SV40) large T-antigen, and hence was thought to be an oncogene itself[6-8]. It has since been established that it has a critical role as a tumour-suppressor gene[9-11]. *p53* inactivation predisposes cells to malignant transformation in rodent models and in human clinical diseases such as Li-Fraumeni syndrome; the latter being characterized by germline mutations of *p53*[12-14]. The tumour suppressive role of *p53* is so crucial that it is referred to as "the guardian of the genome"[15,16]. It is the most common mutation found in cancers and is present in half of all solid tumours thus emphasising its importance in protecting cells from carcinogenesis[3]. The frequency of mutation varies in individual cancers ranging from 5%-12% in cervical and haemopoietic malignancies to 40%-50% in colorectal and ovarian cancer[1,5]. Additionally, the remaining cancers with no detectable *p53* mutation are still thought to have dysfunctional *p53* caused by mechanisms other than mutation[9,17-20]. The most recent advances in colorectal cancer (CRC) treatment have been in the field of immunology with the use of antibodies against potent growth factors including epidermal growth factor receptor (EGFR) and vascular endothelial growth factors (VEGF)[21,22]. As such, *p53*, with its diverse immuno-regulatory role maintains a vital role in future of management of cancer and benign diseases. This review begins with the description of the normal *p53* gene function and mechanisms of *p53* inactivation in cancer, followed by a systematic review of the association between the anti-*p53* auto-antibody response and underlying *p53* mutations, and finally a clinical focus on the current evidence and potential future role of anti-*p53* auto-antibody in colorectal cancer.

p53 GENE AND GENE FUNCTION

p53 acts as a tumour suppressor by preventing propagation of defective cells. It is up-regulated by various upstream factors in response to cellular stress or damage such as DNA damage, hypoxia, telomere shortening and oncogenic stimulation or radiation[2,11]. Activated *p53* modifies downstream gene expression and co-factor transcription, which in conjunction with *p53*, lead to growth arrest (*e.g.*, *via* $p21^{WAF1}$) or apoptosis (*e.g.*, *p53*-upregulated modulator of apoptosis, PUMA)[19,23,24].

The *p53* gene encodes for a 393 amino-acid, 53 kDa, phospho-protein which is divided into 3 domains-an amino (-NH_2) terminal region (approximately amino acids 1-100), a central "core" domain (amino acids 100-300) and a terminal carboxyl (-COOH) region (amino acids 320-360)[25-27]. Almost all mutations are harboured in the central "core" which contains the DNA-binding regions. Thus *p53* dysfunction is most likely caused by mutations that alter DNA binding behaviour. However, most anti-*p53* auto-antibodies do not recognise central core mutations but rather recognise epitopes in the 2 terminal regions. An interesting observation is that these terminal regions which contain the least mutations are also found on the wild-type and the mutant p53 protein[25,27,28]. This suggests anti-*p53* is not only produced in response to mutation but also elevated levels of normal *p53*. This is discussed later (see Anti-*p53* Auto-antibody).

Wild-type p53 protein expression is intra-nuclear with a half-life of 5-30 min and is subject to complex regulation[29]. The most important regulator is thought to be Murine Double Minute 2 (Mdm2)[29,30]. Mdm2 is an ubiquitin-dependant E3 ligase which targets wild-type p53 protein for nuclear and cytoplasmic proteasome-mediated degradation[31,32]. When up-regulated *p53* binds to the Mdm2 promoter leading to increased levels of mdm-2 transcription; the *Mdm2* gene product then inhibits *p53* thus creating a negative feedback loop. This feedback process is complex and regulated by multitude of factors. Mdm-2 in itself is subject to modifications mainly self-degradation by (1) auto-ubiquitination[33,34]; (2) small Ubiquitin-like Modifier (SUMO)-ylation[35]; (3) acetylation[36]; (4) post-translational upstream kinases (*e.g.*, ATM kinase phosphorylation of Mdm2 and Mdm-X)[37-39]; (5) Mdm-2 in conjunction with Mdm-X (also known as Mdm-4) can form a Mdm2-MdmX-p53 complex which represses *p53* activity; and (6) Mdm-X can furthermore act independently of Mdm-2 and repress *p53*-bound chromatin, without Mdm2, most likely by direct binding. The combinations of these mechanisms, regulate *p53* accumulation in response to various cellular stresses (Figure 1).

p53 increases in response to cellular stress caused by a variety of insults including DNA damage, oncogene activation, ribosomal stress and hypoxia[11] by several mechanisms: (1) increased transcription; (2) increased intra-nuclear accumulation of active *p53*; (3) increased extra-nuclear export of Mdm-2[40,41]; (4) down-regulation of Mdm2-Mdmx which usually represses chromatin-bound-*p53*[24]; (5) various downstream post-translational modifications of both *p53* and its regulators *e.g.*, Mdm2[17]; and (6) raised cytosolic *p53*[42-45].

Active *p53* has tumour suppressive activity by causing cell cycle arrest, apoptosis and autophagy. Cell cycle arrest initially provides additional time for the cell to repair damaged DNA. However, cells unable to repair damage are directed towards apoptosis by shifts in the balance between pro-arrest, pro-autophagy and pro-apoptotic factors severe cellular damage, the cell pushed directly towards apoptosis by the relative increase in pro-apoptotic markers relative to cell-cycle arrest promoters[19].

Autophagy is an evolutionary catabolic process of mass lysosomal self-degradation of cytosol/proteins/organelles which are sequestered into a double membrane vesicle which is then fused with lysosomes for bulk degradation[42,43]. *p53* plays a dual role in activating and/or inhibiting autophagy by transactivating numerous autophagy regulators including mammalian target of rapamycin (mTOR)[46], activated protein kinase (AMPK) and tuberous sclerosis protein (TSC2)[43,47]. *p53* is also able to influence the decision between apoptosis and autophagy by selectively activating pro-autophagy proteins such as AMPK, death-associated protein kinase 1 (DAPK-1) and damage-regulated autophagy modulator (DRAM)[48]. Alternatively, *p53* also promotes apoptosis by activating pro-apoptotic markers such as B-cell Lymphoma 2

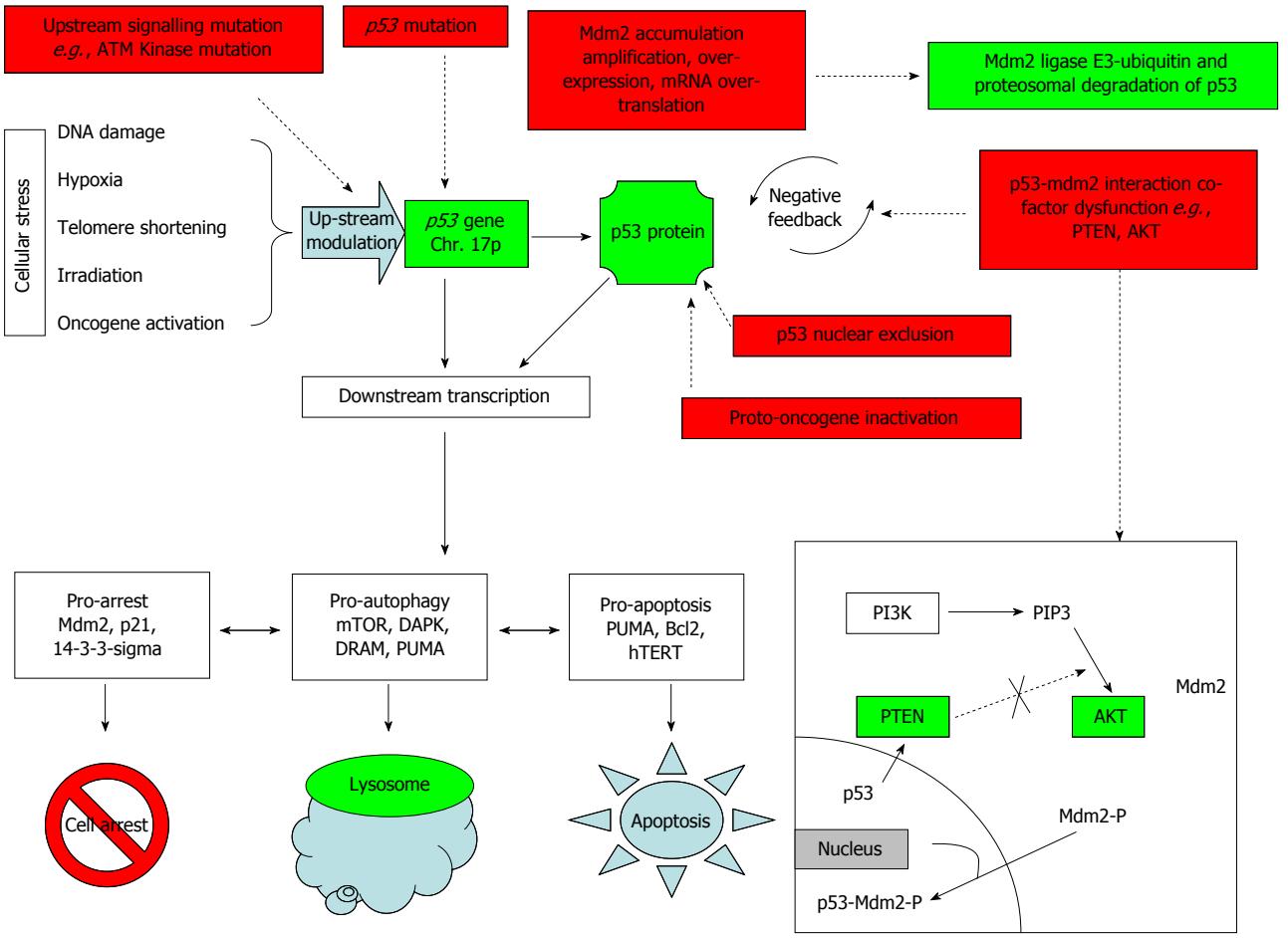

Figure 1 Normal *p53* function highlighted in green boxes and blue figures as described. Red boxes show mechanisms for inactivation; Normal *p53* function highlighted in green and blue figures. PI3K: Phosphatidylinositol-3-kinase; PTEN: Phosphatase and tensin homolog; AKT: Protein kinase B; ATM: Ataxia telangiectasia mutated kinase; mTOR: Mammalian target of rapamycin; DAPK: Death-associated protein kinase; DRAM: Damage-regulated autophagy modulator; PUMA: p53-upregulated modulator of apoptosis; hTERT: Human telomerase reverse transcriptase.

(Bcl-2), Bcl-2 associated death protein (BAD), Bcl-2 associated X-protein (BAX), *p53*-upregulated modulator of apoptosis (PUMA)[49] and autophagy inhibitors such as TP-53 induced glycolysis and apoptosis regulator proteins (TIGAR)[42].

Disruption of *p53* gene transcription function and subsequent production of an inactive mutant p53 protein allows cells to escape the cellular arrest/apoptosis controls. This allows unregulated propagation of abnormal cells and a predisposition to malignant transformation. It is important to be aware that *in vivo p53* behaviour can be different from *in vitro* response. This could be due to different stress types[50,51], cell types[52] and immune responses[24,53,54].

MECHANISMS OF *p53* INACTIVATION

The most common cause of *p53* inactivation is mutation which most frequently occurs within the *p53* core, and furthermore 70% occur at "hot spots"-amino acids 132-142, 151-159, 172-179, 237-249 and 272-286[26,55]. The International Agency for Research on Cancer (IARC) TP53 database similarly reports the most frequent *p53* mutations at codons 175, 245, 248, 249, 273 and 282; which is further corroborated by the UMD-TP53 mutation database, another international database that spans over 25 years[1,3,56]. The most common type of mutation is mis-sense (73%) followed by frame shift (9%), non-sense (8%), silent (4%) and others (6%)[1,3,57].

p53 is also inactivated by mechanisms other than *p53* gene mutation and are described below (Figure 1).

Proto-oncogene activation

Proto-oncogenes are normal proteins that become oncogenic with relatively minor modifications[6]. These proto-oncogenes are usually important cell cycle regulators (*e.g.*, $p14^{ARF}$)[58]. Human papilloma virus (HPV) 16/18 E6 protein which causes cervical cancer is able to inactivate *p53* without mutation. This explains the relatively low incidence of *p53* mutation in cervical cancer[59,60]. The SV40 large-T-antigen is another viral oncogene which is able to inactivate wild-type *p53*[61].

Mdm2 over-expression

Mdm2 is a negative regulator of *p53* and reduces the cell's ability to trigger the pro-arrest/apoptotic pathway in the event of cellular damage[62,63]. Mdm2 over-expression can occur by gene amplification, gene over-expression or

mRNA over-transcription[20,56]. Mdm2 over-expression is classically observed in soft tissue sarcomas[64,65]. Interestingly, instead of a decrease in p53 expression, the levels of both Mdm2 and p53 expression are increased. This suggests Mdm2 may have an additional *p53*-independent oncogenic mechanism (in addition to *p53* suppression by negative feedback) which can promote tumour growth.

Dysfunction of regulators of the p53-Mdm2 loop

Mutations of in the *p53*-Mdm-2 feedback loop such as AKT Kinase, Phosphatidylinositol-3-kinase (PI3K), Phosphatase and Tensin Homolog (PTEN) and Ataxia Telangiectasia Mutated (ATM)-Kinase can inappropriately influence levels of *p53* (see detailed description below). *p53* disruption has also been associated with inactivation of other tumour suppressors *e.g.*, BRCA1, Bcl-2, transforming growth factor (TGF)-β. AKT-kinase not only influences p53 levels but forms an apoptotic pathway with mTOR, an autophagy marker, in the PI3K/AKT/mTOR pathway demonstrating the complex interplay between *p53* and the relative levels of its regulators in deciding cell fate[19,42,43]: (1) AKT-kinase phosphorylates Mdm2 and induces migration of phosphorylated-Mdm2 into the nucleus where it inactivates *p53*. AKT over-expression has been shown to occur in cancer cells[66,67]; (2) PTEN is tumour-suppressive and activated in response to stress leading to *p53* up-regulation. Wild-type PTEN inhibits AKT-kinase phosphorylation of Mdm-2 and thus, intra-nuclear Mdm2 migration which suppresses *p53* activity[29,68,69]. In contrast mutated PTEN is unable to inhibit AKT-kinase which leads to continuous Mdm2- phosphorylation and Mdm-2 intra-nuclear migration leading to reduced *p53* tumour suppressive ability[69,70]; and (3) Cell stress (*e.g.*, irradiation) activates factors up-stream of *p53* such as ATM kinase and checkpoint Kinase-2[54,65]. Mutated ATM-kinase is unable to activate *p53* in response to radiation-induced stress.

Nuclear exclusion and cytoplasmic p53

Extrusion of *p53* into cytoplasm has been observed in certain tumours such as breast[71], colon[72], neuroblastoma[73] and malignant melanoma[74]. Nuclear extrusion prevents *p53* from performing its intra-nuclear interactions.

Gain of oncogenic function

Mutant *p53* has an impaired ability to regulate cell cycle which is referred to as "loss of function"[3,6,75]. In addition to this, mutated *p53* can also exhibit conformational changes which result in acquisition of new pro-oncogenic abilities; this is known as "gain of function". Such functions include increased transcription of tumour-promoting factors such as MYC and VEGF[76] and disruption of protective pro-apoptotic factors such as p73[7,77].

ANTI-*p53* AUTO-ANTIBODY

An anti-*p53* auto-antibody response was first reported by Crawford *et al*[78] in 1982 in 9% (14/155) of patients with breast cancer. Further interest in this anti-*p53* auto-antibody response declined due to the lack of accurate quantification methods and no observable clinical relevance. Research into the auto-antibody was invigorated in the 1990s when the critical role of *p53* gene in carcinogenesis was recognized. The exact cause of induction of anti-*p53* auto-antibody production is unknown but is thought to be associated with the presence of *p53* mutation and p53 protein over-expression.

An anti-*p53* auto-antibody is not normally produced wild-type p53 protein induces tolerance of the host[32,79]. In abnormal cells, mutant p53 protein is stabilised as discussed above which cause relatively high intra-nuclear p53 protein accumulation which then escapes into the cytoplasm. The resulting high cytoplasmic p53 levels increase the likelihood of p53 protein being degraded by proteasomes and presented on cell surfaces to be recognised by T-cells in a MHC I response[16]. Auto-antibodies recognises epitopes on the terminal regions of the protein, and hence auto-antibody production can theoretically be triggered by either the wild-type or the mutant *p53*, provided sufficiently high levels of these immunodominant epitopes are present at the cell surface[80]. Another probable antigen presentation mechanism is where cancer cells containing high cytoplasmic concentrations of *p53* undergo necrosis and release *p53* into the blood and lymphatic system where appropriate B-cells can interact. These antigens are also captured by Antigen Presenting Cells (APC) in their normal scavenging role and are presented in association with MHC class II response causing a Th2-like cell response[16] (Figure 2).

P53 mutation alone is insufficient to trigger anti-*p53* auto-antibody production as evidenced by several observations. Firstly, only 20%-50% of patients with detectable *p53* mutations produce detectable auto-antibodies[81,82]. This is attributed to the type of mutation, *e.g.,* mis-sense mutations are associated with higher auto-antibody production compared with other mutations[5,56,57,83]. This is probably because mis-sense mutations are more likely to produce a stable mutant p53 protein which is more likely to accumulate to sufficient levels to increase the likelihood of antigen presentation. Other mutations such as non-sense, frameshift and deletions often lead to truncated mRNA and unstable protein sequences which are less likely to accumulate, and thus less likely to induce auto-antibody production[84]. Secondly, anti-*p53* auto-antibodies most frequently recognise terminal epitopes but not the central domain with the majority of mutations[25,27,28,81]. Thirdly, large SV40 T-antigen stabilises p53 protein leading to accumulation of the wild-type protein which also induces auto-antibody production. Together these observations suggest that humoral response is triggered by elevated p53 protein levels *per se* (mutated and/or wild-type) rather than specifically directed at a mutated sequence.

Discrepancy between anti-p53 auto-antibody and p53 mutation

There are discrepancies between the presence of *p53* mutation, p53 protein product expression and anti-*p53*

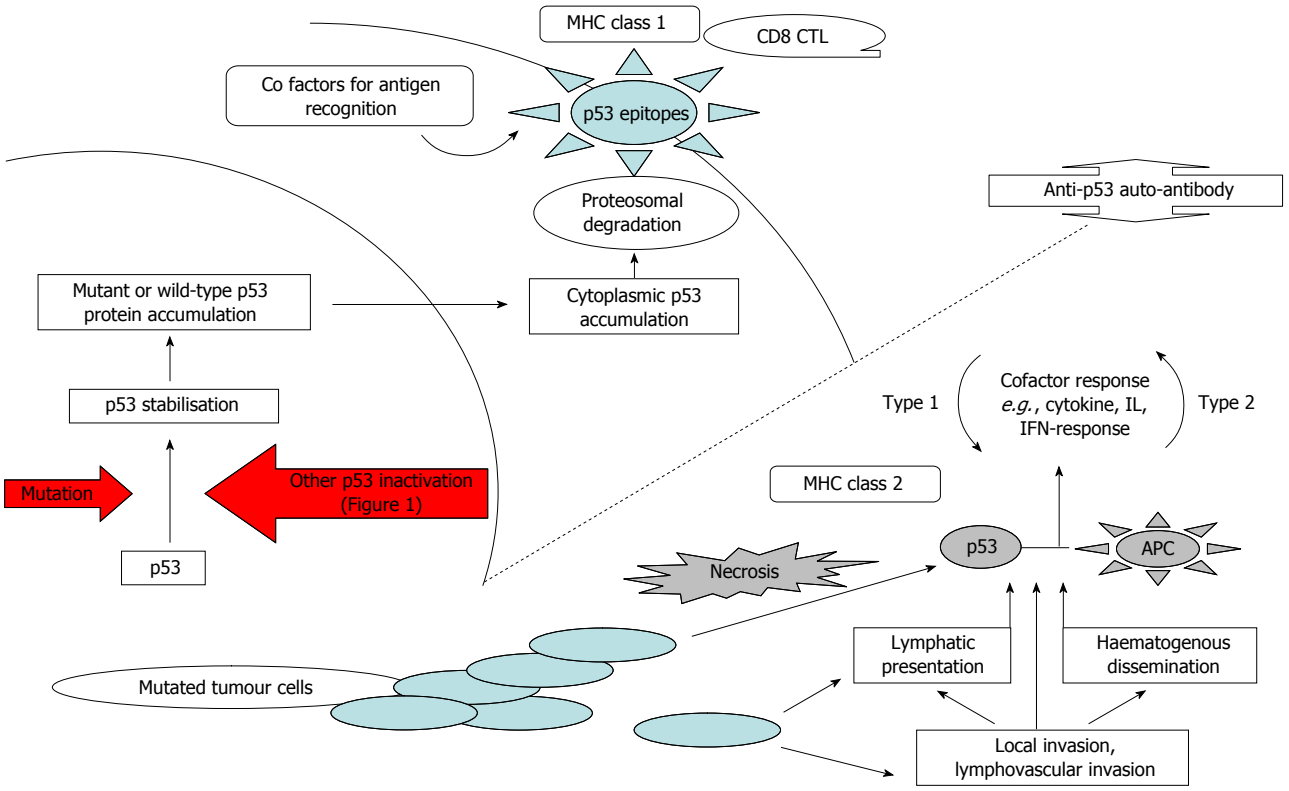

Figure 2 Proposed mechanisms of anti-*p53* auto-antibody induction. MHC: Major histocompatibility complex; CD8: Cluster of 8 differentiation; CTL: Cytotoxic T-cell; IL: Interleukin; APC: Antigen presenting cell.

auto-antibody production. This is largely attributed to the methodological differences of detection. Initial gene screening studies reported that most *p53* mutations were localised to exons 5-8 and to a lesser extent 4, 9, 10. Subsequent studies then only tended to screen these regions leading to substantial screening bias. It is now known that at least 10% of *p53* mutations occur outside these areas[84,85]. Another source of methodological difference is p53 protein detection which is inherently subject to tissue sampling and biopsy errors. Older studies (pre-1999) had different immuno-histochemical, fixation, paraffinization, antigen and antibody retrieval and observer scoring techniques. Finally, the antibody used to detect the mutant p53 protein affects sensitivity of p53 protein detection in IHC and also the detection of anti-*p53* auto-antibody in ELISA as described below.

Historically, the auto-antibody was initially detected using immunoblots or in-house enzyme linked immunosorbent assay (ELISA). These ELISA used different cut-off values leading to a vast range of reported frequencies of anti-*p53* auto-antibody within individual cancers in many older studies. Although standardised commercial ELISA are now widely available leading to an increase in anti-*p53* ELISA studies (2000 onward), auto-antibody detection can still vary depending on different manufacturers' product[86]. Most importantly, these ELISAs only measure an antibody response against those *p53* epitopes, which are expressed by the recombinant proteins used as the coating antigen. This may account for the reason that there are minor variations in commercial ELISA studies in different populations but when the same ELISA is used in the same population, inter and intra-coefficient of variations of 0.3%-2.7% are extremely reliable[82].

Finally, the differences in individual's immune systems cannot be ignored. The humoral response is dependent on an individual's unique MHC presentation as shown by several observations. Firstly, patients with similar cancers containing the same *p53* mutations do not necessarily mount the same immune response[81]. Secondly, whilst anti-*p53* auto-antibody titres increase in response to tumour load, all patients who are initially sero-negative do not develop an auto-antibody response despite disease progression and metastases. Conversely, patients who are sero-positive at diagnosis do not sero-convert to a negative anti-*p53* auto-antibody status even after the cancer is completely excised. It seems that once the patient's immune system has been primed, there is sufficient *p53* antigen available to maintain a long-term anti-*p53* humoral response[28,87,88].

MATERIALS AND METHODS: SYSTEMATIC REVIEW

Literature searches were performed using Medline and PubMed up to January 2012. Keywords used were "*p53*", "anti-*p53*", "antibody", "auto-antibody", "cancer" and combinations. No language or time restrictions were applied. All abstracts were reviewed and the relevant articles retrieved. The results of all published anti-*p53* auto-antibody cancer studies were accumulated and compiled in Table 1 with relevant key findings. The anti-*p53* auto-

Table 1 Cumulative reported frequencies of anti-*p53* auto-antibody (anti-*p53*) in controls and individual cancers *n* (%)

Group	Ref.	Anti-p53 positive	Summary of study and tumour type
Healthy/Benign	Park *et al*[107]	4/79 (5)	Comparative study with lung cancer
	Wu *et al*[133]	9/879 (1)	Case-control study of anti-*p53* in various cancers
	Kulić *et al*[134]	1/20 (5)	Comparative study with breast carcinoma
	Suppiah *et al*[130]	0/28 (0)	Comparative study with colorectal carcinoma
	Cai *et al*[125]	0/30 (0)	Comparative study with oesophageal carcinoma
	Atta *et al*[135]	5/29 (17.2); 13/26 (50)[1]	Comparative study with hepatocellular carcinoma
	Mattioni *et al*[136]	0/64 (0)	Comparative study with gastric carcinoma
	Akere *et al*[137]	4/45 (8.9)	Comparative study with hepatocellular carcinoma
	Müller *et al*[123]	0/57 (0); 0/379 (0)[2]	Single study of anti-*p53* in various cancers
	Chang *et al*[85]	0/40 (0)	Comparative study with colorectal carcinoma
	Fonseca *et al*[95]	0/15 (0)	Comparative study with glioma
	Shimada *et al*[82]	10/205 (6.3); 13/189 (7)[3]	Multi-institutional study of anti-*p53* in various cancers
	Neri *et al*[138]	0/51 (0)	Comparative study with lung carcinoma
	Numa *et al*[139]	0/9 (0)	Comparative study with uterine, ovarian, cervical carcinoma
	Mack *et al*[140]	1/46 (2.2)	Comparative study with SCLC
	Chow *et al*[141]	1/28 (3.6)	Comparative study with head and neck carcinoma
	Moch *et al*[142]	2/130 (1.5)	Comparative study with skin carcinoma (SCC/BCC)
	Hofele *et al*[143]	0/80 (0)	Comparative study with oral SCC
	Hagiwara *et al*[144]	0/13 (0)	Comparative study with oesophageal carcinoma
	Ralhan *et al*[145]	4/50 (8)	Comparative study with lung carcinoma
	Bielicki *et al*[111]	0/28 (0)	Comparative study with colorectal carcinoma
	Soussi[90]	35/2404 (1.5)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	102/4924 (2.1)	
Oesophageal	Blanchard *et al*[146]	24/97 (28)	Correlates with decreased overall and disease free survival
	Wu *et al*[133]	4/29 (13.8)	Case-control study of anti-*p53* in various cancers
	Cai *et al*[125]	18/46 (39.1)	Correlates with advanced histological grade, stage, lymph node metastases and decreased tumour response following radiotherapy
	Müller *et al*[123]	10/50 (20)	No correlation with stage or prognosis
	Bergström *et al*[147]	31/42 (73.8)	No correlation with clinico-pathological parameters, tumour size or survival
	Shimada *et al*[82]	90/301 (29.9)	Multi-institutional study of anti-*p53* in various cancers
	Kozłowski *et al*[148]	20/75 (26.6)	No correlation with stage, lymph node metastases or size.
	Shimada *et al*[99]	14/35 (40)	Correlates with tumour p53 protein expression but not clinico-pathological parameters
	Hagiwara *et al*[144]	13/46 (28)	Correlates with increased stage and tumour p53 protein expression but not prognosis
	Ralhan *et al*[145]	36/60 (60)	Correlates with tumour p53 protein expression and missense mutations but not clinico-pathological parameters.
	Soussi[90]	85/274 (31)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	345/1055 (32.7)	
Head/Neck[16]	Wu *et al*[133]	1/20 (5.0)	Case-control study of anti-*p53* in various cancers
	Shimada *et al*[82]	10/31 (32.3)	Multi-institutional study of anti-*p53* in various cancers
	Chow *et al*[141]	23/75 (31)	Correlates with nodal metastases but not prognosis
	Total	34/126 (27.0)	
Oral	Wu *et al*[133]	5/15 (33.3)	Case-control study of anti-*p53* in various cancers
	Hofele *et al*[143]	19/102 (18.6)[4]; 12/24 (50)[5]	Correlates with poor prognosis
	Castelli *et al*[149]	3/61 (18.7); 9/13 (69.2)[3]	Serum anti-*p53* is useful as a screening tool in pre-malignant lesions
	Soussi[90]	309/1062 (29.1)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	348/1219 (28.5)	
Ovary	Wu *et al*[133]	5/12 (41.6)	Case-control study of anti-*p53* in various cancers
	Qiu *et al*[150]	36/92 (39.1)	Correlates with p53 expression, not clinico-pathological parameters
	Shimada *et al*[82]	2/27 (7.4)	Multi-institutional study of anti-*p53* in various cancers
	Numa *et al*[139]	8/30 (27)	Correlates with *p53* tumour expression and poor prognosis
	Abendstein *et al*[151]	28/113 (25); 21/113 (19)[6]	Correlation between serum and ascitic anti-*p53*. No correlation with stage or grade. Anti-*p53* in ascites associated with poor prognosis
	Soussi[90]	140/635 (22)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	219/909 (24.1)	
Colorectal (detailed results in Table 2)	Wu *et al*[133]	11/66 (16.7)	Case-control study of anti-*p53* in various cancers
	Suppiah *et al*[130]	20/92 (21.7)	No correlation with stage or prognosis
	Nozoe *et al*[97]	17/36 (47.2)	Correlates with advanced lymph node status and stage
	Müller *et al*[123]	63/197 (32)[7]; 7/46 (15.2)[8]	No correlation with stage or prognosis
	Chang *et al*[85]	47/167 (28.1)	*p53* mutation, not anti-*p53*, correlates with poor prognosis
	Lechpammer *et al*[88]	40/220 (18.2)	? Correlation with stage or prognosis in Dukes' A/B1
	Shimada *et al*[82]	46/192 (23.9)	Multi-institutional study of anti-*p53* in various cancers
	Forslund *et al*[84]	24/88 (27.3)	Correlates with *p53* mutation
	Tang *et al*[89]	130/998 (13)	Correlates with advanced lymph node involvement but not prognosis
	Broll *et al*[152]	20/130 (15.4)	No correlation with stage or prognosis
	Takeda *et al*[98]	17/27 (63)	95% negative sero-conversion within 3 wk post-surgery
	Shiota *et al*[112]	18/71 (25.4)	Correlates with advanced stage and poor prognosis

	Bielicki *et al*[111]	30/145 (20.7)	? Correlation with Dukes' A →B
	Soussi[90]	307/1244 (24.7)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	797/3719 (21.4)	
HCC	Wu *et al*[133]	15/93 (16.1)	Case-control study of anti-*p53* in various cancers
	Atta *et al*[135]	28/41 (68.3)	Correlates with advanced stage and shorter survival.
	Akere *et al*[137]	5/41 (12.2)	Correlates with increased Okuda stage
	Müller *et al*[123]	19/80 (23.8)	Non-significant trend towards poor prognosis
	Charuruks *et al*[153]	26/141 (18.4)	Correlates with stage but not tumour p53 protein expression
	Tangkijvanich *et al*[154]	16/121 (13.2)[17]	Preliminary report of Charuruks *et al* (2001). No correlation with severity, stage or prognosis. Survival too short for survival analysis (3 mo *vs* 4 mo)
	Sitruk *et al*[155]	19/159 (12)	Correlates with multinodular, infiltrative tumour but not survival
	Soussi[90]	82/387 (1.2)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	210/1063 (19.8)	
	Wu *et al*[133]	0/11 (0)	Case-control study of anti-*p53* in various cancers
Bladder	Müller *et al*[123]	3/24 (12.5)	No correlation with prognosis
	Watanabe *et al*[156]	17/63 (27)[9]	Correlates with higher grade, stage, lymph node metastases and tumour p53 protein expression, but not prognosis
	Gumus *et al*[157]	14/80 (17.5)	Correlates with tumour p53 protein expression and poor prognosis.
	Gumus *et al*[158]	25/76 (33)	Negative sero-conversion post-treatment (35%, 8/23) associated with good prognosis.
	Shimada *et al*[82]	4/33 (12.1)	Multi-institutional study of anti-*p53* in various cancers
	Morita *et al*[159]	12/100 (12)	Correlates with stage, and p53 protein expression but not prognosis
	Wunderlich *et al*[160]	4/32 (12.5)	Correlates with tumour protein p53 expression but not stage.
	Soussi[90]	8/29 (27.6)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	70/385 (18.2)	
Lung	Park *et al*[107]	28/82 (34.1)	Sensitivity study with other markers for lung cancer
	Wu *et al*[133]	13/95 (13.7)	Case-control study of anti-*p53* in various cancers
	Bergqvist *et al*[161]	14/84 (16.6)	No correlation with tumour volume. Correlates with survival in adenocarcinoma, but not SCC
	Bergqvist *et al*[162]	12/58 (20.7)	No correlation with tumour volume or lymph node metastases
	Neri *et al*[138]	2/30 (6.7)[10]; 8/48(16.7)[11]	No correlation with stage, histology or prognosis. Non-significant increased survival in LC but not MM
	Cioffi *et al*[163]	35/109 (32.1)	Low sensitivity, but high specificity (100%) and accuracy (69%). Only 14% agreement with other tumour markers (CEA/TPA, CYFRA21-1, NSE.)
	Zalcman *et al*[126]	20/97 (20.6)	Correlates with poor prognosis in limited stage SCLC, but not all SCLC
	Mack *et al*[140]	4/35 (11.1)[12]; NSCLC 13/99 (13.3)[13]	Correlates with stage and prognosis in NSCLC but not SCLC
	Shimada *et al*[82]	18/125 (14.4)	Multi-institutional study of anti-*p53* in various cancers
	Soussi[90]	219/1282 (17.1)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	373/2049 (18.2)	
Cervix	Shimada *et al*[82]	10/53 (18.9)	Multi-institutional study of anti-*p53* in various cancers
	Numa *et al*[139]	12/86 (14)	No correlation with tumour p53 protein expression or prognosis
	Total	22/139 (15.8)	
	Wu *et al*[133]	7/43 (16.3)	Case-control study of anti-*p53* in various cancers
Gastric	Qiu *et al*[150]	19/61 (31.1)	Correlates with tumour size but not prognosis.
	Mattioni *et al*[136]	17/111 (15.3)	Correlates with tumour p53 protein expression, prognosis and survival
	Lawniczak *et al*[164]	16/71 (22.5)	Correlates with tumour type and age, but not stage or prognosis
	Müller *et al*[123]	14/122 (11.5)	No correlation with prognosis
	Shimada *et al*[82]	13/123 (10.6)	Multi-institutional study of anti-*p53* in various cancers
	Nakajima *et al*[165]	13/81 (16)	Correlates with lymph node metastases but not stage or prognosis
	Maehara *et al*[166]	23/120 (19.2)	Correlates with increased stage and tumour p53 protein expression but not prognosis
	Soussi *et al*[90]	105/727 (14.1)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	227/1459 (15.6)	
	Nozoe *et al*[167]	15/42 (35)	Correlates with grade 3 and triple negative cancer
Breast	Wu *et al*[133]	9/25 (16)	Case-control study of anti-*p53* in various cancers
	Kulić *et al*[134]	21/61 (35)	Correlates with decreased 5 year survival
	Müller *et al*[123]	17/50 (34)	Non-significant trend towards poor prognosis
	Gao *et al*[168]	31/144 (21.5)	Correlates with stage, lymph node metastases, ER negative, c-erb-2 and tumour p53 protein expression
	Shimada *et al*[82]	13/71 (18.3)	Multi-institutional study of anti-*p53* in various cancers
	Volkmann *et al*[169]	18/165 (10.9)	Poor concordance between recombinant/native *p53* ELISA, immunoblot and immunofluorescence
	Metcalfe *et al*[87]	155/1006 (15.4)	No correlation with stage and prognosis
	Soussi[90]	296/2006 (14.8)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	539/3467 (15.5)	
Uterus	Wu *et al*[133]	1/13 (7.7)	Case-control study of anti-*p53* in various cancers
	Shimada *et al*[82]	5/22 (22.7)	Multi-institutional study of anti-*p53* in various cancers
	Numa *et al*[139]	5/41 (12)	No correlation with tumour p53 expression/prognosis (see Cervix, Ovary)
	Total	11/79 (13.9)	
Pancreas	Wu *et al*[133]	0/17 (0)	Case-control study of anti-*p53* in various cancers

	Müller *et al*[123]	5/22 (22.7)	Increase sensitivity in conjunction with CA19-9. No correlation with prognosis.
	Shimada *et al*[82]	3/28 (10.7)	Multi-institutional study of anti-*p53* in various cancers
	Ohshio *et al*[170]	19/82 (23.2)	No correlation with tumour *p53* expression or prognosis
	Soussi[90]	60/650 (9.2)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	87/799 (10.9)	
Lymphoma	Messmer *et al*[171]	19/120 (15.8)	Associated with 17p deletions
	Wu *et al*[133]	0/18 (0)	Literature review of anti-*p53* in various cancers (1979-1999)
	Soussi[90]	19/248 (14.3)	Case-control study of anti-*p53* in various cancers
	Total	38/386 (9.8)	
Biliary tract[16]	Wu *et al*[133]	1/8 (6.3)	Correlates with tumour p53 protein expression but not stage
	Limpaiboon *et al*[172]	6/49 (12.2)	Multi-institutional study of anti-*p53* in various cancers
	Shimada *et al*[82]	1/6 (16.7)	Correlates with tumour *p53* mutation
	Tangkijvanich *et al*[173]	6/82 (7.3)	
	Total	14/145 (9.7)	
Haematological	Wu *et al*[133]	8/33 (25)	Case-control study of anti-*p53* in various cancers
	Shimada *et al*[82]	32/364 (6.3)[14]	Multi-institutional study of anti-*p53* in various cancers
	Soussi[90]	14/428 (3.3)[15]	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	54/825 (6.5)	
Glioma	Wu *et al*[133]	1/24 (4.2)	Case-control study of anti-*p53* in various cancers
	Fonseca *et al*[95]	5/24 (20.8)	No correlation with *p53* protein but increased in patients < 16 years
	Shimada *et al*[82]	2/31 (6.5)	Multi-institutional study of anti-*p53* in various cancers
	Soussi[90]	6/144 (4.2)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	14/223 (6.3)	
Prostate	Wu *et al*[133]	1/8 (12.5)	Case-control study of anti-*p53* in various cancers
	Shimada *et al*[82]	4/23 (17.4)	Multi-institutional study of anti-*p53* in various cancers
	Soussi[90]	4/148 (2.7)	Literature review of anti-*p53* in various cancers (1979-1999)
	Total	9/179 (5.0)	
Skin	Moch *et al*[142]	3/105 (2.9)	No difference between controls and patients. Increased in aggressive SCC (8%) *vs* slow-growing BCC (1.5%)
Testicular	Soussi[90]	0/144 (0)	Literature review of anti-*p53* in various cancers (1979-1999)
Melanoma	Soussi[90]	0/58 (0)	Literature review of anti-*p53* in various cancers (1979-1999)
Total		3419/18595 (18.4)	All cancers (1979-2012)

Cancer types are listed in order of decreasing anti-*p53* auto-antibody frequency. The reported studies within each cancer type are listed in reverse chronology. Squamous cell carcinoma (SCC); basal cell carcinoma (BCC), hepatocellular carcinoma (HCC), carcino-embryonic antigen (CEA). Tissue polypeptide antigen (TPA), CYFRA21-1, Neurone-specific enolase (NSE), Oestrogen receptor (ER), c-erb-2. [1]Cirrhosis; [2]Benign disease; [3]Oral pre-malignant lesions-excluded from calculation; [4]Primary carcinoma; [5]Secondary/recurrent carcinoma; [6]Ascitic titre, not included in calculation of serum titres; [7]Colon; [8]Rectum; [9]Upper renal tract tumours, excluded from anti-*p53* titres in bladder carcinoma; [10]Malignant mesothelioma (MM); [11]Lung carcinoma (LC); [12]Small cell lung carcinoma (SCLC); [13]Non-small cell lung carcinoma (NSCLC); [14]Myeloma; [15]Leukaemia; [16]Tumour type not specified; [17]Excluded as is preliminary report of the same cohort (duplicate) reported in Charuruks *et al*.

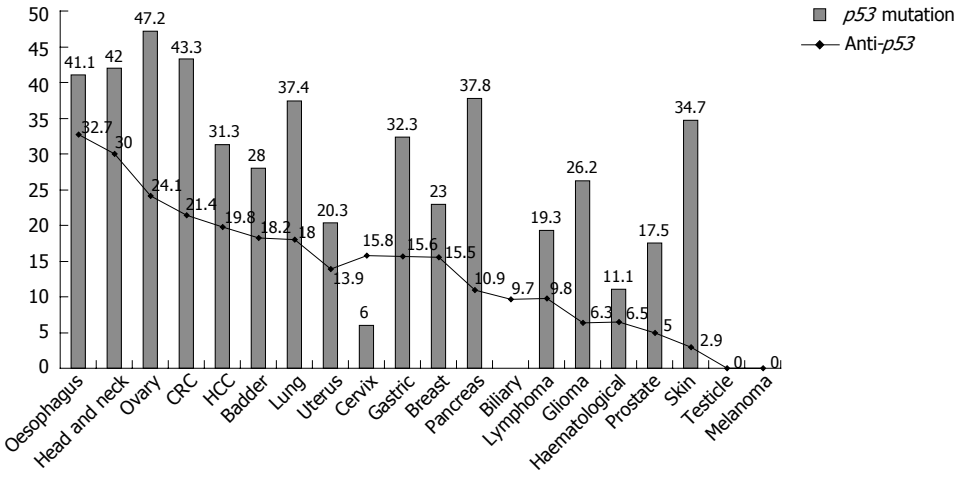

Figure 3 Percent *p53* mutations (International Agency for Research on Cancer, 2008) and percent anti-*p53* auto-antibody incidence calculated in this review. r^2 = 0.45, Correlation = 0.59. CRC: Colorectal cancer; HCC: Hepatocellular carcinoma.

antibody frequency from all published studies in each cancer type was calculated in this review. This calculated anti-*p53* auto-antibody frequency was then correlated with reported *p53* mutation rates to determine the associated between anti-*p53* auto-antibody presence and mutation in each cancer (Figure 3).

Methodological quality of anti-p53 and CRC publications

All published studies on anti-*p53* auto-antibody (1979-2012) were retrospective or cross-sectional case

control series with relatively small sample size (27-220 subjects tested) with a heterogeneous mix of cancer stages. The largest single study was published by Tang *et al*[89] that included a cohort of 998 CRC patients with anti-*p53* present in only small numbers (n = 130) for stage-specific analysis. An earlier non-systematic review by Soussi in 2000 recruited large numbers from various anti-*p53* studies but was study quality was limited by different cancers at various stages and different auto-antibody detection methods[90]. The primary outcome was not stated in most studies, and none was powered appropriately for survival outcomes.

ANTI-*p53* AUTO-ANTIBODY IN ALL CANCER TYPES

The reported frequency of anti-*p53* auto-antibody in individual cancer studies vary significantly due to small sample sizes, stage bias (usually a greater proportion of advanced stage tumours were included and different detection methods used. Anti-*p53* auto-antibody is usually measured in patients' sera but has also been measured in ascitic fluid of patients with ovarian cancer[68], saliva of patients with oral cancer[91] and in pleural effusions (12.5%) associated with lung, colon and pancreatic cancer[92]. In a landmark review, Soussi compiled results of 80 anti-*p53* auto-antibody studies in 18 cancer types over a 20 year (1979-1999) period[90]. The mean serum sero-positivity across all cancer types was 16.9% (1600/9489 patients, range 0%-31%) compared with 1.45% (35/2404) in controls thus demonstrating remarkable specificity (98%) but poor sensitivity. The specificity would be even higher as half the false positive subjects (17 out of 35) were from a single study reporting an extra-ordinarily high sero-positivity (24%, 17/70)[93](Table 1). When this study was excluded anti-*p53* auto-antibody specificity is near 100% for any cancer, which is confirmed by most recent reports.

Relationship between anti-p53 auto-antibody and p53 mutation

The 30-year cumulative sera anti-*p53* auto-antibody frequencies in individual cancers were calculated in this review to provide the most comprehensive anti-*p53* auto-antibody frequency in each cancer to date (Table 1). The auto-antibody frequencies are plotted (point) against the *p53* mutation rate (bars) as reported by the IARC TP53 Mutation Database to ascertain a relationship between anti-*p53* auto-antibody and *p53* mutation rates in each cancer.

The graph shows moderate correlation (r^2 = 0.45, correlation 0.59) between anti-*p53* auto-antibody and *p53* mutation (Figure 3). In general, cancers with the highest *p53* mutation rate such as oesophageal, head and neck, and colorectal demonstrate highest anti-*p53* auto-antibody rates[82,90]. Conversely, melanoma and testicular carcinoma with the lowest mutation rate have the lowest serum anti-*p53* auto-antibody rates (< 1%)[90]. The two exceptions are gliomas and skin cancers which have moderate *p53* mutation rate and low anti-*p53* auto-antibody rate (Figure 3). Proposed reasons for the low anti-*p53* auto-antibody production are poor brain antigenicity, poor *p53* antigen-presentation across the blood-brain barrier, and use of immuno-suppressive steroids (dexamethasone) in the majority of glioma cases[90,94,95]. Similar arguments about poor antigen presentation across an epithelial barrier are made for the majority superficial skin cancer. In summary, anti-*p53* auto-antibody has up to 35% sensitivity, depending on cancer type, nearly 100% specific for any malignancy but varies in individual cancer types; and demonstrates moderate correlation with *p53* mutation rate of each cancer.

ANTI-*p53* AUTO-ANTIBODY AND COLORECTAL CANCER VARIATIONS

CRC has the second highest anti-*p53* auto-antibody sero-positivity rates due in part to the high frequency of *p53* mutation. Pre-1999, eight studies used an "in-house" developed ELISA, 1 used Western blotting (WB), 1 used immuno-precipitation (IP) and another used all 3 detection methods (ELISA, WB, IP)[90]. Despite these methodological differences, most studies, 10 out of 11, reported a sero-positivity rate between 12.5% and 32%. The only study to report a discrepant and much higher sero-positivity rate (68%) used WB thus demonstrating the potential bias caused by non-standardised detection methodology[96]. New standardised commercial ELISA kits have since been developed with less variation in sero-positivity (13%-27%) with intra- and inter-assay coefficient of variation of 1.85%-2.37% and 0.3%-3.32% respectively (MESACUP anti-*p53* Test; Medical and Biological Laboratories, MBL, Nagoya, Japan)[82].

The mean sero-positivity from ELISA-only CRC studies calculated in this review was 19.9% (479/2409) with individual studies reporting of 13%-27% (Table 2). Only two studies reported inconsistently high rates of 47% and 63% in patients, and also in controls (2.6%), which suggests a lower cut-off value was used[97,98]. The same authors then reported an unusually high (40%) sero-positivity in superficial oesophageal carcinoma in another series, compared with 20%-30% in the majority of other studies. These studies used the same ELISA (Pharmacell, France)[99]. Interestingly, when the same authors later used a different ELISA (anti-*p53* EIA Kit Ⅱ, MESACUP) in a similar population, they reported a much lower sero-positivity of 30% (oesophageal cancer) and 24% (CRC) which was more consistent with other published ELISA studies[82]. This highlights potential methodological biases with anti-*p53* auto-antibody quantification even with commercially standardised ELISA kits.

Anti-p53 auto-antibody in diagnosis and screening

Cancer screening is used when early detection and intervention can lead to improved outcome for example CRC where 5 year survival in Dukes' A is 95%-100% com-

Table 2 Anti-*p53* auto-antibody in all published colorectal cancer studies and key findings *n* (%)

Ref.	Method and manufacturer	Samples	Follow-up	Key findings
Suppiah *et al*[130]	ELISA (*p53* ELISAPLUS, Calbiochem, Darmstadt, Germany)	20/92 (21.7); 0/20 (0)[1] 0/8 (0)[2]	Median 97 mo	No correlation with tumour stage, differentiation or location. Multivariate analysis show only Stage (Dukes' and TNM) to be independent prognostic factors
Nozoe *et al*[97]	ELISA (Pharmacell, France)	17/36 (47.2)	Not stated	Anti-*p53*-ab (+) associated with greater lymphatic invasion (94.1%; 16/17 *vs* 68.4%; 13/19), nodal involvement (70/6%; 12/17 *vs* 17.6%; 3/17) and advanced stage (P = 0.02). Anti-*p53* frequency higher in *p53* protein expressing tumours (74%; 14/19 *vs* 18%; 3/17). Only 3 patients with Dukes' A CRC, all sero-negative
Muller *et al*[123]	Immunoblot	Colon 63/197 (32); Rectum 7/46 (15.2); 0/57 (0)[1] 0/379 (0)[2]	CRC patients enrolled into trial with 5 year follow-up	No correlation with clinico-pathological parameters or prognosis. Trend toward higher anti-*p53* sero-positivity in N2/3 disease, poor differentiation and metastases. There were no patients with Dukes' A in this study. Anti-*p53* independent of CEA and CA19-9 with 16% information gain. This is the only study to report negative to positive sero-conversion (3.6%, 11/303)
Chang *et al*[85]	ELISA (*p53*-AK, Dianova, Hamburg, Germany)	47/167 (28.1); 0/40 (0)[1]	Median 36.3 mo (4-58)	Anti-*p53* correlates with *p53* mutation (43% *vs* 18%) but not tumour *p53* expression, clinico-pathological features or prognosis. *p53* mutations, advanced stage and pre-operative CEA > 5 ng/mL were independent prognostic factors (in that order). *p53* mutation strongly associated with advanced stage and poor differentiation
Lechpammer *et al*[88]	ELISA (ELISAPLUS Oncogene Research Products, Cambridge, United States)	40/220 (18.2); 0/42 (0)[1]	40 patients up to 20 wk; 8 patients up to 48 wk	Anti-*p53* had higher tumour *p53* expression (70% *vs* 52%). Anti-*p53* frequency shows highest increase in Dukes' A (0%, 0/28) → Dukes' B: (24%, 21/87) but no increase in progression to Dukes' C (18%,19/105). No correlation with overall tumour grade or metastases. Anti-*p53* reflects tumour load following surgery, during chemotherapy and with disease recurrence
Shimada *et al*[82]	ELISA (Anti-*p53* EIA Kit II, MESACUP anti-*p53* Test; MBL; Nagoya, Japan)	46/192 (23.9); 10/205 (4.9)[1]; 13/189 (6.9)[2]	Not reported	Validation study for MESACUP ELISA using prevalence of anti-*p53* in various cancers. Good intra- and inter-assay coefficient of variation of 1.85-2.37% and 0.3-3.2% respectively. Demonstrates stability of anti-*p53* titres at room temperature for 7 d and following 10 freeze-thaw cycles. No comment on correlation with clinico-pathological parameters or prognosis
Forslund *et al*[84]	ELISA (Dianova, Hamburg, Germany)	24/88 (27)	Not reported	Cross-sectional study on relationship between *p53* mutations and anti-*p53* presence. Frequency of *p53* mutation higher in anti-*p53* sero-positive group (92%, 22/64 *vs* 34%, 22/64) Correlation with clinico-pathological and survival parameters not reported
Tang *et al*[89]	ELISA (Calbiochem-Novabiochem, Darmstadt, Germany)	130/998 (13); 2/211 (1)[3]	Not reported	Anti-*p53* sero-positivity increases in progression from N2→ N3 (2.9%-10.6%); but not N0→N1 (11.7%-12.3%), N1→N2 (12.3%-10.6%) or M0→M1 (12%-17%). No correlation with CEA, overall TNM stage or metastases. Anti-*p53* associated with shorter survival in uni- but not multi-variate analysis. Largest study on anti-*p53* in CRC
Broll *et al*[152]	ELISA (*p53*-autoantikorpfer ELISA, Dianova, Hamburg, Germany)	20/130 (15); 0/44 (0)[1]	Median 25.5 mo	Anti-*p53* positive predictive value of 100%, but accuracy 37% and negative predictive value 29% due to poor sensitivity (15%). Anti-*p53* correlated with *p53* expression (P < 0.05), but not TNM stage, grade or location (exact numbers not shown). Approximately 70% of series Stage Ⅰ/Ⅱ CRC
Takeda *et al*[98]	Anti-*p53* EIA (PharmaCell, Paris, France)	17/27 (63); 1/38 (2.6)[3]	Up to 2 years Median not reported	Anti-*p53* correlates with *p53* protein expression and independent of CEA and CA-19-9. Sero-conversion in 94% (16/17) within 3 wk of endoscopic resection. No correlation with clinico-pathological parameters or prognosis/recurrence as all patients had early superficial CRC (23 mucosal, 4 submucosal invasion). This study reports exceptionally high anti-*p53*, especially considering very early CRC
Takeda *et al*[174]	ELISA (anti-*p53*-EIA kit, Pharmacell, Paris, France)	40 patients with anti-*p53* ab from previous studies	Up to 29 mo	No correlation between post-operative anti-*p53* sero-positivity and histological (depth, lymphatic or venous invasion) or clinico-pathological features of lymph node or liver metastases. High (96%; 27/28) sero-conversion in patients with complete tumour resection. No sero-conversion in patients with residual disease.
Shiota *et al*[112]	ELISA (GIF, Munster, Germany)	18/71 (25); 1/18 (6)[3]	Not stated, median survival 56 mo anti *p53* ab negative	Anti-*p53* correlates with TNM stage (Stage Ⅰ-Ⅲb: 9%, 4/45 *vs* Ⅳ: 56%, 14/25), Dukes' stage (A-C: 9%, 4/45 *vs* D: 56%, 14/25), CEA, CA19-9 and tumour p53 protein expression. Anti-*p53* associated with shorted survival (56 mo *vs* 20 mo) and is weak poor prognostic indicator. Anti-*p53* prognostic significance secondary to other factors, including weak factors *e.g.*, CEA and CA19-9. Only small number of Stage Ⅰ-Ⅲb patients

Bielicki *et al*[111]	ELISA (Dianova, Hamburg, Germany)	30/145 (21); 0/20 (0)[2]; 0/8 (0)[3]	Not stated. Cross sectional study	No correlation with Dukes' Stage (A/B: 22%, 16/73 *vs* C/D 19% 14/72), size, location, CEA. Highest increase in anti-*p53* frequency from Dukes' A (0%, 0/6) to Dukes B1 (28%, 5/18) but no further difference in progression to Dukes' C (19%, 7/36). Only 6 Dukes' A patients in study, all sero-negative
Soussi[90]	ELISA/WB/IP	307/1244 (24.7)	ELISA/ WB/IP	Review combining all studies with different methodologies from 1979-1999. Range of sero-positivity (12.5%-68% in 11 studies)
Total (1999-2009)		479/2409 (19.9)		All modern studies (1999 onwards) using commercial ELISA only, with one exception using Immunoblot (Muller *et al*, 2006)
Review Total (1979-2009)		786/3653 (21.5)		All studies on anti-*p53* in CRC (1979-2009)

Studies prior to 1999 used different methodology and not included (see above). Enzyme-linked immunosorbent assay (ELISA); Western blotting (WB); Immunoprecipitation (IP) [1]healthy, [2]benign disease, [3]adenoma. The study by Muller *et al* was included despite using immunoblot technique as it was a recent study with relatively large sample size. CRC: Colorectal cancer; CEA: Carcino-embryonic antigen.

pared with 5% in Dukes' D. Colonoscopy is the current gold-standard diagnostic tool but is painful, expensive and is associated with life-threatening complications such as colonic perforation (0.01%-0.3%) and haemorrhage (0.6%)[100,101]. The United Kingdom Flexible Sigmoidoscopy Screening Trial has provided evidence that one-off screening flexible sigmoidoscopy between age 55 and 64 was beneficial in reducing CRC incidence by 23%-33% and reducing mortality by 31%-43%[102,103]. Similar mortality reduction has been reproduced in other screening trials such as Norwegian Colorectal Cancer Prevention (NORCCAP)[104]. A recent meta-analysis similarly confirmed benefits of screening (endoscopy or stool-based screening) over an unscreened population in increasing detection and prognosis[105].

There are intuitive benefits of screening with serum anti-*p53* auto-antibody compared to CT, barium enemas and colonoscopy. The titre is not subject to tumour sampling error, is quicker, cheaper, easier and less traumatic, thus making it more repeatable in the general population. The auto-antibody titre itself is remarkably stable, showing no significant change when stored at room temperature for up to 7 d, or when stored at -80 ℃ for 3 years[81]. Repeated freeze-thaw cycles (up to 10 cycles) have minimal or no effect on serum levels as immunoglobulins are generally robust proteins[82]. Also, anti-*p53* auto-antibody appears to be independent of other conventional CRC tumour markers such as carcino-embryonic antigen (CEA) which means it could detect CRC in CEA-negative patients. The combined advantages of serum testing and the characteristics of anti-*p53* auto-antibody (serum stability, 95%-100% specificity, independent of current tumour markers), makes anti-*p53* auto-antibody a potentially valuable screening modality.

The role of *p53* in screening is promising its specificity for cancer, but this enthusiasm is tempered by a low sensitivity (20%-30%). It would thus be required as part of a panel of tumour markers. This panel could then be used to guide more invasive investigations such as colonoscopy. Combined serum immuno-testing for 6 markers (CEA, anti-*p53* auto-antibody, CYFRA 21-1, osteopontin, separase and ferritin) has been reported to have comparable sensitivity (> 80%) to faecal immuno-testing[106]. A similar tumour marker panel using CYFRA-21, CEA and anti-*p53* has been used in lung cancer also with 80% sensitivity[107]. Combined biomolecular and endoscopic strategies[108] are being investigated, and in conjunction with other new diagnostic non-invasive modalities (*e.g.*, CT-colonography)[109] may be able to further broaden the screening programmes for CRC and other cancers in the general population.

Anti-p53 auto-antibody and clinico-pathological parameters of CRC

p53 mutation is usually a late event in the adenoma-carcinoma sequence and hence anti-*p53* auto-antibody is unlikely to be present in early pre-invasive lesions where *p53* mutations have not occurred[110]. The largest study reports 1% (2/211) sero-positivity in adenomas which increased to 6% in carcinoma *in-situ*[89]. This 1% could be due to undetected microfoci of invasive cancer within adenoma or changes that predate microscopic detection. The increase prevalence of anti-*p53* auto-antibody to 6% in carcinoma *in situ* can be expected in these tumours which are at the end of the adenoma-carcinoma sequence with greater proportion of *p53* mutation. This would then suggest that anti-*p53* auto-antibody should increase with further growth (CRC stage) but this is not seen. Almost all studies reported no association between anti-*p53* auto-antibody and CRC stage (Tables 1 and 2). This was reported in the largest cross-sectional series, and confirmed by other long-term follow-up studies[89] (Table 2). Only a handful of studies have suggested an association between anti-*p53* and T-stage[88,111], selected nodal disease[89] and metastases[112]

Tumour depth

Two studies reported increased anti-*p53* in progression from Dukes' A to B, but not with progression from Dukes' B to C[88,111]. Lechpammer *et al*[88] reported 0% (0/28) anti-*p53* in Dukes' A which increased significantly to 9.6% (21/87) in Dukes' B, but did not increase further with Dukes C (8.6%, 19/105). Bielicki *et al*[111] similarly reported increased anti-*p53* auto-antibody from Dukes' A (0%) to Dukes' B (28% Dukes' B1, 22% Dukes' B2); but no increase in progression to Dukes C (19%). This suggests auto-antibody production is stimulated by early (Tis to T2) local invasion such as microvascular basement membrane invasion leading to antigen presentation; but not further progression. Further studies are required to

Table 3 Prevalence of anti-*p53* auto-antibody and carcino-embryonic antigen in studies reporting the presence of both tumour markers in colorectal cancer *n* (%)

	CEA	Anti-*p53*
Tang *et al*[89]	408/943 (43.3)	130/998 (13.0)
Shibata *et al*[96]	23/47 (48.9)	32/47 (68.0)
Bielicki *et al*[111]	46/148 (31.1)	29/148 (19.6)
Hammel *et al*[175]	20/54 (37.0)	14/54 (25.9)
Overall	497/1192 (41.7)	204/1247 (16.4)

CEA: Carcino-embryonic antigen.

understand the precise series of events in anti-*p53* auto-antibody production.

Nodal involvement

Anti-*p53* auto-antibody is produced in part due to response to *p53*-antigen presentation. Thus nodal involvement should also increase anti-*p53* auto-antibody production by increasing probabilities of antigen presentation to the humoral system. However, there is no correlation between anti-*p53* auto-antibody and nodal involvement in any of the studies (Table 2). Tang *et al*[89] suggested increased anti-*p53* with "advanced" nodal disease (N3: > 10 regional nodes or systemic nodal metastases) compared to N0-2 CRC in selected analysis. We re-classified the data into node "positive" and "negative" disease and found no difference in sero-positivity of 12% *vs* 14% with nodal involvement (calculation not shown).

Metastases

It would be expected that haematogenous cancer cell dissemination should invoke a further immune response but there has been no association between anti-*p53* auto-antibody status and metastatic disease except one study[112]. In this study, anti-*p53* auto-antibody had extremely high prevalence (56%, 14/25) in Stage Ⅳ disease, and unusually low prevalence in Stage Ⅰ-Ⅲ (9%, 4/45) leading to strong anti-*p53* bias towards Stage Ⅳ disease. This is the only study to report anti-*p53* auto-antibody association with stage and adverse prognosis which is discussed later (anti-*p53* auto-antibody in prognosis in CRC).

Summary of anti-p53 auto-antibody and CRC Stage

Anti-*p53* auto-antibody production is initially most likely to be produced in the final stages the adenoma-carcinoma sequence (in keeping with *p53* mutation being a relatively late event. It is likely that anti-*p53* auto-antibody production is no longer dependant on antigen-presentation, but rather now dependant on immune-recognition by (1) tumour factors *e.g.*, *p53* mutation type and conformation, presence of co-factors; and (2) patients' immune-specific factors such as MHC expression required for recognition. This response is not sufficiently consistent to justify a separate clinico-pathological parameter of its own. In the future, anti-*p53* auto-antibody may have some benefit in refining CRC stage if there is influence on prognosis or treatment, similar to k-RAS status in anti-EGFR and anti-VEGF therapy for CRC and liver metastases, or oestrogen- or progesterone-status in breast cancer.

Anti-p53 auto-antibody and carcino-embryonic antigen

CEA is the most common serum tumour marker used in CRC. It is a 180 kDa serum glycoprotein which is present at low levels in normal cells but over-expressed in adenocarcinoma, especially of the colon, rectum, breast and lung[113]. Pre-operative CEA presence has been associated with aggressive CRC and poor prognosis[114,115]. CEA has also been used as an adjunct in CRC screening, monitoring for disease recurrence following resection, or as part of tumour marker panel for metastases of unknown primary origin. CEA has high specificity (80%) with false elevations in smokers, inflammatory diseases, cirrhosis, obstructive jaundice, gastric ulcers, emphysema, diabetes and collagen vascular diseases[116-118].

CEA in isolation is not recommended for screening or detection of recurrence due to its variable sensitivity (30%-80%)[114,119]. CEA sensitivity can be modulated by changing the cut-off values for "positivity" but sensitivity has still remained low despite variations in the cut-off value used[120,121]. Despite this, The American Society of Surgical Oncology (ASCO) guidelines suggest serial CEA measurements every 3 mo in Stage Ⅱ/Ⅲ CRC for at least 3 years following diagnosis, and during treatment of metastatic disease[122].

Tumour markers used in conjunction with CEA could increase the efficacy of CRC screening in selected populations. Such tumour markers should be independent of CEA as to detect the CEA-negative CRC population and thus increase sensitivity of the tumour marker panel. The majority of studies have shown that anti-*p53* auto-antibody is independent of CEA (Table 3). The two studies which report a positive correlation had results inconsistent with other studies, with the first study having an unusually strong association between anti-*p53* auto-antibody and Stage Ⅳ disease (as discussed earlier)[112] and the second reporting the highest anti-*p53* auto-antibody frequency (68%) and used WB, not ELISA[96]. Methodological difference and sample bias are most likely responsible for the results observed.

In this review, information is compiled from all studies reporting CEA and anti-*p53* in Table 4. This shows that when used in isolation, anti-*p53* auto-antibody can CEA can detect CRC in 17% and 42% respectively. If both tumour markers are used, the sensitivity increases to 51% (as both markers are absent in 48.9%). This results in information/sensitivity gain of +9% (compared with CEA alone); and +34% (compared with anti-*p53* auto-antibody alone). The only other study to report "information gain" using anti-*p53* auto-antibody in CRC confirmed reported mean increased sensitivity of 16% with individual increased sensitivity from 55% to 71% in colon cancer and 78% to 83% in rectal cancer[123]. This report is consistent with our calculation using data from all other published anti-*p53* auto-antibody and CEA studies in CRC (Tables 3 and 4).

Table 4 Combined carcino-embryonic antigen and anti-*p53* auto-antibody rates from all studies reporting the presence of both markers (*n* = 1192) *n* (%)

	CEA normal	CEA elevated
Anti-*p53* ab present	112 (9.4)	90 (7.6)
Anti-*p53* ab absent	584 (48.9)	406 (34.1)

CEA: Carcino-embryonic antigen.

The clinical utility of this "information gain" requires examination. CRC has low prevalence in the general population and thus pick-up rates would remain low despite the use of both tumour markers. Both markers also have preponderance towards later stage CRC (as opposed to Stage I) which reduces impact of earlier detection and thus screening efficacy. It is thus likely that these 2 markers alone are insufficient and additional markers would be required, i.e panel of 6 tumour markers was used to form a panel with sensitivity similar to faecal occult testing in population screening[108]. In post-operative surveillance, small studies have demonstrated overall 4 tumour markers (CEA, TPA, CA19-9, CA72.4) panel sensitivity of 81% compared with 9%-45% using individual markers[124]. Hence, the optimal strategy would be to use other markers in addition anti-*p53* and CEA to select patients for investigations. The cost-effectiveness of these immunological-targeted strategies for general population screening, high risk population screening or post-operative surveillance requires further evaluation.

Anti-p53 Auto-antibody and monitoring for recurrence or metastases

Anti-*p53* auto-antibody may have its most promising role in post-operative monitoring for disease recurrence or distant metastases. Several, but not all, studies have demonstrated that anti-*p53* reflects tumour load, with increasing serum titres corresponding with disease recurrence/progression and decreased titres following surgery/chemotherapy[81,88,120]. Lechpammer *et al*[88] produced the most convincing series demonstrating clear decreases with post-surgery and during chemotherapy. More importantly, increases, especially during chemotherapy, predated clinical diagnosis of recurrence. Smaller subset analysis in other studies has also demonstrated fluctuations in serum titres with disease load. Similar fluctuations with resection and radiotherapy have been reported in oral, oesophageal, lung, ovarian and breast cancer[125-127].

In almost all cases, the anti-*p53* auto-antibody persists but at a much lower level. Only one study has reported complete absence of the anti-*p53* auto-antibody in a series of patients with superficial (mucosal and submucosal) CRC treated with endoscopic resection[98]. This may be because the early stage CRC had a smaller mutant *p53* load which may not have adequately stimulated the humoral system to produce a prolonged immune response following CRC removal. The other studies had more advanced CRC where there would have been prolonged antigen exposure to the humoral system[81,88,98,128].

This cost efficacy of serial anti-*p53* auto-antibody for surveillance must be considered in the light of only 20%-30% prevalence at presentation and subsequent sero-conversion (Table 5). Assuming 1% future sero-conversion and 3-monthly serum measurements for 3 years as per ASCO recommendations, this would result in 20-30 initial positives at diagnosis; and an additional 1 positive over the subsequent 3 years. This results in an initial yield 20-30 positives followed by only 1 positive out of 960 samples over next 3 years (remaining 80 patients × 4 samples per year × 3 years). We then consider this 1 positive sero-conversions out of 960 samples may not alter treatment as serum measurements may predate clinical evidence of disease, and treatment cannot be offered based anti-*p53* auto-antibody titres alone.

An alternative more cost-effective strategy of screening would be to screen all patients for anti-*p53* auto-antibody at diagnosis with further serial measurements only in patients sero-positive at diagnosis. Post-operative patients with rising titres could be selected for expedited investigations and thus increase diagnostic yield, compared to current blanket strategy of routine investigations for surveillance at fixed time intervals. Preliminary studies in small groups using tumour marker panel (CEA, TPA, CA19-9 and CA72.4) demonstrated 81% sensitivity for recurrence with mean lead times of 5.3 mo prior to radiological confirmation of recurrence[124]. A cost efficacy study would be required to ascertain the ability of anti-*p53* auto-antibody as part of a tumour marker panel to guide post-operative surveillance, improve resource allocation and prolong survival.

Anti-p53 auto-antibody and prognosis in CRC

p53 mutations have been associated with poor prognosis, possibly in part due to chemo-resistance against *p53*-dependant chemotherapy (*e.g.,* 5-fluorouracil) but reports of its prognostic significance are inconsistent[90,129]. As the anti-*p53* auto-antibody response has been associated with *p53* mutations and serum testing is easier than DNA sequencing, studies have focused on using anti-*p53* auto-antibody to predict prognosis. The majority of studies report that anti-*p53* auto-antibody response has no independent prognostic value. This was confirmed in the study with the longest follow-up which reported CRC stage, but not anti-*p53* auto-antibody, to be an independent prognostic marker in multivariate analysis[130], and also by the study with the second longest follow-up but larger sample size[85] (Table 6).

Four studies report an adverse prognostic significance but in 3 of these, the prognostic significance was in selective univariate analysis where anti-*p53* was associated with advanced stage, and prognostic significance was lost when stage was incorporated in multivariate analysis[112,131,132]. The fourth, and only study, to report anti-*p53* auto-antibody as an independent prognostic indicator in multivariate analysis strongly associated anti-*p53* auto-antibody with Dukes' D to an extent that median survival of anti-*p53* positive patients was extremely low (20 mo) compared to other studies reporting median survival up

Table 5 Anti-*p53* auto-antibody and sero-conversion in colorectal cancer

Ref.	Patients, method	Follow-up	Findings
Müller *et al*[123]	303 patients, 197 colon, 46 rectal	Median 6 mo	All cancers: 3.6% (11/303) sero(-)→ (+); 3.6% (11/303) sero(+)→ (-); Total 7.2% (22/303) sero-conversion. Colon cancer: 3% (4/137) sero(-)→ (+); 3.6% (5/137) sero(+)→ (-); Total 6.6% (9/137) sero-conversion. Rectal cancer: 6.5% (2/31) sero(-)→ (+); 3.2% (2/31) sero(+)→ (-); Total 12.9% (4/31) sero-conversion
Lechpammer *et al*[88]	Immunoblot 32 , ELISA (Oncogene, Research Products, Cambridge, United States)	Up to 20 wk; 8 patients-48 wk	Non-significant decrease at 4 wk (pre-first cycle chemo) and significant decrease at 12 wk post-surgery Significant decreases during chemotherapy and 2 patients with anti-*p53* increase at 12 wk (during chemotherapy) developed recurrence 8 patients with extended follow-up: 7/8 had decreased anti-*p53* with no recurrence. 1/8 anti-*p53* decrease post-surgery/chemotherapy but increased at 12 wk corresponding with liver metastases. Anti-*p53* fluctuates in response to tumour load but does not disappear. Anti-*p53* levels reflects tumour load even during chemotherapy
Takeda *et al*[174]	30 CUR A, 5 CUR B, 5 CUR C, anti-*p53* EIA, Pharmacell	Median 26 mo (13-144)	CUR A (n = 30): 28/30 sero(+)→(-) in 6 mo; 2 no sero-conversion: 1 recurrence CUR B (n = 5): 2 sero(+)→(-) no recurrence. 3 no sero-conversion, 2 had metastases CUR C: No sero-conversion Correlation between post-operative negative conversion and operative curability
Takeda *et al*[98]	17 mucosal/submucosal, ELISA (anti-*p53* EIA, Pharmacell, France)	Up to 2 years	94%, 16/17 sero(+)→(-) within 3 wk post-surgery No recurrences as early stage tumours and hence not able to comment on anti-*p53* and recurrence rates
Polge *et al*[128]	10, ELISA (Dianova, Hamburg, Germany)	Up to 6 mo	8 followed-up: 5/8 remained sero(+) post-operatively. All developed metastases 3/8 decreased anti-*p53* titres. No metastases or recurrence. Anti-*p53* titres decreased within 1 mo of surgery/chemotherapy but no sero-conversion to anti-*p53*(-)
Angelopoulou *et al*[81]	6, "In house" immunofluorometric assay	Up to 17 mo	Anti *p53* decreases with surgery/chemotherapy but persists at low levels Anti-*p53* increases with recurrence Anti-*p53* reflects tumour load more sensitively than CEA (n = 5) and in non-CEA producing tumour (n = 1)
Hammel *et al*[175]	12, "In house" ELISA	Up to 20 mo	Anti-*p53* in 5/8 patients decrease by > 25% within 1 mo. At 1 year, 3 with normal anti-*p53* levels and 3 with substantial decrease in anti-*p53* remain disease-free 2 patients with post-operative increased anti-*p53*: 1 developed recurrence and 1 developed metastases Anti-*p53* decreased again following surgery in both patients. CEA and CA19-9 were normal in both cases

Sero(-): Sero-negative; Sero(+): Sero-positive; CUR A: No residual tumour macroscopically; CUR B: No residual tumour but not as evaluable as CUR A; CUR C: Definite residual tumour; CEA: Carcino-embryonic antigen.

to 60 mo and 5-year survival > 50%[89,123,130]. Remarkably, anti-*p53* auto-antibody prognostic significance was even weaker than CA19-9, a pancreatic tumour marker considered unsuitable for pancreatic cancer screening by ASCO[112,122]. The results of this study are hard to credit. As such, anti-*p53* auto-antibody has no independent prognostic value.

CONCLUSION

The anti-*p53* auto-antibody response is the end-point of a complex multi-factorial humoral response to the accumulation of p53 protein which is a product mainly of *p53* gene mutation, but also mutation of *p53* regulators and non-mutative pathways. Anti-*p53* auto-antibody has low (13%-32%) sensitivity in CRC but is nearly 100% specific for malignancy. The auto-antibody frequency may increase with early local invasion or late nodal progression but is not sufficiently consistent to form a separate stage classification. There may be a promising future role of anti-*p53* auto-antibody in screening and monitoring for disease recurrence. The characteristics of the immunoglobulin and the benefits of serum testing provide a promising role in guiding the radiological and endoscopic screening of high risk populations in conjunction with other current tumour markers. The most promising future focus of anti-*p53* auto-antibody lies in being part of a bio-molecular panel of tumour markers to guide endoscopic and radiological screening in general population and high-risk population screening; and in post-operative

Table 6 Anti-p53 auto-antibody and prognosis in colorectal cancer

Ref.	*n* (%)	Follow-up	Findings
Suppiah *et al*[130]	20/92 (21.7)	Median 97 mo	No difference in overall survival (62 mo *vs* 60 mo) or disease-free survival (73 mo *vs* 82 mo)
Müller *et al*[123]	70/243 (28.8)	5-year trial protocol	No survival difference with anti-*p53* in CRC and other cancers. Trend towards decreased survival in anti-*p53* positive patients with HCC and breast carcinoma
Tang *et al*[89]	130/998 (13)	Recruitment 1995-2000	Anti-*p53* associated with decreased survival in univariate analysis but not multivariate analysis. Anti-*p53* associated with advanced nodal disease (Stage N2→N3) and metastases (M1)
Chang *et al*[85]	147/167 (28)	Median 36.3 mo (22-85)	*p53* mutation associated with poor differentiation and advanced stage. Multivariate analysis shows *p53* mutation most significant survival predictor, followed by CRC stage. No prognostic significance of p53 protein expression or anti-*p53*
Shiota *et al*[112]	18/71 (25)	Not stated	Anti-*p53* associated with shorter overall survival (20 mo *vs* 56 mo) but highly significant association with metastases (M1). Cox regression showed prognostic significance with liver metastases, TNM stage, Dukes stage, Ca19-9 and anti-*p53* (in that order)
Kressner *et al*[131]	59/184 (32.1)	Median 6 years	Anti-*p53* associated with decreased survival in univariate, but not multivariate analysis. Anti-*p53* is independent prognostic indicator in Dukes' A-C with curative surgery (*i.e.*, when metastases excluded)
Houbiers *et al*[132]	65/255 (25.5)	36 mo	Anti-*p53* associated with reduced overall (75% *vs* 88%) and disease-free survival (56% *vs* 64%) at 3 years in subgroup analysis of Dukes' A and B1. No difference in overall survival (61% *vs* 68%) or disease-free survival (51% *vs* 58%) when all stages included

CRC: Colorectal cancer; HCC: Hepatocellular carcinoma.

cancer surveillance to guide earlier detection of cancer and cancer-recurrence; and finally with more significant impact on cost-efficacy and survival.

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