Hematology Meeting Reports 2007; 1(5):33–42

The role of alemtuzumab in chronic lymphocytic leukaemia patients with p53 defects

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A B S T R A C T

Among the biological prognostic factors so far identified in chronic lymphocytic leukaemia (CLL), the most powerful predictor of short survival is mutation or deletion of the TP53 gene at 17p13, which encodes the p53 protein. p53 is a transcription factor that regulates cell growth and survival, contributes to the action of DNA-damaging chemotherapy, and plays a pivotal role in maintaining genomic integrity. TP53 defects are rare at diagnosis but more common in patients with progressive and chemo-resistant disease. Furthermore, p53 dysfunction can arise through alternative mechanisms such as inactivation of ATM, which activates p53 in response to double-strand DNA breaks, or extrinsic suppression of the p53 pathway by basic fibroblast growth factor, which is abundant in the CLL-cell micro-environment. However, mutation or loss of p53 itself is associated with the worst prognosis. Such patients do badly due to rapid clonal expansion, clonal instability and resistance to chemotherapy. Fortunately, two treatments in routine clinical use, glucocorticoids and alemtuzumab, have established activity against p53-defective CLL and work independently of p53. Although each of these agents has its limitations, it is hoped that these can be overcome by using both agents in combination. Early data involving alemtuzumab in combination with high-dose methylprednisolone look promising, and this approach is being formally investigated within the NCRI CLL206 trial. A similar study involving alemtuzumab in combination with dexamethasone is under development by the German CLL Study Group.

Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common haematological malignancies and results in significant morbidity and mortality. It is caused by the clonal expansion of antigen-experienced B cells with a distinctive morphological appearance and surface immunophenotype.[1] One of the most striking features of CLL is the extent of its clinical variability, and one of the most significant developments in CLL research in recent years has been to understand this variability in terms of biological heterogeneity. A number of biological variables have been identified that predict short survival. These include IgVH mutation status,[2,3] CD38 expression,[2,3] ZAP-70 expression[4-6] and certain chromosomal abnormalities.[7] In general, adverse prognostic factors overlap with one another but only to a partial extent. Among the biological prognostic factors so far identified, deletion or mutation of the TP53 gene encoding p53 at chromosome band 17p13 is by far the most ominous predictor of short survival.[8-17] This obser-

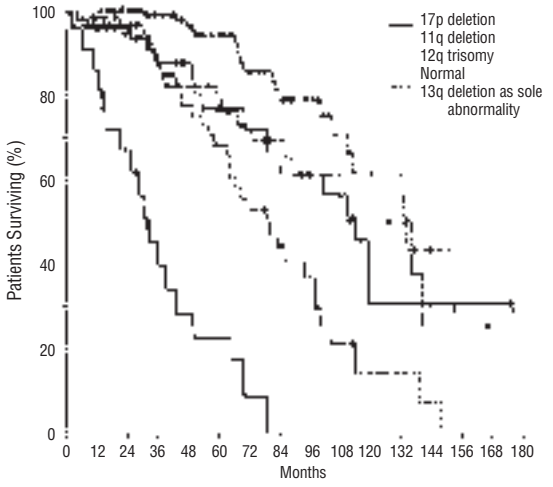

Figure 1: Overall survival in newly diagnosed patients is reduced in patients with deletion or mutation at 17p.[7]

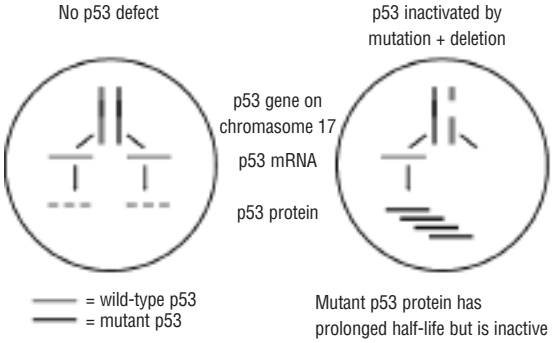

Figure 2: Typical pattern of p53 activation.

vation applies to cohorts of both newly diagnosed patients and those who have progressed to the point of requiring therapy (Figure 1) (German CLL4 trial data).[7]

p53 is a transcription factor that is activated by DNA damage and co-ordinates the cellular response to such damage by triggering apoptosis or cell-cycle arrest.[18,19] This transcription factor also contributes directly to the repair of some forms of DNA damage.[20] p53 can be activated by factors other than DNA damage such as hypoxia, nucleotide depletion and activated oncogenes and, in doing so, it fulfils an important function in regulating cell growth and survival in the face of a diverse range of cellular stresses while playing a pivotal role in maintaining genomic integrity following DNA damage.[21] In keeping with this, p53 is inactivated by mutation/deletion in about half of all human cancers,[22] with the usual defect being mutation of one allele and deletion of the other.[23] Despite lacking wild-type function, mutant p53 is present at increased levels owing to its prolonged half-life.[24,25] This is summarised in Figure 2.

First principles dictate that the outcome of any cancer is determined by three main variables intrinsic to the malignant clone: rate of clonal expansion, degree of clonal instability, and sensitivity to available treatments. Based on its known biological functions, wild-type p53 should have a beneficial effect on all of these variables. Conversely, cancer cells lacking wild-type p53 should be predisposed to rapid growth, clonal evolution and chemoresistance. There is evidence that this is indeed the case in CLL. For example, p53 defects are associated with advanced clinical stage, short lymphocyte doubling time, and short time to first treatment – all features of rapid clonal expansion.[12,13,15] p53 defects are also associated with large-cell transformation indicative of clonal instability,[12,13] and with resistance to chemotherapy (*see below*).

p53 defects in patients with chronic lymphocytic leukemia

In CLL, TP53 is mutated or deleted in only a minority of cases at diagnosis, although a higher proportion acquire p53 defects during the course of the disease. Cordone et al.[13] examined CLL cells from 181 patients for evidence of p53 protein over-expression using immunocytochemistry and found strong expression of p53 protein in 15% of cases. The coexistence of p53-positive and p53-negative cells within the same CLL population suggested that p53

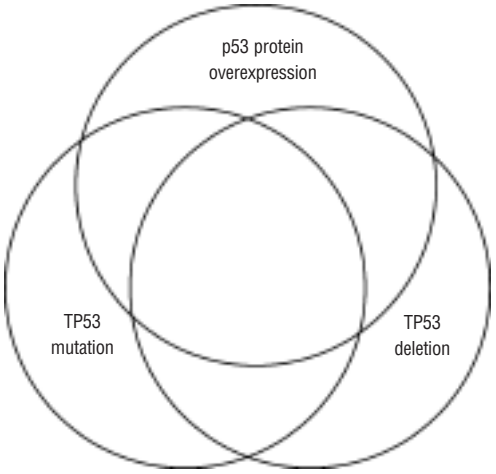

Figure 3: Detection of p53 defects.

defects were probably arising as late event during disease evolution. p53 positivity correlated with disease stage, the proportion of p53-positive cases increasing significantly from Binet stage A (7.4%) to stage B (24.4%) and C (29.2%) (p=0.002). p53 positivity also correlated with disease phase, p53 over-expression being observed in 7.1% of newly diagnosed patients, in 20% of patients studied during the course of the disease, and in 35.3% of patients with overt disease progression (p=0.0001). TP53 mutations were found in 15 of 17 cases that over-expressed p53 protein (88%).

Thornton et al.[17] studied 115 patients with CLL (90 untreated and 25 heavily pretreated/refractory) for allelic loss at chromosome 17p by FISH and for p53 protein over-expression by flow cytometry. A total of 17 cases were identified with TP53 deletion and/or protein over-expression. Ten out of the 17 patients were found to have both types of p53 abnormality, 6 had one or the other abnormality, and flow cytometry failed in one case with a TP53 deletion. Material for direct DNA sequencing was available in 14 of 17 cases. Mutations were found in 7 cases (50%), all of which displayed over-expression of p53 protein and 5 of which had allelic loss of TP53. Mutations were not found in 13 of 14 other cases without TP53 deletion or p53 protein over-expression. The overall frequency of p53 abnormalities in this series was 15%, with a significant difference between untreated patients (7%) and those with pretreated/refractory disease (50%; p<0.01). Abnormal p53 was predictive for shorter survival, regardless of the method used. In summary, TP53 deletion, TP53 mutation and p53 protein over-expression each predict for poor outcome and overlap with one another (Figure 3). In routine clinical practice, the most simple and reliable way of detecting p53 defects is to look for deletion of the TP53 gene at 17p13 by FISH. This technique should be within the capabilities of any experienced genetics laboratory.

Functional impairment of the p53 pathway can arise through mechanisms other than structural abnormalities of the TP53 gene. There appear to be two ways in which this can occur: intrinsic defects of components of the p53 pathway other than p53 itself, and extrinsic suppression of the pathway by factors in the *in vivo* micro-environment. To investigate intrinsic defects of the p53 pathway, CLL cells from 43 patients were treated with ionising radiation (IR) and examined by Western blotting for changes in the expression of p53 and p21, an important transcriptional target of p21. Wild-type p53 is normally present at low levels owing to its short half-life but accumulates in the nucleus following its activation and regulates gene expression. Thirty of the 43 cases (70%) demonstrated a normal p53 response, with an increase in levels of both p53 and p21 following IR. However, p21 up-regulation was impaired in the remaining 13 cases (30%), indicating p53 dysfunction. In 6 of the p53-dysfunctional cases, baseline levels of p53 were increased (*type A* p53 dysfunction); each of these cases was found to have a TP53 mutation. In the remaining 7 cases with p53 dysfunction, p53 failed to increase following IR

(*type B* p53 dysfunction); each of these cases was found to have mutation in ATM (ataxia telangiectasia mutated), a kinase encoded at 11q23 that phosphorylates and activates p53 in response to double-strand DNA breaks. CLL cells with type A p53 dysfunction were completely resistant to radiation-induced apoptosis, whereas those with the type B defect were partially resistant.[26] Although ATM mutations were previously known to occur in CLL,[27,28] this was the first direct demonstration in human cancer cells that functional impairment of the p53 pathway could arise through a mechanism other than TP53 gene mutation.

CLL patients with p53 dysfunction detected in this way were found to have relatively unmutated IgVH genes (<5%), preferentially use the VH3-21 gene segment and have a poor prognosis.[29] The "p53 function test" has subsequently been adapted for flow cytometry,[30] and when detected in this way p53 dysfunction was found to correlate only imperfectly with deletion of TP53 and ATM, suggesting that chromosomal and p53 functional analysis might provide complementary prognostic information.[31] The prognostic value of p53 dysfunction and its relationship with other prognostic factors is currently under further investigation within the UK CLL4 trial. Other studies have confirmed that ATM mutations in CLL are associated with impaired p53 activation and adverse clinical outcome.[32,33] CLL cells probably depend heavily on ATM for p53 activation as they express very low levels of ATR (ATM-related) protein, which has structural and functional homology with ATM (Jones et al., 2004).[34]

The second mechanism through which p53 dysfunction can arise in CLL in the absence of TP53 gene defects involves extrinsic suppression of the p53 pathway by factors in the tumour-cell microenvironment. CLL cells were cultured in the presence or absence of basic fibroblast growth factor (bFGF) and exposed to IR to induce p53 accumulation. bFGF is greatly increased in the plasma of CLL patients and can suppress p53 activation in some experimental models. IR induced a marked increase in p53 levels in 28 samples from 24 patients. bFGF inhibited IR-induced p53 accumulation to some extent in most of these samples and by more than 50% in 7 samples from 7 patients. Suppression of p53 activation by bFGF was frequently but not always accompanied by up-regulation and/or activation of the p53-inhibitory protein MDM2 and was associated with impaired transcriptional activation of the p21 gene. Thus, CLL cells with no intrinsic defects of the p53 pathway appear to have the potential, at least in some cases, to acquire p53 dysfunction in their *in vivo* micro-environment.[35] The clinical significance of this observation remains unclear but it seems likely that, *in vivo*, functional impairment of the p53 pathway may be more common than has previously been suspected.

Resistance to treatment in chronic lymphocytic leukemia patients with p53 defects

Numerous studies of thymocytes, spleen cells and haemopoeitic cells have shown that anti-cancer agents that cause DNA damage exert their cytotoxic effects at least in part through the induction of p53-dependent apoptosis.[36-40] In keeping with this, p53 defects in CLL have been strongly linked to resistance to alkylating agents and purine analogues in in-vitro studies,[41-44] in retrospective clinical studies,[8-10,45] and most importantly in prospective clinical trials of first line therapy.[46-49]

The German CLL4 study evaluated first-line treatment with fludarabine or fludarabine in combination with cyclophosphamide (FC) in CLL patients aged ≤65 years (n=475).[48] FC produced a significantly higher overall

response rate (ORR) (94% *vs*. 83%, *p*=0.001), longer median progression free survival (PFS) (48 *vs*.20 months, *p*=0.001), but no difference in overall survival (OS) compared with fludarabine alone. Analysis of prognostic factors within the trial has shown that the ORR was significantly lower in patients with 17p- (53.8% vs 89.6%, *p*=0.001). 17p- was also associated with a significantly shorter PFS (median 11.0 vs 24.1 months, p=0.002) and OS (median 15.9 months vs not reached, 75% survival at 43.8 months; *p*<0.001) (Figure 1). Multivariate analyses confirmed 17p- as the most significant adverse prognostic factor for both PFS (*p*=0.001) and OS (*p*<0.001).

Further evidence for resistance to first-line chemotherapy in CLL patients with p53 defects comes from the study by Bosch et al.[49] This study assessed the use of fludarabine (25 mg/m^2 iv on days 1 to 3), cyclophosphamide (200 mg/m^2 on days 1 to 3) and mitoxantrone (6 mg/m^2 iv on day 1) (FCM), given at a 4-week intervals in untreated patients aged <65 years with a diagnosis of CLL.[49] For evaluable patients (n=64, median age: 58 years), the ORR was 88%, with a response duration at 36 months of 55%. Ten percent of patients had a 17p deletion detected by FISH. All patients without 17p- responded to treatment with FCM, whereas none of the patients with 17p- achieved a response (*p*=0.003).

Effective treatment approaches in chronic lymphocytic leukemia patients with p53 defects

In the light of the marked resistance to chemotherapy observed in CLL patients with p53 defects, one of the most pressing therapeutic challenges in CLL is to develop novel and effective ways of treating these patients. Such a development would represent a major advance in the treatment of CLL.

In theory, two approaches could be employed to overcome chemo-resistance due to p53 dysfunction: bypassing the defective pathway or restoring functionality to it. There is currently no reliable way of achieving the latter. With regard to bypassing the p53 pathway, two approaches could be used. First, strategies could be developed to induce *catastrophic*' un-repaired DNA damage in the hope of activating p53-independent DNA-damage response pathways. For example poly(ADP-ribose) polymerase (PARP), an enzyme involved in DNA repair that is activated by single-strand DNA breaks, can induce depletion of cellular NAD and ATP resulting in necrotic cell death. Indeed, this mechanism underpins the cytotoxicity of purine analogues in rare cases of CLL.[50,51] Potent and specific inhibitors of DNA repair enzymes are currently under development, and the hope is that such agents will act synergistically with DNA-damaging chemotherapy to effect cell death in this way.

An alternative strategy to circumvent p53 defects is to use cytotoxic agents that do not cause DNA damage. This approach has the theoretical advantage of being potentially less toxic and less likely to facilitate clonal evolution – an important consideration given that CLL clones with p53 defects are likely to be genomically unstable. Two such agents with established activity against p53-defective CLL are, in fact, already in clinical use: corticosteroids and alemtuzumab.

Glucocorticoids induce apoptosis of lymphoid cells independently of p5336–40 and are cytotoxic to cultured CLL cells irrespective of their p53 status.[44] At the clinical level, high-dose methylprednisolone (HDMP), alone or in combination with other agents, produced an ORR of 77% in a cohort of 25 patients with advanced refractory CLL.[52] Responders included 5 out of 10 patients with p53 defects, two of whom achieved a nodular partial

Table 1: Summary of data: alemtuzumab treatment in CLL patients with p53 deletions.

Authors	*Number of patients*	*Number of patients with p53 deletions*	*Overall treatment response in p53 deleted group*
Osuji et al (2005)	28	8	50%*
Lozanski et al (2004)	36	15	40%
Stilgenbauer et al (2005)	50	13	53.8%*

**No complete remissions documented.*

response (nPR). In addition, HDMP was effective at inducing regression of bulky lymphadenopathy. There were no differences in response irrespective of whether HDMP was used alone or in combination with chemotherapy, and the response rate was not significantly different between cases with or without p53 defects.

Alemtuzumab, a humanised anti-CD52 monoclonal antibody that has been approved for clinical use in patients with fludarabine-refractory CLL, is another agent of value in the treatment of p53-deleted CLL. Alemtuzumab induces responses in around 30–40% of previously treated patients with CLL when given via the intravenous route.[53-56] Lundin et al.[57] reported an ORR of 87% in previously untreated patients when alemtuzumab was given subcutaneously, and two thirds of patients achieved a CR or nodular PR. When deciding on the treatment schedule it is important to bear in mind the limited activity of alemtuzumab in patients with bulky lymphadenopathy.[58]

The cytotoxicity of alemtuzumab results from complement-mediated cell lysis and antibody-dependent cell-mediated cytotoxicity,[59] neither of which should in theory involve the p53 pathway. In keeping with this, alemtuzumab was reported to induce a durable CR in a patient with chemo-resistant CLL who had a deletion of one TP53 allele and a point mutation of the other.[60] Subsequent reports have shown response rates in the order of 40–50% among pre-treated patients with p53 defects, with most responses being partial. These response rates were similar to those seen in patients without TP53 defects (Table 1).[61-63]

Lozanski et al. sought to determine whether alemtuzumab was effective in CLL patients with p53 defects.[61] Thirty-six patients with fludarabine-refractory CLL were treated with alemtuzumab, 15 (42%) of whom had p53 mutations or deletions. Clinical responses in patients with TP53 mutations, deletions, or both were noted in 6/15 patients (40%) compared with 4/21 (19%) of patients without these genetic abnormalities.[61] The median response duration for patients with p53 defects was 8 months (range: 3–17 months).

Osuji et al. reviewed the efficacy of alemtuzumab in the treatment of 28 patients with refractory CLL in whom p53 status was known. TP53 deletions were present in eight patients (28.6%) and affected >20% of cells in 6 of these 8 cases.[63] The overall response rate was 53.6% (CR 18%, PR 36%, stable disease 36%, progressive disease 10%) with no significant difference between patients with (ORR 50%; all PR) or without (ORR 55%: CR 25%, PR 30%) TP53 deletions (p=0.214). Four of the six patients with >20% p53-deleted cells achieved a PR and two had stable disease.

Emerging data from the German CLL2H trial of alemtuzumab in fludarabine-refractory CLL indicate that ORR and PFS were unaffected by p53 deletion status.[62] The CLL2H trial of the GCLLSG was initiated to evaluate the subcutaneous administration of 3×30 mg alemtuzumab weekly for a maximum of 12

weeks in patients with fludarabine-refractory CLL following intravenous dose escalation. An interim analysis (n=50) has reported an ORR of 36% (CR 2%, PR 34%), a median progression free survival time of 9.7 months and a median overall survival time of 13.1 months. Analysis of genetic risk factors showed high-risk genomic aberrations in the majority of patients, including 17p- in 29%. However, responses (CR or PR) were observed in 7 of the 13 of 17p- cases (53.8%). This interim data therefore confirms the effectiveness of subcutaneous alemtuzumab in fludarabine-refractory patients with 17p- CLL.

Although single-agent therapy with alemtuzumab or HDMP appears effective in some patients with p53-deleted CLL, response durations and the depth of response remain suboptimal. Given that the two drugs work by separate mechanisms and preferentially target different body compartments, a protocol has been developed to combine these agents in an attempt to overcome their individual limitations in p53-defective CLL and maximise any potential mechanistic synergy between them. This regimen, referred to as *CAM-PRED*, has been designed to achieve maximal cytoreduction as rapidly as possible. This is likely to be an important consideration to in p53-defective CLL as it should in theory reduce the risk of drug-resistant clones emerging in this setting of genomic instability.

CAM-PRED consists of up to four 28-day cycles with each cycle consisting of intravenous methylprednisolone 1.0 g/m^2 on days 1–5 and alemtuzumab 30 mg three times per week on days 1–28 following an initial week of dose escalation. Alemtuzumab is initially administered intravenously during the first treatment cycle to ensure that therapeutic plasma levels are rapidly achieved, and then given via the subcutaneous route from the second cycle onwards. All patients receive prophylaxis with co-trimoxazole and acyclovir, together with granulocyte-colony stimulating factor (G-CSF) and ciprofloxacin if the neutrophil counts fall below 1.0×10^9/L. In addition, regular surveillance for cytomegalovirus (CMV) reactivation is carried out on a weekly basis.

Early data are available on CLL patients with p53 defects who were treated with the CAM-PRED regimen during the development of a phase II clinical trial.64 All treated patients (n=5) had clinically aggressive CLL with bulky lymphadenopathy and clear-cut p53 abnormalities. The latter consisted of TP53 deletion affecting at least 20% of leukaemia cells in 4 cases, protein over-expression in 4 cases and functional impairment in 3 cases. Four patients had a p53 gene deletion plus at least one other type of p53 defect in keeping with the remaining allele being mutated. The TP53 gene could not be analysed in one patient who had relapsed with internal lymphadenopathy only. However, immunohistochemical staining of fixed biopsy tissue showed unequivocal over-expression of p53 protein indicative of gene mutation. Two patients were previously untreated, whereas the other three had received a median of three lines of prior therapy (range 3–6), which included autologous and allogeneic stem-cell transplantation and alemtuzumab monotherapy.

All five patients responded to CAM-PRED treatment with three patients achieving a CR, two of whom had no residual disease detectable in the marrow by a sensitive flow cytometric technique. The remaining two patients achieved a PR or nPR, respectively. Previously untreated patients appeared to respond particularly well, both achieving a CR. All five patients experienced infective episodes during treatment, including CMV reactivation and bacterial chest infections, although four of the five patients were at high risk of infection due to being heavily pre-treated or having a history of prior infective problems. All infections resolved with appropriate

treatment. In addition, haematopoietic toxicity was transient and modest.

In view of the adverse event profile of the CAM-PRED regimen, stringent antimicrobial surveillance and prophylaxis is obligatory in all patients receiving this treatment. Any patient displaying symptoms consistent with infection should be investigated thoroughly and promptly in order to obtain a firm microbiological/virological diagnosis, and appropriate antimicrobial/antiviral therapy should be instituted without delay. For this reason, CAM-PRED should not be administered outside well-equipped medical centres experienced in the management of serious opportunistic infections. The regimen should ideally be given within the framework of an approved clinical study. In the UK, a phase II trial (NCRI CLL206) has recently opened to investigating the efficacy an toxicity of CAM-PRED in untreated and previously treated CLL patients with >20% TP53 deletion, and a similar study is being developed by the GCLLSG. Outside clinical trials, the use of CAM-PRED should probably be confined to fludarabine-refractory CLL with bulky (>5 cm) lymphadenopathy and >20% TP53 deletion, as treatment options for such patients are very limited and the potential risks of the regimen therefore more easy to justify.

Conclusions

p53 dysfunction in CLL can arise through several different mechanisms and patients with these defects do badly. However the most ominous predictor of adverse outcome and resistance to chemotherapy in CLL is deletion or mutation of the TP53 gene itself. The poor prognosis of such patients is due, in part, to resistance to chemotherapeutic agents such as alkylating agents and purine analogues. Treatments with established activity against p53-defective CLL include steroids and alemtuzumab, and combining these agents may overcome the limitations of monotherapy. Early data indicate that the CAM-PRED regimen may be an effective treatment in this setting, particularly in previously untreated patients. Infectious complications were considerable but appear manageable with appropriate antimicrobial prophylaxis and surveillance. If the encouraging preliminary data are confirmed in the NCRI CLL206 trial, the scene will be set for a more tailored and logical approach to the first-line treatment of CLL patients with p53 defects.

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