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# Anti-p53-directed immunotherapy of malignant disease

Matthias Theobald and Rienk Offringa

**Mutation and aberrant expression of the p53 tumour suppressor protein are the most frequent molecular alterations in human malignancy. Peptides derived from the p53 protein and presented by major histocompatibility complex molecules for T-cell recognition could serve as universal tumour-associated antigens for cancer immunotherapy. Because p53 normally functions as a ubiquitously expressed self-protein, controlling cell-cycle progression and apoptosis, it also represents a paradigm target molecule for tumour-reactive yet self-antigen-specific T cells. Tailoring p53-based cancer immunotherapy thus requires both interference with p53-specific self-tolerance and induction of the entire repertoire of p53-reactive T cells. Transferring selected T-cell receptor genes into human T cells offers a novel and appealing strategy to meet these requirements.**

Cytotoxic T lymphocytes (CTLs) specific for tumour-associated antigens (TAAs) are capable of mediating efficient antitumour immune responses in vivo (Refs 1, 2). The majority of TAAs bound to major histocompatibility complex (MHC) class I that have been identified are expressed uniquely by a given type or limited set of tumours (Ref. 1). Universal TAAs (UTAAs) presented at elevated amounts by cells of a wide variety of human malignancies and derived from proteins that are inherently involved in the process of malignant transformation would provide attractive targets for broad-spectrum immunotherapy of malignant disease. However, CTL epitopes that are representative of UTAAs have only rarely been identified (Refs 3, 4). A major barrier to the design of UTAA-specific immunotherapeutics has been the observation that presentation of such TAAs at low copy numbers by nontransformed cells and tissues, including thymus, results in a peripheral T-cell repertoire that is devoid of high-avidity, UTAA-specific CTLs due to self-tolerance (Refs 5, 6, 7). The p53 tumour suppressor protein falls into the category of ubiquitous self-proteins inherently involved in the process of malignant transformation (Ref. 8) and therefore potentially provides a feasible target for immunotherapy of malignant disease.

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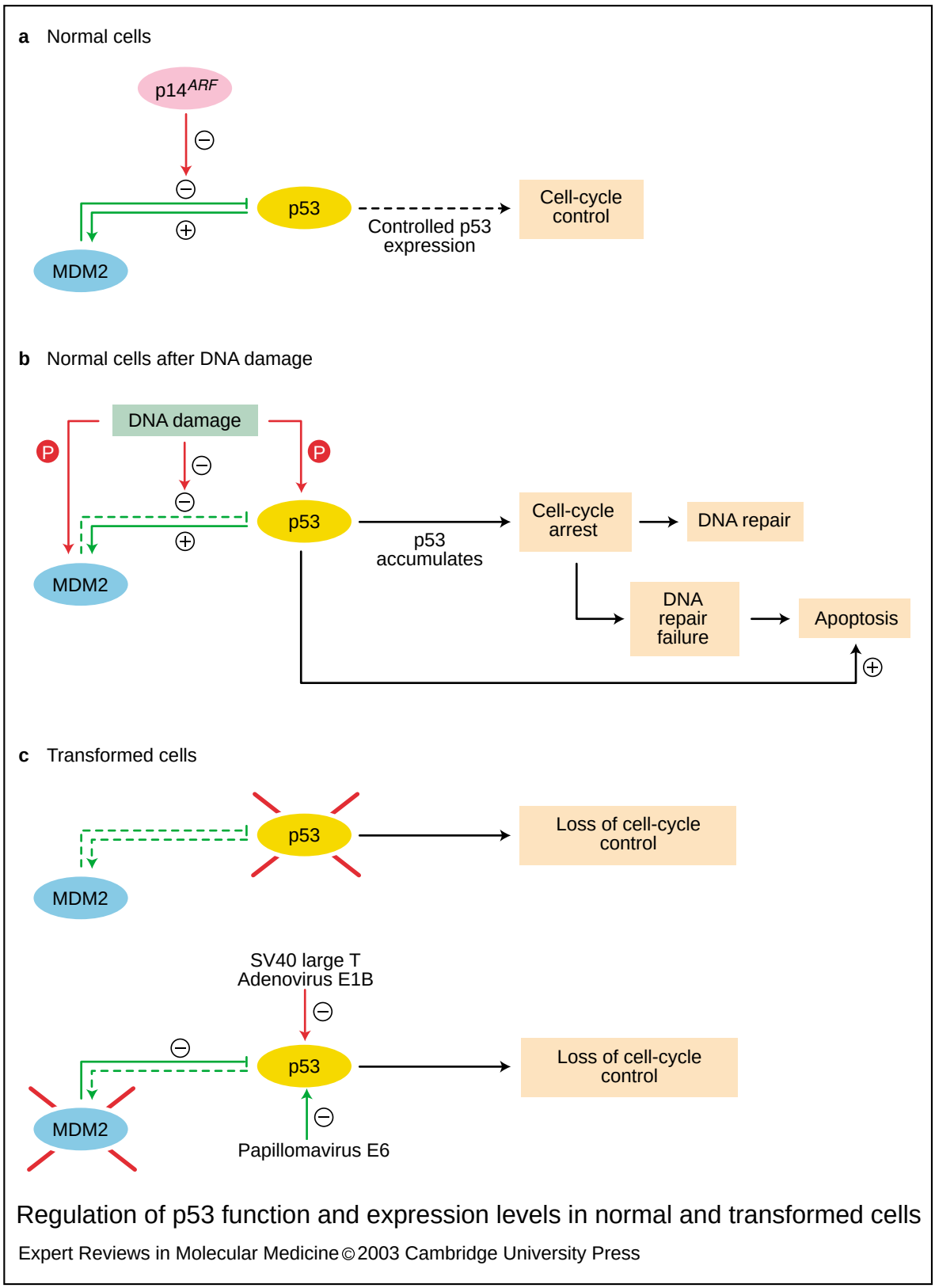


**Figure 1. Regulation of p53 function and expression levels in normal and transformed cells** *(see next page for legend)* **(fig001mtm).**



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**Figure 1. Regulation of p53 function and expression levels in normal and transformed cells.** Mechanisms resulting in a decrease in p53 steady-state levels are indicated with green arrows, those resulting in increased p53 levels with red arrows. Solid lines designate active mechanisms, broken lines designate inactivated mechanisms. (a) Normal somatic cells exhibit a tight regulation of the cell cycle, involving the interaction of many different proteins. One of these, p53, can induce arrest of cell growth and apoptosis. Under normal circumstances, p53 turnover is maintained at a high level by the MDM2 protein, which inhibits its function as a transcriptional activator and targets p53 for proteasome-mediated degradation. In turn, p53 upregulates MDM2 protein levels, forming a negative autoregulatory loop. In addition, the $p14^{ARF}$ protein stabilises p53 by inhibiting MDM2-dependent p53 degradation, thus specifically activating the p53 pathway. Together, these proteins maintain p53 at a steady-state level, allowing the cell cycle to be controlled. (b) Following DNA damage and other stress signals, the cell is required to arrest its cell cycle to allow DNA repair to take place. DNA damage induces phosphorylation (P) of MDM2, which prevents it from inhibiting p53 function. Similarly, stress signals induce phosphorylation of p53, which protects p53 from MDM2-mediated targeting for degradation, and p53 therefore accumulates. As a result of the increased p53 levels, cells will not enter the cell cycle until the DNA damage has been repaired. If repair fails, p53 will eventually trigger apoptosis. (c) In transformed cells, any abnormal modulation of p53 function and metabolism profoundly affects cell growth by loss of cell-cycle control. For instance, functionally mutated p53 can fail to induce cell-cycle arrest and apoptosis. Other mutations in p53 can prevent binding of MDM2, resulting in protection of p53 from degradation, leading to increased cell cycle arrest. Mutated p53 can also fail to stimulate MDM2 expression, decreasing the MDM2-mediated targeting of p53 for degradation. Tumours also frequently show overexpression of MDM2, constitutively suppressing p53 levels and thereby preventing cell-cycle arrest and apoptosis. Expression of $p14^{ARF}$ is also often lost in tumour cells, resulting in constitutive suppression of p53 levels by MDM2. In addition, the viral oncoproteins SV40 large T antigen and adenovirus E1B can inactivate the regulatory functions of wild-type p53 through sequestration of p53 and/or by increasing the phosphorylation of p53. As a consequence, transformed cells express high levels of functionally inactive p53. By contrast, the human papillomavirus E6 oncoprotein functionally inactivates p53 by strongly increasing degradation of this protein in a manner similar to MDM2 **(fig001mtm)**.

## The p53 tumour suppressor protein

### Function of p53

The p53 protein functions as a potent inhibitor of cell growth by arresting the cell cycle and preventing uncontrolled cell division (Ref. 9) (Fig. 1); this role of p53 is of critical importance for tumour suppression and p53 is therefore defined as a tumour suppressor protein. The inhibitory activity of p53 must be kept tightly controlled in order to allow normal cell growth and development to take place, and one component of this is the control of p53 turnover and stability (Ref. 10). The MDM2 protein is central to the regulation of p53 (Fig. 1); MDM2 binds to the N-terminus of p53 in the same region that p53 normally binds components of the transcriptional machinery, and thereby inhibits the activity of p53 as a transcription factor (Refs 9, 11). MDM2 also targets p53 for degradation through its ubiquitin ligase activity.

In order to provide a fine balance in p53 activity, another component of p53 regulation is its phosphorylation to protect it from MDM2-mediated degradation, and several kinases have been implicated in this process (Refs 9, 10). Not all p53-activating signals depend on direct phosphorylation, as shown by the activity of another tumour suppressor protein, $p14^{ARF}$ ($p19^{ARF}$ in mice), which binds to MDM2 in a region distinct from the p53-binding domain such that it inhibits MDM2-mediated degradation of p53 (Refs 9, 10) (Fig. 1). Many other proteins are involved in the regulation of p53, including the retinoblastoma protein Rb, which inhibits p53 degradation by direct binding to MDM2, and the E2F family of transcription factors, one member of which (E2F1) stabilises p53 (Refs 9, 10).

### Alterations of p53 in human cancer

In many cases, mutation of the p53 tumour suppressor gene leads to an increased half-life of the otherwise very unstable p53 molecule and thereby to accumulation of the protein, which abrogates the regulatory function of p53 on cell-cycle control (Ref. 8). The persistent overexpression of mutated p53 in tumours was the first feature that marked this protein as a potential target antigen for immunotherapy of cancer. Interestingly, tumours that express non-mutated p53 can exhibit abnormalities in p53 function and metabolism, for instance as a result of expression of the E6 oncogene of high-risk types of human papillomavirus (HPV) (Ref. 12). Such abnormalities can also arise from



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alterations in the expression or function of an impressive repertoire of cellular gene products, including MDM2 and $p14^{ARF}$ (Ref. 10). Indeed, perturbation of the p53 regulatory pathway and p53 metabolism occurs in most, if not all, types of human cancer (Ref. 13). This makes p53 an almost universal target for the immunotherapy of cancer.

### Targeting p53 by T cells

Since p53 is an intracellular protein, p53-specific antibodies are unlikely to exert immunotherapeutic antitumour effects. Targeting of tumours through the p53 protein thus relies on recognition of p53-derived peptide epitopes by T cells in the context of MHC class I molecules at the surface of tumour cells (Fig. 2). As mutation of p53 is a frequent event in carcinogenesis, one possibility is to direct the T-cell attack against peptide epitopes that comprise the mutated residue(s). Given that p53 is a ubiquitously expressed autoantigen, this approach would have the advantage of avoiding immunological tolerance (the mechanism that prevents destruction of cells carrying normal p53) without inducing autoimmunity. Mutant p53 peptides would constitute true tumour-specific target molecules. However, unlike mutations in

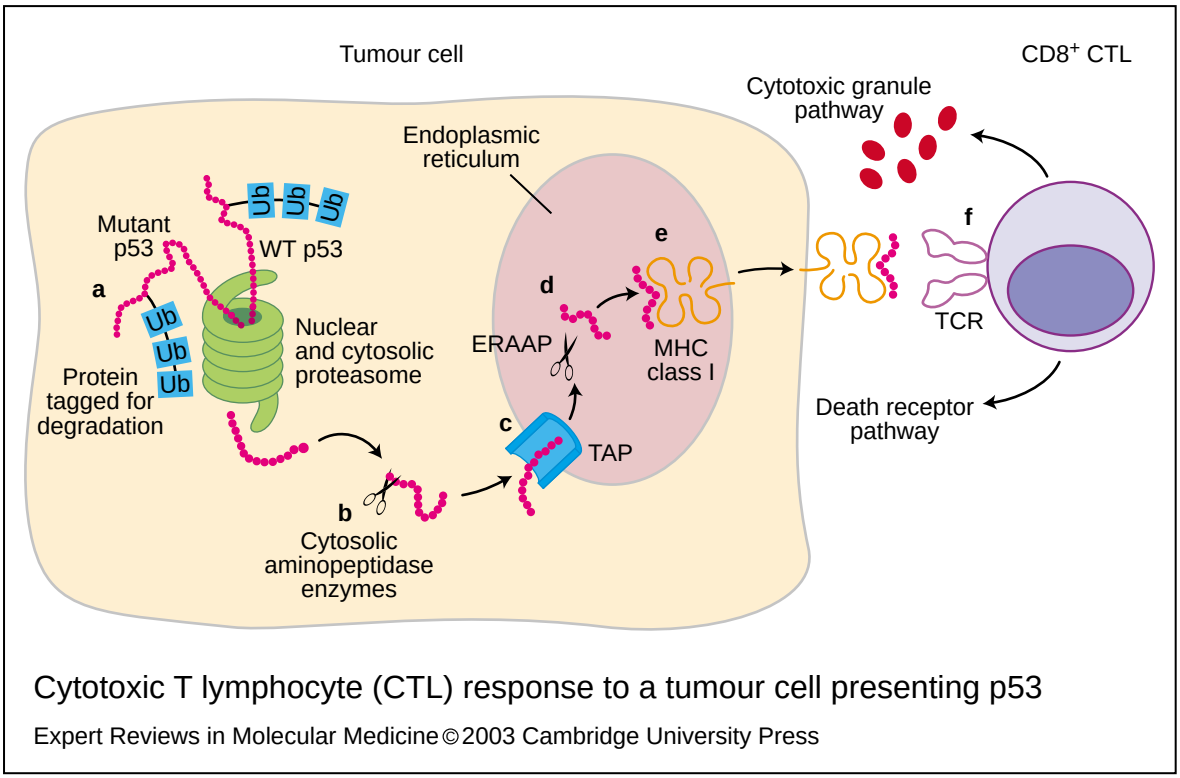


**Figure 2. Cytotoxic T lymphocyte (CTL) response to a tumour cell presenting p53**. (a) Mutant or wild-type (WT) p53 protein is marked for degradation by ubiquitination (shown as Ub chains), and is fragmented into peptides of up to 15 amino acids in length by nuclear and cytosolic proteasomes. (b) Peptides are further shortened by aminopeptidase enzymes in the cytosol and (c) are transferred to transporters associated with antigen processing (TAP), which translocate the p53-derived peptides into the endoplasmic reticulum (ER). (d) Here, peptides are again trimmed by ER-resident enzymes, such as the ER aminopeptidase associated with antigen processing (ERAAP). (e) Peptides of approximately 9 amino acids in length are rescued from continuous degradation if they fit into the peptide-binding groove of nascent major histocompatibility complex (MHC) class I molecules in the ER. (f) MHC class I–peptide complexes traverse the Golgi and are presented on the cell surface of the tumour cell for recognition by the T-cell receptor (TCR) of p53-specific, MHC class I-restricted $CD8^+$ effector CTLs. Cognate interaction of high-affinity, WT p53 peptide-specific and MHC class I-restricted TCRs with these complexes results in the release of cytolytic granules containing perforin and granzymes by the CTL. Furthermore, CTLs can trigger death receptors at the target cell surface (i.e. CD95). These triggers induce death of the tumour cell through apoptosis **(fig002mtm)**.



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the ras oncogenes, those in p53 are highly diverse (Ref. 8). Immunotherapy based on the mutated part of p53 would therefore require prior identification of the p53 mutation in patient tumours, followed by generation of a patient-tailored vaccine or T-cell-induction protocol (the ex vivo expansion of antigen-specific T cells for subsequent adoptive transfer into cancer patients). Furthermore, the mutated region of a given p53 allele might not encode a peptide that is processed into one of the patient MHC molecules, or it might not be of sufficient immunogenicity to serve as target antigen for the T-cell immune repertoire. In fact, the known examples of mutant p53-derived T-cell epitopes are extremely scarce (Refs 14, 15).

As a result of these limitations, research on p53-targeted immunotherapy has primarily focused on epitopes comprising wild-type (WT) p53 sequences (Refs 3, 6, 16, 17, 18, 19). This is based on the concept that aberrant expression of p53 in tumour cells provides a window of opportunity for T cells to discriminate between malignant and normal somatic cells. Accumulation and overexpression of mutant p53 in tumours, as opposed to low-level expression of WT p53 in normal cells, leads to elevated levels of MHC class I-restricted presentation of peptides representative of WT p53 sequences. Combating these WT p53 epitopes also has the advantage that it allows targeting of tumours that exhibit abnormalities in p53 metabolism caused by mutations in proteins other than p53 (e.g. MDM2 or $p14^{ARF}$ mutations).

## p53-specific cancer immunotherapy

### Tumour eradication by p53-specific CTLs

A wealth of published data demonstrates the efficacy of MHC class I-restricted CTLs in the eradication of tumours (Ref. 20). Investigations concerning p53-specific immunotherapy of cancer have so far been predominantly focused on p53-specific CTL immunity rather than T helper (Th)-cell immunity because the majority of solid tumours lack expression of MHC class II (which is recognised by $CD4^+$ Th cells).

Over the past few years, several laboratories, including ours, have found evidence that WT p53-derived peptides can be presented in the context of both murine and human MHC class I molecules. Because p53 is subject to ubiquitin/proteasome-mediated degradation (Ref. 10), it is conceivable that this degradation generates a repertoire of p53-derived peptides available for translocation by transporters associated with antigen processing (TAP) into the endoplasmic reticulum (ER), followed by binding to nascent class I MHC molecules (Refs 16, 17) (Fig. 2). Accordingly, tumour cells presenting such epitopes can be recognised by WT p53-specific CTLs in vitro (Refs 3, 5, 16, 17, 18). Importantly, it has been demonstrated that such CTLs are able to eradicate tumours efficiently in vivo (Refs 6, 16, 19). These experiments further revealed that p53-specific CTL-based tumour eradication in p53-proficient mice (e.g. mice with normal WT p53 expression) occurred without inflicting damage to normal tissues, indicating that these CTLs were truly tumour specific (Ref. 6).

High steady-state levels of p53 were not a prerequisite for tumour regression by p53-specific CTLs. Instead, p53 turnover appeared to be an important factor governing the susceptibility of malignant cells to CTLs (Ref. 19). As a result, tumours expressing non-mutated p53 could also serve as targets for killing by p53-specific CTLs. It is conceivable that this feature relates to the observation that the function and metabolism of p53 can also be profoundly modified by the expression of viral oncogenes or alteration of other cellular regulatory genes. Both overexpression and slow turnover of mutant p53 protein, as well as aberrant expression/metabolism and high turnover of WT p53 protein, result in presentation of high copy numbers of WT p53 peptide–MHC class I complexes on tumour cells as compared with low copy numbers on normal cells (Refs 16, 17, 19). Thus, targeting p53 with CTL-mediated immunotherapy is not associated with a high risk for autoimmune disease and can be extended to a wider range of tumours than has so far been anticipated.

### p53-specific self-tolerance

Once obtained, p53-specific CTLs appear to be fully efficient in mediating antitumour responses. However, self-tolerance to this ubiquitously expressed molecule (Ref. 21) is a major barrier for generating such immune cells. In five studies in p53-proficient mice, p53-specific vaccination was reported to induce protective immunity against a subsequent challenge with p53-overexpressing tumours (Refs 22, 23, 24, 25, 26). Although these results were interpreted to indicate that the protective antitumour response involved p53-specific CTLs,



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implying that efficient CTL immunity to p53 can be raised in p53-proficient subjects (e.g. mice and individuals with normal WT p53 expression), in only one of these studies was limited evidence provided to support this notion directly (Ref. 22). Other reports that demonstrate the induction of p53-specific CTLs in p53-proficient mice or in cultures of human lymphocytes do not present direct proof for the efficacy of such CTLs in response to tumours in vivo (Refs 27, 28, 29, 30, 31, 32, 33).

In contrast to these reports on the induction of p53-specific CTLs, studies by three independent laboratories involving adoptive transfer of p53-specific CTLs into tumour-bearing mice report CTL-mediated antitumour responses in vivo. In two of these studies, the CTLs were capable of delaying but not prohibiting tumour outgrowth (Refs 34, 35), whereas two other reports show that such CTLs can entirely clear tumours from mice (Refs 6, 19). Although multiple discrepancies between the experimental settings could explain the different outcomes, it should be emphasised that CTLs isolated from p53-proficient mice were employed in the two former reports, whereas the latter studies took advantage of CTLs generated in p53-deficient animals.

It is tempting to speculate that the difference in the source of CTLs is crucial. In fact, it has been unambiguously demonstrated that a considerable degree of p53-specific self-tolerance exists in p53-proficient, as opposed to p53-deficient, mice at the level of MHC class I-restricted T cells (Refs 5, 18). This p53-specific self-tolerance results in a peripheral p53-reactive T-cell repertoire that is devoid of high-avidity, MHC class I-restricted CTLs with only low-avidity, if any, T cells remaining (Refs 5, 6, 18, 33, 36). Similar observations have been made for CTL responses to artificial autoantigens in transgenic mouse models (Refs 37, 38, 39).

The WT p53 protein is expressed in the thymus (Ref. 21) and high-avidity, MHC class I-restricted, p53-specific CTL precursors might be subject to T-cell deletion during thymic T-cell selection mechanisms (Fig. 3a). The steady-state level of p53

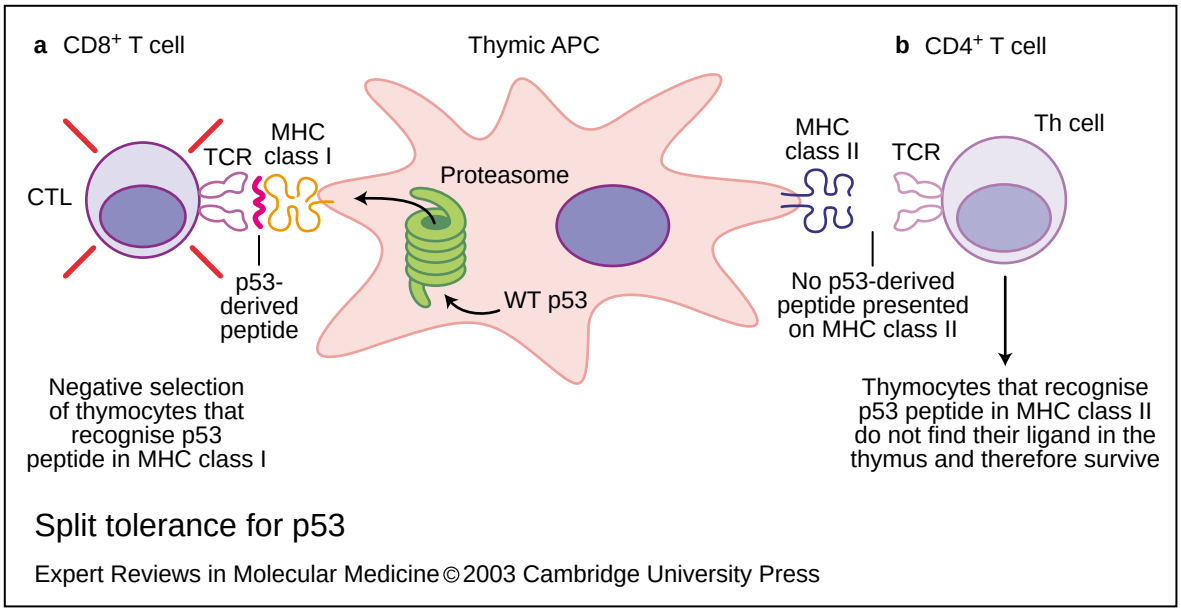


**Figure 3. Split tolerance for p53.** (a) In thymic antigen-presenting cells (APCs), as in the vast majority of normal somatic cells, wild-type (WT) p53 exhibits a very short half-life of fewer than 30 minutes. Degradation of p53 is mediated through the ubiquitin/proteasome-dependent pathway, which results in efficient delivery of p53-derived peptide antigens for loading onto major histocompatibility complex (MHC) class I molecules. During T-cell selection in the thymus, those thymocytes that recognise p53 epitopes in the context of MHC class I with high avidity are likely to be deleted, in order to maintain self-tolerance in the periphery. (b) Because of the prevalence of p53 presentation via MHC class I, p53-derived peptides are unlikely to become available either for direct presentation in MHC class II on this APC or for cross-presentation on other APCs. Thus, thymocytes that would be capable of recognising p53 in the context of MHC class II do not find their ligand in the thymus and can make it to the periphery **(fig003mtm)**.



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in thymic antigen-presenting cells (APCs) is low, owing to the fact that newly synthesised WT p53 is particularly sensitive to ubiquitin/proteasomal degradation (Ref. 10). Although this prevents accumulation of p53 protein, it is also likely to result in processing of p53-derived peptide epitopes and binding to MHC class I molecules of thymic APCs. It is therefore conceivable that high-avidity immature T cells encounter WT p53 epitopes in the context of MHC class I in the thymus and thus become negatively selected (deleted) from the repertoire (Fig. 3a). By contrast, WT p53-derived peptides are not available in sufficient amounts for binding by MHC class II in the thymus, because of the prevalence of their presentation via class I (Fig. 3b). As discussed in further detail below, MHC class II-restricted Th-cell immunity to p53 is therefore not limited by p53-specific self-tolerance.

Thus, high-avidity, MHC class I-restricted, p53-specific CTL immunity might be affected by negative selection in the thymus and/or by peripheral mechanisms of tolerance induction (Ref. 40). Regardless of which mechanism occurs, it is clear that the immune repertoire in p53-proficient subjects is mostly limited to low-avidity CTLs. Consequently, sufficient numbers of CTLs with high avidity for naturally processed p53 peptides presented by self-MHC class I on tumour cells are not present in humans and are thus not available to attack cancer cells.

## Future directions of p53-specific immunotherapy

### Bypassing p53-specific self-tolerance

The T-cell receptor (TCR) repertoire appears to be the limiting factor determining p53-specific self-tolerance (Refs 5, 6, 18), thus it is unlikely that this tolerance can be bypassed by potent vaccines that provide strong costimulatory events while inhibiting tolerogenic signals. Although such approaches used for melanoma-specific immunisation resulted in both high-avidity and high-frequency melanoma-antigen-specific CTL responses, high-frequency CTL activities were of low avidity in the case of p53 (Refs 32, 34, 36, 41, 42, 43, 44). Likewise, immunisation with modified CTL epitopes that provide increased MHC class I binding affinity is also expected to fail because low-avidity (as opposed to high-avidity) CTLs would predominantly be induced (Ref. 45).

However, self-MHC-restricted tolerance can be circumvented both in HLA-transgenic mice and by taking advantage of allogeneic MHC class I-restricted, yet epitope-specific, CTLs (Refs 3, 5, 7, 46, 47) (Fig. 4). Indeed, a tumour- and leukaemia-associated CTL epitope derived from the widely expressed and p53-dependent human MDM2 oncoprotein was recently identified and used to bypass self-tolerance to this UTAA in HLA-A*0201 (A2.1)-transgenic mice and by generating $A2.1^{-}$, allo-A2.1-restricted human T cells (Ref. 7). A broad range of malignant, as opposed to nontransformed, cells were killed in vitro using high-avidity transgenic murine and allogeneic human CTLs specific for the A2.1-presented MDM2 epitope. Whereas the self-A2.1-restricted human T-cell repertoire gave rise only to low-avidity CTLs unable to recognise the natural MDM2 peptide, human $A2.1^{+}$ T cells were turned into efficient MDM2-specific CTLs upon expression of WT and partially humanised high-affinity TCR genes derived from the transgenic mice (Fig. 4a). These results demonstrate that transfer of TCR genes can be used to circumvent self-tolerance of autologous T cells to UTAAs and thus provide the basis for a broad-spectrum immunotherapy of malignant disease based on TCR gene transfer (Ref. 7).

A similar example of this approach is the adoptive transfer of murine T cells transduced with a TCR that recognises the immunodominant H-$2D^{b}$-restricted CTL epitope of the influenza A/NT/60/68 nucleoprotein (NP) encompassing residues 366–374; this strategy was able to protect recombination-activating gene (RAG)-deficient mice against challenge with a tumour that expressed the A/NT/60/68 NP epitope (Ref. 48). There was an impressive, up to 1000-fold, expansion of transduced cells in vivo following influenza infection. TCR-transduced T cells were capable of surviving for at least 80 days after transfer and were functional after viral challenge (Ref. 48). Delivery of genes encoding tumour-specific TCR molecules into T cells thus allows the efficient generation of an immune response.

The ability to bypass self-tolerance by induction of $A2.1^{-}$ and allo-A2.1-restricted human CTLs specific for WT p53-derived peptides is likely to offer an opportunity to provide TCR genes of primary human origin for therapeutic transfer (Refs 7, 46, 47) (Fig. 4b). Yet another appealing option is to turn low-affinity human TCRs restricted to self-MHC class I into high-affinity, WT p53-specific TCR molecules by in vitro mutagenesis (Refs 49, 50, 51) (Fig. 4c).



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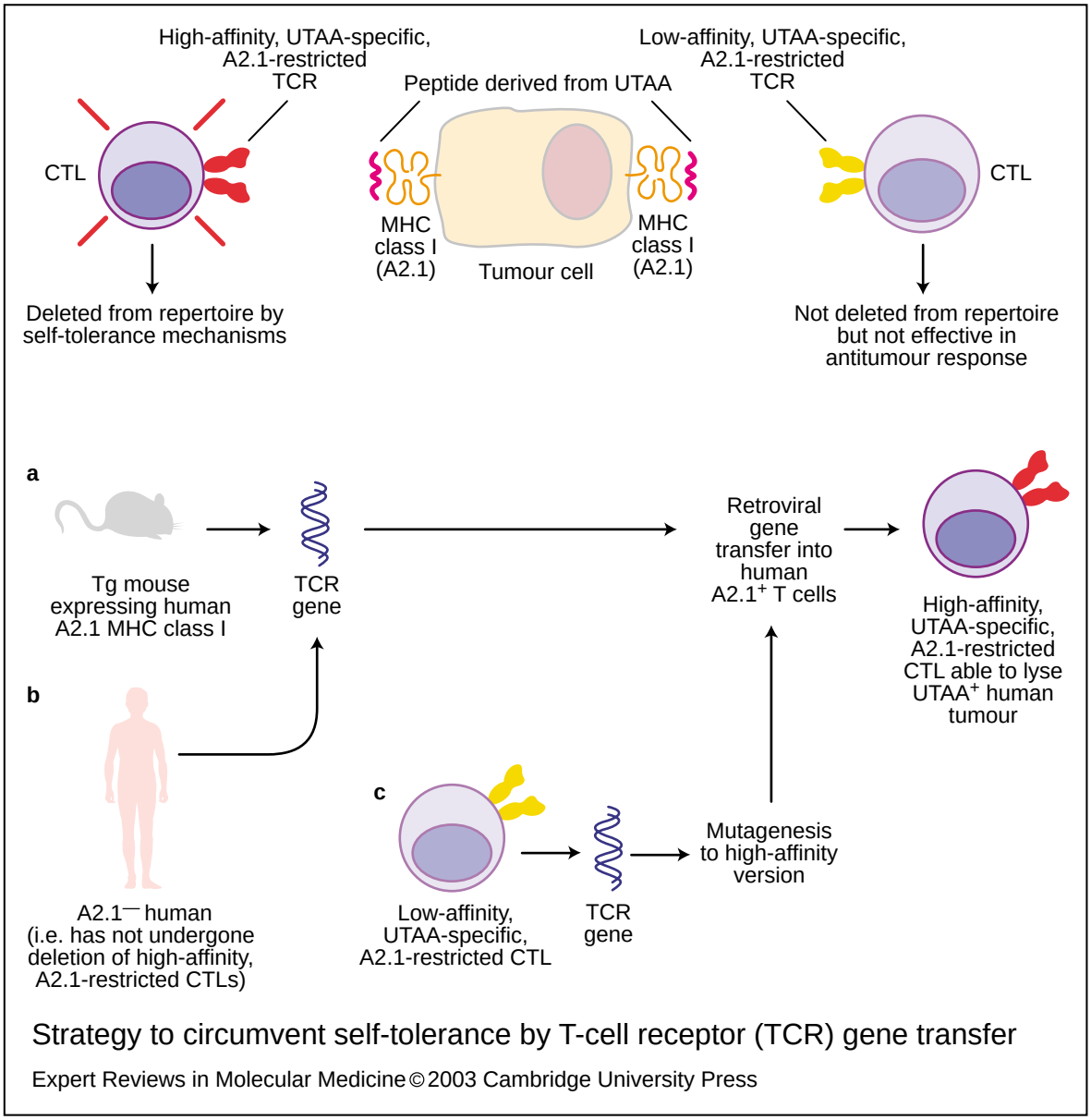


Strategy to circumvent self-tolerance by T-cell receptor (TCR) gene transfer



**Figure 4. Strategy to circumvent self-tolerance by T-cell receptor (TCR) gene transfer.** In this example, showing haplotype HLA-A*0201 (A2.1), the autologous self-MHC class I, A2.1-restricted, T-cell repertoire is devoid of high-avidity, universal tumour-associated antigen (UTAA)-specific CTLs [i.e. those CTLs recognising antigens that correspond to wild-type (WT) p53 epitopes or the MDM2 epitope] because of self-tolerance mechanisms. This could be circumvented in three possible ways. (a) T cells derived from transgenic (Tg) mice expressing human A2.1 recognise sequence differences between human and murine UTAAs and can thus be used as a source of CTLs that carry high-affinity, UTAA-specific TCRs that recognise p53 or MDM2 in the context of human MHC class I A2.1. The TCR genes can be cloned from these cells and, by transferring into a retroviral vector, can be used as the basis of an adoptive anti-p53/MDM2 immunotherapy. Alternatively, (b), as self-tolerance is restricted by self-MHC, A2.1-restricted human T cells of high avidity for UTAAs can also be raised from allogeneic (i.e. $A2.1^{-}$) individuals. The genes obtained from allogeneic human CTLs encoding high-affinity, UTAA-specific TCRs can also be cloned into a retroviral vector and transduced into $A2.1^{+}$ human T cells. Another alternative, (c), would be to mutate low-affinity, UTAA-specific, human TCR genes in order to obtain high-affinity counterparts and clone these into a retroviral vector. Using one of these strategies, the autologous T-cell repertoire of a cancer patient could thereby be equipped with effective UTAA-specific and tumour-reactive CTLs that they themselves had lost because of self-tolerance **(fig004mtm)**.



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It therefore seems possible to replenish the human T-cell repertoire with high-affinity, p53-specific TCR molecules obtained either by: (1) circumventing self-tolerance to p53 in p53-proficient and p53-deficient HLA-transgenic mice; (2) using allorestricted human CTLs; or (3) mutagenesis of low-affinity, p53-specific human TCR genes (Refs 7, 48, 49, 52, 53). A limited selection of cloned TCRs recognising defined WT p53 epitopes presented by common HLA class I molecules would be sufficient to cover the majority of the patient population.

### Th-cell immunity to p53

Self-tolerance appears to be less profound in the case of p53-specific Th-cell responses. It has long been observed that the serum of tumour-bearing mice and cancer patients contains p53-specific IgG-type antibodies (Refs 54, 55). This points at the presence of an underlying p53-specific Th-cell response. Indeed, evidence for anti-p53 Th-cell immunity in humans has recently been obtained (Refs 56, 57, 58). It appears that accumulated mutant p53 protein released from dying tumour cells and taken up by professional APCs can serve as potent immunogens for B-cell and Th-cell activity. By contrast, the very low steady-state levels of WT p53 protein in normal cells, although sufficient for MHC class I-restricted tolerance induction, are likely to be inadequate for Th-cell tolerisation by (cross)presentation via MHC class II, therefore enabling these T cells to survive negative selection and/or peripheral tolerisation, and be available for anti-p53 immunity at a later stage (Fig. 3b).

Recent experiments confirm that WT p53-specific Th-cell immunity can be raised in p53-proficient mice and humans (Refs 59, 60). Despite the fact that most cancer cells lack expression of MHC class II, precluding direct attack on these malignancies by Th cells, cumulative evidence has shown that specific Th-cell activity is of pivotal importance for efficient eradication of such tumours (Ref. 61). For instance, vaccination with a tumour-specific Th-cell epitope has been reported to induce partial protective immunity in response to MHC class II$^-$ tumours while synergising with CTL epitope immunisation in inducing complete protection (Ref. 62).

In several other mouse models, similar studies have found that tumour-specific CD4$^+$ Th cells critically contribute to the development and efficacy of antitumour immune responses (Ref. 63). Th cells were also shown to exert their tumour-reactive effect independently of CD8$^+$ CTLs by recruitment of innate immune effector cells, such as tumouricidal macrophages and eosinophils (Ref. 63). In other cases, Th cells were found to drive CTL-based antitumour immunity. By triggering professional APCs through CD40–CD40 ligand interaction (Refs 64, 65, 66) and by interferon γ release (Ref. 67), Th cells deliver an essential activation signal to APCs. As a result, the APC will display its entire array of antigen-presenting and costimulatory functions, of which the latter are considered key for providing naive CTLs with a license to kill. Whereas the delivery of this type of T-cell help to CTLs requires the APC to present both the MHC class I-restricted CTL epitope and the MHC class II-restricted Th-cell peptide, these antigens do not have to be derived necessarily from the same protein (Ref. 62). As a result, p53-specific Th cells can also provide help to CTLs directed against other antigens whose expression coincides with aberrations in p53, such as carcinoembryonic antigen (CEA) in colorectal cancer and HER2/neu in breast cancer (Refs 68, 69).

## Concluding remarks and clinical implications

In conclusion, it is evident that WT p53-specific CTLs are a highly desirable tool for the targeted immunotherapy of a wide array of cancers. Current awareness of the importance of tumour-specific Th-cell responses has boosted the search for Th-cell-specific peptides associated with human malignancy, as well as the development and testing of clinical-grade antitumour vaccines that comprise tumour-associated Th-cell antigens in addition to CTL epitopes. Although self-tolerance appears to hamper the generation of high-avidity, anti-p53 CTLs under various conditions, alternative strategies, such as TCR gene transfer into human CD8$^+$ T cells, offer hope that these hurdles can be bypassed.

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## Further reading, resources and contacts

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DeVita, V.T., Jr, Hellman, S. and Rosenberg, S.A. (eds) (1991) Biologic Therapy of Cancer, Lippincott, Philadelphia

Cancer Immunity, a journal of the Acadamy of Cancer Immunology, provides a peptide database authored by Benoit Van den Eynde and Pierre van der Bruggen that gives sequence information on human T-cell-defined tumour antigens:
http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm

The National Center for Biotechnology Information (NCBI) features a Genes and Disease database that includes an entry for p53, which gives a general description of the gene and links to specific resources such as 3D structure of the protein and OMIM entry:
http://www.ncbi.nlm.nih.gov/disease/p53.html

## Features associated with this article

### Figures

Figure 1. Regulation of p53 function and expression levels in normal and transformed cells (fig001mtm).
Figure 2. Cytotoxic T lymphocyte (CTL) response to a tumour cell presenting p53 (fig002mtm).
Figure 3. Split tolerance for p53 (fig003mtm).
Figure 4. Strategy to circumvent self-tolerance by T-cell receptor (TCR) gene transfer (fig004mtm).

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