

## P53 and microRNAs in chronic lymphocytic leukemia

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### Abstract

There has been considerable progress in characterising chronic lymphocytic leukemia with respect to the underlying biology and the corresponding clinical presentation. Most patients with CLL live for years without the need for therapy. This does not hold true for patients that exhibit inactivation of the tumour suppressor p53 or, possibly, its pathway members. Deletions of chromosome 17p and mutations of TP53 are the main characteristic of aggressive and refractory disease. In this review we will outline the current understanding of mechanisms known to contribute to dysfunctional p53 and its connection to microRNAs.

### Introduction

Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in the western world<sup>1</sup> characterized by the accumulation of small, non-dividing B-lymphocytes in various lymphatic tissues, the bone marrow and the blood. Novel findings additionally underline the potential impact of the tumourenvironment on disease progression and refractoriness to therapy. Specifically, the interaction with surrounding T-cells and so called nurse-like cells seems to provide anti-apoptotic and proliferative stimuli.<sup>2</sup>

In most cases the patients live for years without symptoms. However, a considerable portion of patients show a more severe or progressive disease course. Extensive research on the underlying mechanisms has dissected this leukemia with respect to epidemiologic data, biology and the heterogeneous clinical presentation.<sup>3</sup> Nowadays, patients can be stratified to different risk-groups by utilising prognostic markers such as recurrent genomic aberrations<sup>4</sup> and mutational status of TP53<sup>5</sup> or possibly ATM.<sup>6</sup> The status of somatic mutations in the variable regions of the immunoglobulin heavy chain (IGHV) genes,<sup>7,8</sup> biased IGHV usage and stereotyped B cell receptors (BCRs)<sup>7,9,10</sup> exhibit important prognostic value as well. Additional insights and potential for risk-stratification came from the discovery of microRNAs (miRs) as key elements of specific gene-regulation. These small non-coding RNAs were shown to modulate the expression and translation of target mRNA and therefore to act as oncogenes or tumour suppressors.<sup>11-13</sup> Subsequent profiling studies revealed the association with clinical features and prognostic markers. For example, low levels of miR-223 and the miR-29-family were found by independent groups to coincide with unmutated IGHV and disease progression.<sup>14-16</sup> MiRs gained additional attention when it became evident that p53 shows considerable integration to the miR level.<sup>17-20</sup> The finding of this new regulatory mechanism extended the understanding of chemotherapy resistance and at the same time offers opportunities to increase the accuracy of risk-stratification and treatment choice.<sup>3</sup>

Impairment of the p53-network by loss or mutation of single components usually results in detrimental effects and has been shown for a variety of cancers. Though mutations are present in the majority of gastrointestinal- or gynaecological cancers or malignancies of the head and neck<sup>21-23</sup> mutations in haematological malignancies are found only in approx. 10% at presentation.<sup>24</sup> The role of p53 and especially its deregulation has been studied intensively for the last three decades and currently places p53 at the center of a complex regulatory network in tumour suppression.<sup>25,26</sup> Deleterious signals such as oncogene activation, stress and especially DNA double strand breaks induced by radiation or cytotoxic drugs, result in an orchestrated activation of the ataxia telangiectasia mutated (ATM) pathways<sup>27</sup> and consecutive increase of p53-activity.<sup>28,29</sup> Convergence of DNA-damage induced signals to p53 and its negative regulators mouse double minute 2 (MDM2) and mouse double minute 4 (MDM4) facilitates a shift towards a p53 dominance.<sup>30,31</sup> Induction or repression of genes with p53 response elements mediate elementary functions like induction of apoptosis by BBC3 and BAX encoding the proteins PUMA and BCL-2 associated X, cell cycle arrest through CDKN1A encoding p21 and activation of senescence.<sup>26,32-35</sup>

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In 2007 the regulatory network of p53 was extended through the observation that members of the miR-34 family were directly regulated by p53. Up-regulation of these miRs mediated cell cycle arrest and apoptosis by repression of various potential targets including CDK4/6, Cyclin E2, MET and Bcl-2.<sup>17-19</sup> Furthermore, p53 was shown to be involved in miR-processing,<sup>36</sup> to increase the own efficacy through a feedback loop via miR-34a and SIRT1<sup>37</sup> or to be directly repressed by miRs.<sup>38,39</sup>

### Characteristics, clinical presentation and therapeutic consequences in CLL with p53 pathway defects

Early evidence for TP53 and ATM involvement in CLL came from the observation of recurring genomic aberrations of chromosome 17p and 11q in the early 90s and consecutive investigations pointed at the prognostic meaning of loss or mutation of the involved genes, namely TP53 and ATM.<sup>5,6,40-43</sup>

Interestingly, the distribution of these high-risk lesions was found with significant predominance in unmutated IGHV CLL cases.<sup>44-47</sup> The poor prognosis in cases with unfavourable genomic lesions which show resistance to purine analogues or alkylating agents and rapid disease progression<sup>4,5,48-51</sup> has led to the development of prognostic models for risk-adapted therapy.<sup>3</sup> Combined treatments including compounds that act independently of ATM and TP53 for example alemtuzumab and possibly lenalidomide as well as flavopiridol are promising in these cases.<sup>50,52,53</sup>

### ATM impairment in CLL

Deletion of chromosome 11q is the second

most frequent aberration in CLL (15-25%) and mutations of the ATM gene occur in 36% of patients with 11q deletions or 12% of all patients irrespective of 11q.<sup>4,54,55</sup> 11q deletions are rarely found in earlier stages whereas advanced CLL patients exhibit this genomic aberration in roughly 25%.<sup>3</sup> There are hints that ATM-mutations in 11q deleted cases may evolve during disease progression<sup>54,55</sup> and biallelic ATM abnormalities therefore might represent a more advanced stage than those with impairment of just a single allele.<sup>55</sup> ATM mutant tumours show an intermediate to poor clinical course with initial response to treatment but recurring relapse and extensive lymphadenopathy. *In vitro* these tumours often retain the capacity for apoptosis at a low level after DNA damage.<sup>56,57</sup> However, in contrast to mouse-models with homozygous ATM-disruption and increased radio-sensitivity,<sup>58,59</sup> 11q deleted CLL cells with ATM-mutations have been reported to show a relative radio-resistance<sup>56,60</sup> implicating additional mechanism in CLL for alternative survival mechanisms and the aggressive phenotype.<sup>61-63</sup> The clinical course of patients with 11q deletion and remaining wild-type ATM appears to be better than that of patients with a coexisting mutation of the other ATM allele.<sup>55</sup> It has been hypothesized that DNA damaging agents in CLL with loss of one allele may provide a selective pressure for the development and proliferation of sub-clones with biallelic ATM defects.<sup>54,55</sup> Studies showing better results after more intensive combination chemotherapy in these patients<sup>63,144,145</sup> seem to support this model since the fraction of sub-lethally exposed cells prone to clonal evolution might be diminished in this setting. Further confirming this, novel results show high efficacy for chemo-immunotherapy in patients with 11q deletions.<sup>64,65</sup> The addition of rituximab to FC-based regimen was reported with CR rates more than three times higher than with standard chemotherapy alone<sup>64</sup> (Figure 1).

### TP53 impairment in CLL

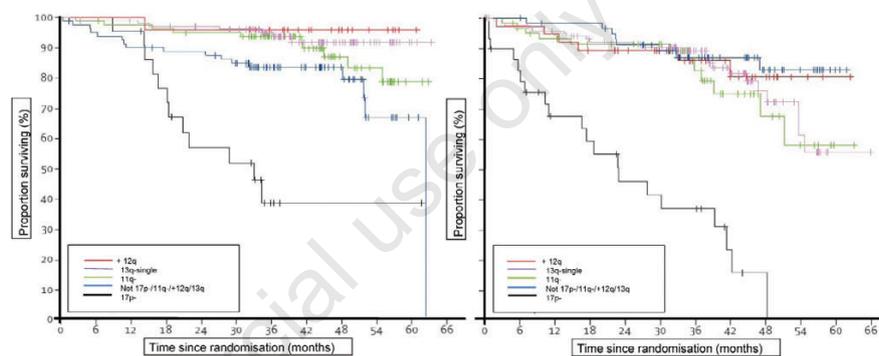
In contrast to 11q deletions, loss of chromosome 17p belongs to the less frequent aberrations in CLL (approx. 7% in untreated patients), its association with TP53 mutation is tight and was found to exceed the 80% rate. Though TP53 mutations are rare in cases with unaffected chromosome 17p,<sup>5,66,67</sup> a preferential selection of clones bearing single TP53 missense mutations has been suggested for clinically advanced patients with unfavourable risk factors.<sup>68</sup> In addition, 17p deletions and TP53 mutations have been found to correlate with genomic complexity which itself is associated with short time to initiation of treatment.<sup>66,67,69</sup> With respect to clinical presenta-

tion, 17p deletions occur in 5-7% of patients with early stage disease and are present in approximately one third of advanced, refractory cases.<sup>5,50</sup> The incidence of TP53 mutations ranges between 4-10% in patients without prior treatment.<sup>5,41,66</sup> Similar to other cancer entities, TP53 mutations in CLL are found to be mostly missense mutations located in the DNA-binding domain which leads to impaired transcriptional activity of p53.<sup>70,71</sup> Beside the frequently affected regions (codon 173, 220, 248, and 273), a high incidence of frameshift mutations has been reported for CLL.<sup>70,71</sup> CLL with abnormality of TP53 is clinically more aggressive, mostly shows primary therapy resistance and lacks DNA damage-induced apoptosis *in vitro*. Cases with single TP53

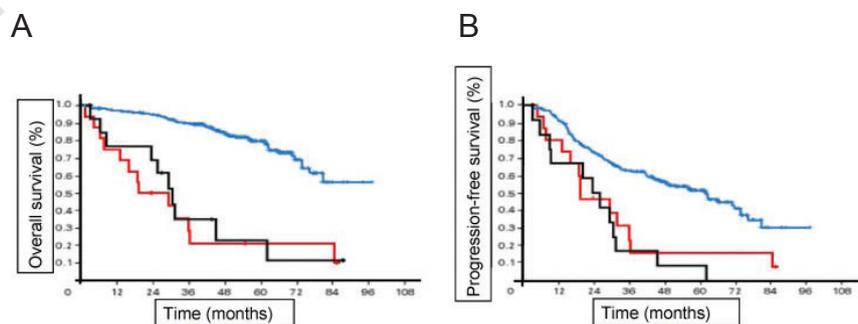
mutations have a clinical course resembling cases with coexisting 17p deletion and mutation of TP53<sup>66,72</sup> (Figure 2). Though chemo-immunotherapy based treatments have been found highly effective in CLL, 17p deleted cases do not substantially benefit from this treatment<sup>64</sup> (Figure 1).

### Other pathway members that control p53 function

Beside mutation and deletion of TP53 or ATM, p53 dysfunction was attributed to MDM2 mediated effects. P53 levels are partly regulated by MDM2 which binds to p53 and promotes its degradation. As a consequence of this function, overexpression of MDM2 was reported to result in p53 impairment.<sup>73,74</sup> Moreover, overexpres-



**Figure 1. Overall survival (OS) of genetic subgroups in the CLL8 trial (FC vs. FCR) of the German CLLSG: Chemoimmunotherapy (fludarabine, cyclophosphamide and rituximab) (A) and chemotherapy (fludarabine and cyclophosphamide) (B). OS at 3 years for FC vs. FCR after randomisation in prognostic subgroups was: 37% vs. 38%, including Del(17p) (n=51); 83% vs. 94%, including Del(11q) (n=142); 86% vs. 96%, including Trisomy 12 (n=61); 89% vs. 95%, including Del(13q) (n=224); 87% vs. 83%, no abnormalities (n=138). (Adapted from [63]).**



**Figure 2. Overall and progression-free survival in CLL patients with respect to TP53 mutation. Patients with TP53 mutations without 17p deletion have an outcome similar to patients with 17p deletion. (A) Overall survival (OS) of patients with 17p deletion (n=16; red), patients with sole TP53 mutation (n=14; black), and the remaining patients (n=277; blue). Median OS is significantly shorter for patients with 17p deletion (19.2 months) and sole TP53 mutation (30.2 months) compared with patients without either abnormality (median OS, not reached; P<.001). (B) Progression-free survival (PFS) of patients with 17p deletion (n=15; red), patients with sole TP53 mutation (without 17p deletion; n=13; black), and the remaining patients (n=260; blue). Median PFS is significantly shorter for patients with 17p deletion (19.2 months) and sole TP53 mutation (23.3 months) compared with patients without either abnormality (61.8 months; P<.001). (Adapted from [70]).**

sion of MDM2 was found to be associated with a single nucleotide polymorphism in the intronic promoter region at position 309 in the first intron.<sup>75</sup> The relevance of SNP309 for the clinical course and MDM2 expression in CLL has been addressed by several studies but remains controversial.<sup>76-79</sup> Therapeutic approaches by inhibition of MDM2 with small molecules, exemplary Nutlin 3a, have been shown to induce apoptosis irrespective of ATM-expression or MDM2-levels, but, as expected, resistance is reported in association with TP53 mutations.<sup>77,80</sup>

### Profiling studies of TP53 and ATM induced gene expression

The advent of profiling techniques enabled the global assessment of ATM and TP53 dependent changes in CLL. Gene expression profiling has previously been successfully applied aiming to decipher constitutively expressed and functionally altered gene-patterns in different cancers.<sup>81</sup> Several novel discoveries, including the association of ZAP-70 expression and IGHV mutation status and its prognostic value became possible by using this method.<sup>82,83</sup> Profiling studies on CLL with mutated or wild-type TP53 or ATM found indistinguishable gene expression patterns for unmanipulated cells while radiation induced changes enabled clear differentiation. Only approximately one third of the genes induced by ATM upon radiation showed changes in a p53 specific manner and were mainly related to apoptosis and cell-cycle regulation while the remaining and therefore ATM-dependent genes were rather involved in promoting cell survival and suppressed if TP53 was unattenuated.<sup>60</sup>

Dynamic changes in CLL cells after fludarabine treatment confirmed specific TP53- and time-dependent changes in CLL similar to gamma-irradiation but were absent in fludarabine resistant cases.<sup>84</sup> Discrepant observations come from another study investigating central genes involved in the regulation of apoptosis, cell cycle, B-cell activation and BCR signalling showing significant deregulation in cases with 17p deletion for genes not specifically located on 17p, moreover this study found ATM to be significantly reduced in 17p deleted cases<sup>85</sup> and therefore supports the hypothesis of related pathogenic pathways involving a common mechanism that leads to ATM inactivation.<sup>56,60,85</sup>

In summary, alterations affecting TP53 occur in 40-50% of refractory cases and the remaining fraction exhibits deletions of 11q only in 20-30% indicating additional mechanisms leading to resistance.<sup>3,55,66,72</sup> Regardless of future options or additional effects that can be achieved with combination therapies, allogeneic stem cell transplantation currently stays the only treatment with potential for cure.<sup>86</sup> Promising approaches may be derived

from the observation that genetic alterations in tumours display specific dependencies and therefore might be selectively used as targets. This circumstance has been used to simulate the synthetic lethal interaction for p53-deficient settings in which consecutive suppression of ATM strongly sensitized tumours to DNA-damaging chemotherapy.<sup>87</sup>

## Connecting the p53 pathway and microRNAs in CLL

### Karyotype-dependent changes

MiRs have been found to contribute to initiation, progression, outcome and clinical characteristics in CLL.<sup>88</sup> Studies investigating expression patterns with regard to specific subgroups found deregulation of single miRs in CLL and especially miR-expression with respect to cytogenetic subgroups revealed significant overlap. Strong association with a specific karyotype, even in cases with coexisting other deletions, was found in one study by utilising miR-155, miR-29b, miR-151-3p, miR-29c, miR-34a, miR-640, miR-148a, miR-146a and miR-146b-5p which were identified and confirmed by using gene expression analysis and qRT-PCR (Table 1).<sup>89</sup>

Specific patterns were confirmed for groups with 17p<sup>89-91</sup> and 11q deletion (Table 1).<sup>16, 89,92</sup> It is interesting that the validated miRs, discriminating cytogenetic groups, do not necessarily map to the deleted regions<sup>89</sup> and one might therefore suspect the genes located in these regions to be involved in miR-regulatory circuits.

One example is miR-34a which maps to chromosome locus 1p36 and shows low expression levels in 17p deleted cases. Evidence for the role of p53 in regulating miRs came from several studies that found the members of the miR-34 family to be directly induced by p53. MiR-34a mediates essential steps in a p53-dependant manner by targeting specific genes involved in the cell-cycle, G1-arrest, proliferation and apoptosis.<sup>17-20,93</sup>

### MiR-34a

The p53-dependence has been proven for miR-34a in the disease specific context of CLL. Cases with 17p deletion or mutation of TP53 were shown to exhibit significantly lower basal levels<sup>90,91,94-96</sup> and miR-34a was downregulated after transfection with siRNA against TP53 in MEG01 cells.<sup>91</sup>

Further confirming the dependence between TP53 and miR-34a, irradiation in the absence of functional p53 did not increase expression. In line with the role as a functional member of the p53-pathway, low levels of miR-34a were found in association with fludarabine-refractory disease, impaired DNA-damage response and resistance to apoptosis in cases with unaffected 17p or TP53. It is important to emphasize that no association with the 11q-status was detectable since low miR-34a levels were equally distributed between cases with and without 11q-deletion.<sup>95</sup> However, this finding was not confirmed in a following investigation where cases with affected ATM were attributed to cause low miR-34a levels.<sup>96</sup>

Since miR-34a maps to the chromosome-locus 1p36 which is deleted in a variety of malignancies including neuroblastomas, cancers of the gastrointestinal tract and the breast, similar changes would be expected for CLL but have not been reported up to now.<sup>97-102</sup> Other mechanisms leading to the deregulation of microRNAs have been found in epigenetic changes. Low levels of miR-34a that are not caused by 17p deletion or TP53 mutation may result from aberrant CpG methylation which has been shown in other cancers.<sup>103</sup> With respect to its biologic function, additional regard has to be paid to the observation that miR-34a is significantly up-regulated together with p21 and PUMA in a B-CLL mouse-model during the transition from pre-leukemic to leukemic phase which suggests an oncogenic activation of the p53-pathway.<sup>95,96</sup> The fact that miR-34a levels are upregulated in steady state both in cases with good<sup>95</sup> and adverse risk features and especially in comparison with healthy B-cells<sup>96</sup> implicates the possibility that

**Table 1. MicroRNA expression with respect to 17p or 11q deletion, mutation of TP53 and overexpression/knockdown of TP53.**

Characteristic	Differentially expressed miRs	Study/Author
17p-deletion (including TP53 mutations/knockdown)	miR-151-3p, miR-29c, miR-34a (down) miR-21, miR-155, miR-15a (up) miR-34a, miR-181b (down) miR-34a, miR-29c, miR-17-5p (down)	Visone 2009 Rossi 2010 Mraz 2009
TP53 overexpression	miR-34b (up)	Fabbri 2011
11q-deletion	miR-29b (down), miR-155 (up) miR-29, miR-181 (down) miR-34b, miR-34c (down)	Visone 2009 Pekarsky 2006 Fabbri 2011

miR-34a needs to act in concert with p21, Bax and Puma to execute its function after DNA damage.<sup>95</sup> Constituting a putative therapeutic option, investigations using overexpression of miR-34a have confirmed the potential by inducing apoptosis in various cancer models<sup>104-106</sup> including CLL<sup>96</sup> which exposes it as attractive candidate for future investigations. Still, miR-34a effects in the context of p53-pathway alterations remain to be specifically investigated due to the supposed feedback on regulators upstream of p53 itself<sup>17,37</sup> (Figure 3).

### MiR-34b/c

Unlike the confirmed impact of miR-34a, the role of the miR-cluster 34b/c remains less well defined in the context of the p53-pathway. Contrasting previous observations,<sup>17-20</sup> miR-34b/c expression in CLL was unaffected upon p53 activation<sup>95</sup> while another study found TP53 to bind a pre-miR-34b/c site on chromosome 11 with significant transactivation effect and miR-34b induction after TP53-transfection of MEG01 cells.<sup>93</sup> Furthermore, the miR-34b/c cluster on chromosome region 11q23 centromeric to ATM is frequently deleted in CLL<sup>4</sup> and current studies draw a heterogeneous picture with respect to karyotype-specific expression levels.<sup>89,93</sup> Reasons for variance in expression might include differences in cohort size, biological characteristics, clinical stage or epigenetic regulation and should be carefully addressed in future investigations.

The tempting connection between 11q deletion and loss of negative regulators mapping to this region remains in focus of research. As TCL1 is overexpressed in 11q deleted CLL one group investigated complementarity of the miR-34b/c cluster members to this genes sequence. MiR-34b\* also known as miR-34b-5p was found to match on 4 different positions and to act as specific regulator of TCL1 in HEK293T cells.<sup>107</sup> More recently the miR-34 family has been proposed as regulators of ZAP-70 with interconnection to TP53, cytogenetics and a newly identified regulatory feedback mechanism between TP53 and the miR-15a/16-1 cluster.<sup>93</sup>

### MiR-15a/16-1

MiR-15a and miR-16-1 were identified as part of the tumour suppressor mechanism that has been suspected at the minimally deleted region (MDR) on chromosome 13q14 and following research unmasked their role as repressors of BCL-2.<sup>108,109</sup> Both miRs have been found to inversely correlate with BCL-2 protein levels in CLL with increased rates of apoptosis after transfection with wild-type miR-15/16.<sup>109</sup>

A recent study revealed a direct feedback of miR-15a/16-1 on TP53 and vice versa. Overexpression of miR-15a and 16-1 resulted

in significant reduction of p53 in MEG01 cells and primary CLL (with consecutive reduction of CDKN1A, BBC3 and BCL2). Additionally, p53 was confirmed to induce the miR-15a/16-1 cluster through specific upstream binding sites. The observation of high TP53 mRNA and protein expression in CLL cases with bi- or monoallelic 13q deletion compared with normal karyotype cases supports this finding in primary samples.<sup>93</sup> However, the functional meaning of previously observed high miR-15a levels in patients with 17p deletion remains to be further elucidated in this context.<sup>91</sup>

### MiR-29 and miR-181

TCL1 was previously shown to result in CLL when overexpressed in murine B cells<sup>110</sup> and to positively regulate AKT<sup>111</sup> which itself restrains activity of p53 by activating MDM2. The fact that miRs-29 and miRs-181, which specifically target TCL1,<sup>16,92</sup> are downregulated in cases with 11q/17p deletion and aggressive CLL<sup>16,89-92</sup> implicates an interconnection to the functionality of the p53-pathway. However, it remains unknown how these miRs are regulated and if ATM or TP53 play a specific role in this context.

In line with this, the miR-29 family was shown to influence the p53-pathway by targeting CDC42 which itself reduces p53 levels<sup>112</sup> and by regulating p85 $\alpha$  which composes the regulatory subunit of PI3K. Reducing activity of PI3K with consecutively lower phosphorylation of downstream targets like AKT and MDM2 therefore stabilises p53.<sup>112</sup>

### MiR-155

MiR-155 is upregulated in lymphomas of B- and T-cell origin<sup>113,114</sup> and shows high expression in CLL compared to normal CD 19<sup>+</sup> B-cells.<sup>15</sup> Mouse-models with forced expression of miR-155 in B-cell precursors develop a pre-B-lymphoproliferative disease that progresses to a B-cell malignancy<sup>115</sup> probably through down-regulation of Ship and C/EBP $\beta$ .<sup>116</sup> Ship represents a major target of mir-155<sup>116,117</sup> and negatively regulates the activity of PI3K effectors such as AKT, which is derepressed in T-cell lymphoma with elevated miR-155 levels.<sup>114</sup> Concomitant deletion of PTEN and SHIP in B-cells was shown to results in spontaneous and lethal mature B cell neoplasm in mouse-models.<sup>118</sup>

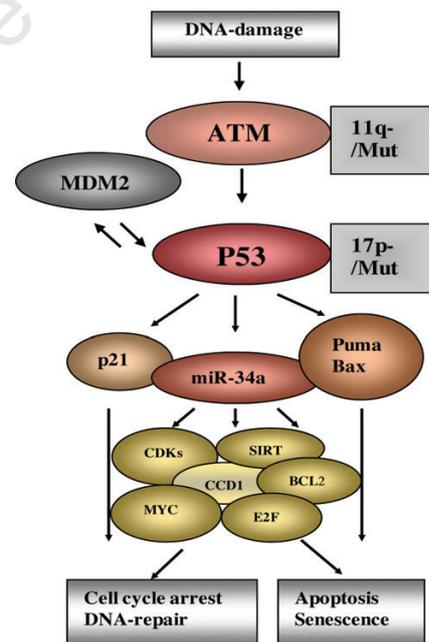
The regulation of miR-155 expression has been attributed to transcription-factors including NF $\kappa$ B, AP1 and MYB.<sup>119,120</sup> Moreover, miR-155 has been found upregulated in CLL after TP53 knockdown, in cases with TP53 mutation/17p-deletion<sup>91</sup> and in cases with 11q deletion.<sup>89</sup> Further hints to an interconnection with p53 come from previous studies that report miR-155 to be transcriptionally repressed in a p53-

dependent manner<sup>121</sup> and that it executes repression on the Tumour Protein 53-Induced Nuclear Protein 1 (TP53INP1) which itself is a pro-apoptotic gene downstream of p53.<sup>122</sup>

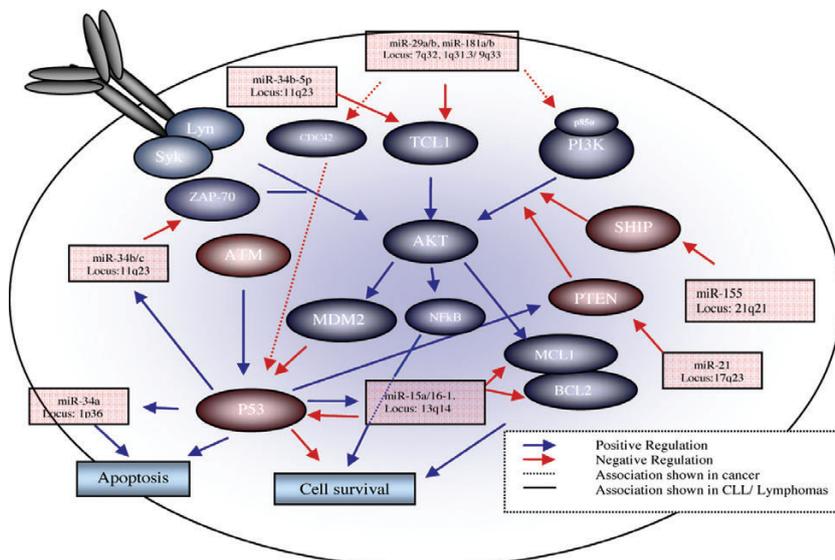
### MiR-21

Mir-21 is a classic example of oncogenic acting miRs in cancer and has been frequently found overexpressed in a variety of malignancies<sup>114,123-128</sup> including CLL.<sup>13,15,91</sup>

Its potential for the development of lymphomas has been shown *in vivo* in a mouse model that conditionally expressed miR-21 and developed a pre-B malignant lymphoid-like phenotype with involvement of lymphoid organs.<sup>129</sup> Interestingly knockdown or loss of miR-21 revert impaired apoptosis, proliferation and decrease tumorigenesis.<sup>130</sup> Amongst the confirmed targets are the tumoursuppressors CDC25A<sup>131</sup>, PDCD4,<sup>114,132,133</sup> BTG2<sup>134</sup> and



**Figure 3.** Schematic model for the p53-miR-34a pathway. P53 is activated by DNA-damage through ATM. Consecutive activation of p21, Puma and Bax enable cell cycle arrest, DNA-repair, senescence and programmed cell death through apoptosis. MiR-34a is a direct transcriptional target of p53 and upregulated upon activation of p53. Cell cycle arrest and apoptosis mediated by p21 and Puma/Bax are fostered by the downregulation of genes targeted by miR-34a. Disruption of this hierarchial model may result from loss or mutation of ATM or TP53 or alterations (overexpression and SNP309) of MDM2. Of note, miR-34a downregulation may occur independently of p53 in activation in CLL and is associated with resistant disease.



**Figure 4.** Role of deregulated microRNAs in CLL with impaired p53-pathway. Altered microRNA levels confer survival and growth advantage by loss of tumor suppressive or gain of oncogenic function. *MiR-34a* is downregulated in CLL with 17p deletion or TP53 mutation. P53 is the major transcriptional regulator of *miR-34a*. Beside p21, Bax and other it composes an important mediator of the p53-effects like cell-cycle arrest and apoptosis. *MiR-34b* and *c* are regulated by p53 and were recently shown to target ZAP-70 in CLL. *MiR-34b\** (also known as *miR-34b-5p*) targets TCL1. The *miRs-34b*, *34b-5p* and *34c* are located on chromosome 11q which is frequently lost in CLL. TCL1 is upregulated in 11q deleted CLL and has been attributed to the loss of a negative regulator in this region. TCL1 is a direct target of *miR-29* and 181 which are downregulated in aggressive and 11q/17p deleted CLL. Moreover the *miR-29* family targets CDC42 and the regulatory subunit of PI3K, namely p85 $\alpha$ . *MiR-15a/16-1* is located on chromosome 13q14 which most frequently shows aberrations in CLL. Feedback regulation conferred by repression of TP53 through *miR-15a/16-1* and their induction through p53 has been recently reported in CLL. Both *miRs* were shown to target antiapoptotic genes like BCL2 and MCL1. *MiR-155* and *miR-21* are over-expressed in lymphomas and target PTEN and SHIP. Impairment of PTEN has been found in several malignancies. PTEN and SHIP collectively suppress PI3K/AKT signalling and concomitant deletion of PTEN and SHIP in B-cells results in spontaneous and lethal mature B cell neoplasms in mouse-models.

PTEN.<sup>114,135</sup> The fact that the above listed miR-21 regulated genes are either downstream targets of p53, interact with the p53-pathway or regulate the cell cycle<sup>132,136-139</sup> accentuates a putative role as a modulator of p53 function in CLL. Especially PTEN, which is a negative regulator of PI3K-signalling and shows tight interaction with p53,<sup>137,140,141</sup> represents an important miR-21 target with relevance in CLL<sup>142, 143</sup>.

Taken together, microRNA deregulation in CLL with impaired p53-pathway seems to confer advantages mostly by impaired apoptosis and increased survival due to loss of tumour suppressive or gain of oncogenic potential. Moreover it is suggestive that the deregulated microRNAs act in concert to additionally impair activity of p53 (Figure 4). Further systemic models as exemplary investigated by Fabbri *et al.* will lead to a better understanding of pathogenesis and explanation of disease characteristics in CLL with a dysfunctional p53-pathway.

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