Fine tuning of p53 functions between normal and leukemic cells: a new strategy for the treatment of chronic lymphocytic leukemia

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doi:10.3324/haematol.2019.230896

Pathophysiology and current therapies in chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in western countries. It is characterized by the accumulation of mature B lymphocytes in the peripheral blood, peripheral lymphoid organs and bone marrow. CLL displays a heterogeneous clinical course, ranging from protracted indolent disease with no requirement for treatment in some patients to rapid disease progression and subsequent treatment refractoriness in others.[1-3]

CLL progression is a reflection of the complex interplay between genomic drivers of disease and interactions with the microenvironment.[4] Whole genome/exome profiling by next-generation sequencing has revealed that the clonal composition of CLL is constantly reshaped during disease progression. It has been proposed that CLL exhibits a stochastic model of progression with the existence of a 'trunk' tumor population and numerous 'branches' that can act as tumor progenitors. According to this model, the subclonal topography of CLL arises over time as a result of an initial driver mutation which leads to malignant transformation and is observed in all tumor cells – the trunk population. This is followed by secondary driver mutations in distinct subclones which are selected by intraclonal competition or treatment, and are likely to contribute to disease progression. Finally, CLL relapse has been attributed to the expansion of highly fit, often treatment-selected subclones (branches) carrying mutations in the DNA damage response (DDR) genes *TP53* and *ATM*, *SF3B1* or *NRAS*.[5,6] As a result, a significant proportion of relapsed/refractory CLL can be attributed to the functional loss of the DDR.

For several decades, alkylating agents and purine analogs were the principal therapies for CLL, augmented by the addition of monoclonal antibodies. The last decade has seen an expansion in the number of compounds targeting specific aspects of the CLL phenotype, from the interactions of tumor cells with the microenvironment and B-cell receptor signaling to anti-apoptotic cellular pathways, heralding a new era of CLL therapy based on targeted treatment approaches.[7-10] In particular, new inhibitors of signaling pathways that are critical to CLL survival and proliferation, such as Bruton tyrosine kinase (BTK), phosphoinositide kinase (PI3K), and the anti-apoptotic protein Bcl-2, have changed the management of many CLL patients.

Despite the array of available therapeutic options, CLL remains, at present, an incurable condition.[11] Firstly, the acquisition of DDR gene defects such as *TP53* deletions and/or mutations renders CLL patients refractory to conventional chemoimmunotherapies. The clinical response to the BTK inhibitor, ibrutinib, is encouraging for some but not all refractory tumors.[12] Secondly, clonal selection and evolution underlies treatment resistance, clinical progression, and disease transformation, particularly in CLL with DDR defects, and efforts are still ongoing to understand and counteract this process.

The p53 pathway as a therapeutic target

The *TP53* tumor suppressor is a transcription factor that responds to various forms of cellular stress imposed by DNA damage, hypoxia, telomere erosion, nucleotide depletion or oncogene activation. In response to genotoxic stress, p53 accumulates in the nucleus and becomes activated through numerous post-translational modifications leading to different outcomes depending on the level of stress and cellular context. Under moderate levels of DNA damage, p53 facilitates growth arrest enabling DNA repair, whereas excessive DNA damage causes p53 to initiate programmed cell death or apoptosis.[13] This ability of p53 to induce apoptosis in cells under genotoxic stress serves as the underlying mechanism of killing by many chemotherapeutic drugs.

A p53-MDM2 feedback loop plays a central role in keeping p53 at a low level in non-stressed cells, thus protecting them from undesirable induction of apoptosis (Figure 1). MDM2 (mouse double minute 2 homolog) is a ubiquitin ligase that facilitates the nuclear export of p53 and targets p53 for proteosomal degradation. Under non-stressed conditions, p53 is continuously targeted by MDM2 for degradation. Consequently, inhibition of the p53-MDM2 interaction is an attractive strategy to activate p53-dependent apoptosis in a non-genotoxic manner, thus facilitating selectivity and efficiency of tumor cell elimination.[14-17]

Indeed, the first-generation non-peptide small molecule MDM2 inhibitors, known as Nutlins, have been shown to activate the p53 pathway in cancer cells harboring wildtype p53 both *in vitro* and *in vivo*. Nutlins inhibit the p53-binding pocket on MDM2, resulting in the accumulation of p53 and restoration of both its transcriptional activity and ability to induce apoptosis. Nutlins have shown preclinical activity in malignancies with elevated MDM2 or MDM4 expression such as sarcomas, neuroblastomas and some leukemias, including CLL.[18-23] Despite these promising initial results, the limited potency and bioavailability of these compounds restrict their clinical use. In addition, the issue of sparing non-tumor tissue from unwanted p53 accumulation and apoptosis remains unresolved.

Selective targeting of a p53-dependent apoptotic defect

In this issue of *Haematologica*, Ciardullo *et al.*[24] offer a novel strategy for the treatment of CLL. They demonstrated that a representative of the new generation of MDM2 inhibitors has a strong anti-tumor effect in CLL.

The authors found that the compound RG7388, a second-generation MDM2 inhibitor with improved potency, stability and pharmacokinetics,[25] decreases the viability of CLL tumor cells, regardless of patients' phenotype or risk status. Importantly, the authors observed that RG7388 affects normal and tumor cells differently. They showed that MDM2 inhibition led to p53 transcriptional activation in both normal and tumor cells. However, while RG7388 treatment of CLL cells induced p53 transcriptional activation and subsequent upregulation of mostly pro-apoptotic genes, *PUMA*,

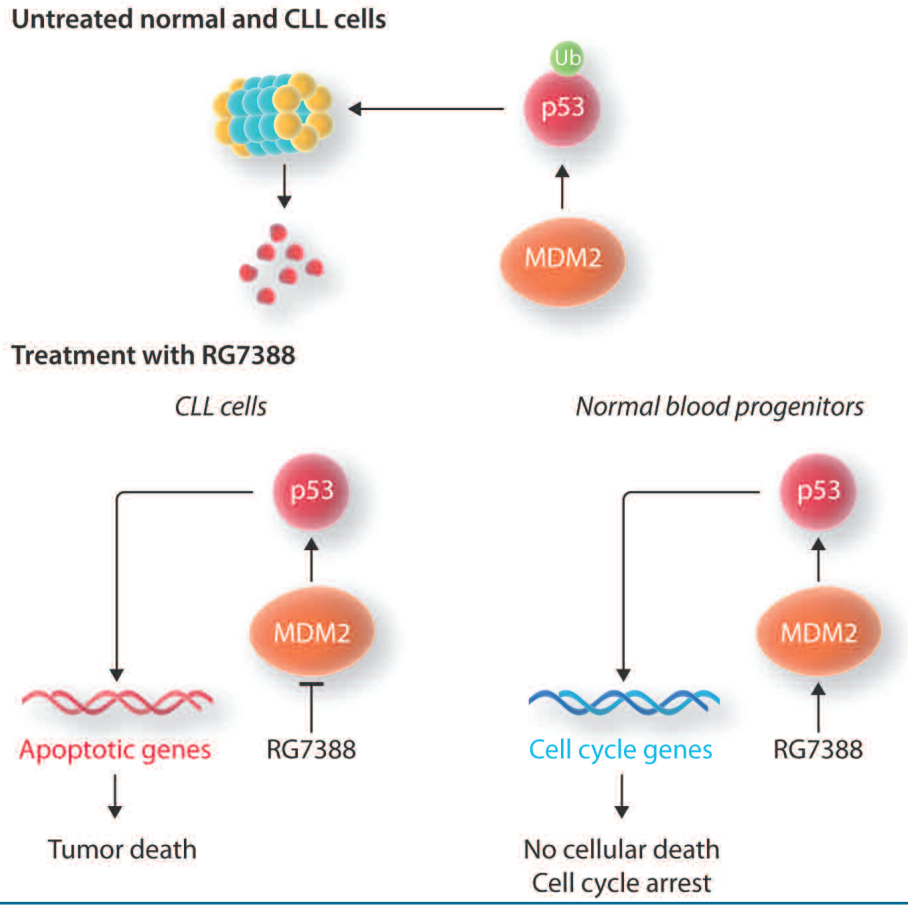

Figure 1. The p53-MDM2 feedback loop and the effect of RG7388 on this loop in normal and tumor cells. (Top) The p53-MDM2 feedback loop plays a central role in keeping p53 at a low level in non-stressed cells, thus protecting them from undesirable induction of apoptosis. MDM2 is a ubiquitin ligase that facilities the nuclear export of p53 and targets p53 for proteosomal degradation. Under non-stressed conditions, p53 is continuously targeted by MDM2 for degradation. (Bottom) A second-generation MDM2 inhibitor, RG7388, affects normal and tumor cells differently. RG7388 leads to p53 transcriptional activation in both normal and tumor cells. However, while treatment of chronic lymphocytic leukemia (CLL) cells induces p53 transcriptional activation and subsequent upregulation of mostly pro-apoptotic genes (left), in mature blood cells and hematopoietic ($CD34^+$) progenitors treatment leads to MDM2 upregulation, thus preventing the induction of unwanted apoptosis coupled with p53 reactivation (right).

BAX, *TNFRSF10B* and *FAS*, such activation was not evident in either mature blood cells or hematopoietic ($CD34^+$) progenitors isolated from patients' bone marrow. In contrast, p53 activation in these non-tumor cells predominantly led to MDM2 upregulation, thus preventing the induction of unwanted apoptosis coupled with RG7388-induced p53 reactivation. This differential effect is very promising and is consistent with the minimal toxicity of RG7388 in normal hematopoietic tissue.

Future prospective

It is well established that *TP53* alterations in CLL are associated with poor outcome following a variety of treatments. Given the clinical heterogeneity of CLL, in which *TP53* alterations even when present at low levels compromise patients' outcome,[26] there is a constant need to invent new therapeutic strategies for this malignancy.

Ciardullo *et al.*[24] also showed that CLL tumors harboring small *TP53* subclones responded well to RG7388, presumably by virtue of debulking the main tumor population that harbors wildtype p53. The authors concluded that *TP53* mutational status is not the determinant of the response to this new generation of MDM2 inhibitors. This observation is encouraging, as it suggests that RG7388 could be effective in a wide range of CLL cases in which other therapeutic options are exhausted. For tumors that harbor small *TP53* mutant subclones, however, additional therapies that specifically target p53 functional loss might be required.

Taken together, in the light of the improved potency and bioavailability of the second-generation MDM2 inhibitors that are now available for clinical use, the study by Ciardullo *et al.* provides the rationale for an additional therapeutic option for patients with CLL.

Acknowledgments

The author is grateful to Bloodwise for financial support (ref 14031).

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