HIF-1α is over-expressed in leukemic cells from TP53-disrupted patients and is a promising therapeutic target in chronic lymphocytic leukemia

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Supplemental Information

**Article title:** HIF-1α is overexpressed in leukemic cells from TP53-disrupted patients and is a promising therapeutic target in CLL

**Running Head:** HIF-1α in TP53-disrupted CLL Cells

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Supplemental Methods

Cells preparation and culture
PBMC were separated using Ficoll-Hypaque (Sigma-Aldrich) and stained with anti-CD19 PerCP Vio700 and anti-CD5-APC antibodies (Miltenyi Biotec, Bologna, Italy). When the CD19+/CD5+ cells were < 90%, CLL cells were purified by negative selection and cultured in RPMI-1640 medium with 10% of fetal bovine serum (FBS) and penicillin/streptomycin. B lymphocytes from healthy donors were purified by positive selection using anti-CD19 micro beads (Miltenyi Biotec). Del(17p) was assessed by fluorescence in situ hybridization and the presence of TP53 gene mutations was evaluated by Sanger sequencing. Immunoglobulin heavy chain variable region gene (IGHV) mutational status was assessed as already described1. Cell lines used in the experiments (i.e. Séraphine, Granta-519 and M2-10B4) were maintained in RPMI-1640 or DMEM medium with 10% fetal bovine serum, glutamine and antibiotics at 37 °C, 5% CO2. A humidified hypoxia incubator chamber was used for hypoxic cultures.

Antibodies used for flow cytometry
Two- and three-color flow cytometry was performed with FACSCalibur and CELLQuest software (Becton Dickinson, Mountain View, CA) and with BD Accuri C6 flow cytometer (BD Biosciences). Data were analyzed with FlowJo software (Tree Star, Inc, Ashland, OR). Antibodies used for flow cytometry were: anti-CD19-PE (BD Biosciences, San José, CA), anti-CD19-PerCP Vio700 (Miltenyi Biotec), anti-CD5-APC (Miltenyi Biotec).

Western blot analysis
Cytosolic and nuclear protein extracts were obtained using the Nuclear Extract Kit (Active Motif, La Hulpe) following the manufacturer’s instructions. Lysates were resolved by SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). The following antibodies were used: anti-HIF-1α (BD Biosciences, San José, CA), anti-ELK3
(Sigma Aldrich); anti-pVHL (EuroClone Spa, Milano); anti p(Thr202/Tyr204, Thr185/Tyr187)-ERK1–2 (Millipore, Bedford, MA); anti-ERK1-2 (Millipore); anti-RAS (Millipore); anti-RHOA (Santa Cruz Biotechnology Inc., Santa Cruz, CA) antibody anti-p(Ser 473)AKT (Millipore); anti-AKT (Millipore); anti-ACTIN (Sigma Aldrich), anti-GAPDH, anti-TUBULIN and anti-TATA-box binding protein (TBP) (Santa Cruz Biotechnology Inc.) used as control of equal protein loading for cytosolic and nuclear fractions, followed by the secondary peroxidase-conjugated antibodies (Bio-Rad, Hercules, CA). To exclude nuclei-cytosol contamination, we verified that GAPDH and TBP were always undetected in nuclear and cytosolic fractions, respectively (not shown). Blot images were acquired with a ChemiDocTM Touch Imaging System device (Bio-Rad Laboratories). Densitometric analysis was performed with the ImageJ software (NIH, Bethesda, MD).

**RNA extraction and quantitative real-time PCR (qRT-PCR)**

Total RNA was extracted and reverse transcribed as described2. The qRT-PCR was performed with the iTaq™ Universal SYBR® Green Supermix (Bio-Rad). The primer sequences were designed with the qPrimerDepot software (http://primerdepot.nci.nih.gov/). The primer sequences were: \( \text{HIF-1A} \): 5′-GGCTGCATCTCGAGACTTT-3′; 5′-GAAGACATCGCGGGGAC; \( \text{ENO1} \): 5′-GCTCCGGGACAATGATAAGA-3′, 5′-TCCATCCATCTCGATCATCA-3′, \( \text{GAPDH} \): 5′-GAAGGTGAAGGTCGGAGT-3′, 5′-CATGGTGGGAATCATATTGGA-3′; \( \text{VEGF} \): 5′-ATCTTCAAGCCATCTCCTGTGC-3′, 5′-GCTCACCGCCTCGGTGTG-3′. The comparative CT method was used to calculate \( \text{HIF-1a} \), \( \text{GLUT1} \), \( \text{ENO1} \) and \( \text{VEGF} \) expression relative to the \( \text{GAPDH} \) product, used as a housekeeping gene, with the Bio-Rad Software Gene Expression Quantitation.

**RAS and RHOA activity**

The isoprenylated membrane-associated RAS or RHOA proteins and the non-
isoprenylated cytosolic forms were detected as previously described. Total RAS and RHOA proteins were analyzed by WB; Briefly, the GTP-bound fraction, taken as an index of active G-proteins was measured, using a pull-down assay (with the RAF-1-GST fusion protein, agarose beads-conjugates, Millipore, Bedford, MA) and an ELISA assay (with the G-LISA™ RHOA Activation Assay Biochem Kit, Cytoskeleton Inc, Denver, CO), respectively.

**Kinase inhibitors titration and kinase activity**

For kinase inhibitors titration experiments we exposed CLL cells (10^6/mL) for 48 hours to PD98059, LY249002 and Y27632 at indicated increasing concentrations. ERK1-2, AKT and RHOA kinases activity was measured by spectrophotometric methods, using the CycLex RHO Kinase Assay Kit (CycLex, Nagano), the MAP Kinase/Erk Assay Kit (Millipore, Bedford, MA) and the AKT Kinase Activity Assay Kit (Abcam, Cambridge), as per manufacturer’s instructions.

**Cell viability assay**

Cell viability was evaluated by flow cytometry using with Annexin-V/Propidium Iodide (Ann-V/PI) staining with the MEBCYTO-Apoptosis Kit (MBL Medical and Biological Laboratories, Naka-ku Nagoya). Normalized cell viability was arbitrarily defined as the ratio between the percentage of AnnV-/PI- CLL cells cultured in the presence of F-ara-A and the percentage of AnnV-/PI- CLL cells that were left untreated. CLL cells characterized by a normalized cell viability ≥0.5 were defined as *fludarabine-resistant*, otherwise, CLL cells were considered *fludarabine-sensitive*. 
**Table 1.** Summary of genetic patient characteristics and treatment status

| Unique Patient Number (UPN) | TP53 subset | del(17)p, clone size in % | TP53\textsuperscript{mut}, allele frequency in % | IGHV mutational status | Previous Treatment |
|----------------------------|-------------|---------------------------|-----------------------------------------------|-----------------------|-------------------|
| UPN01                      | TP53\textsuperscript{dis} | neg | mut, 84 | M | y |
| UPN02                      | TP53\textsuperscript{dis} | 42 | mut, 83 | UM | y |
| UPN03                      | TP53\textsuperscript{dis} | 85 | mut, 83 | UM | n |
| UPN04                      | TP53\textsuperscript{dis} | 80 | mut, 79 | UM | n |
| UPN05                      | TP53\textsuperscript{dis} | neg | mut, 75 | UM | y |
| UPN06                      | TP53\textsuperscript{dis} | 79 | mut, 86 | M | n |
| UPN07                      | TP53\textsuperscript{dis} | neg | mut, nd | UM | y |
| UPN08                      | TP53\textsuperscript{dis} | nd | mut, nd | M | nd |
| UPN09                      | TP53\textsuperscript{dis} | 87 | mut, 81 | UM | n |
| UPN10                      | TP53\textsuperscript{dis} | neg | mut, 88 | M | y |
| UPN11                      | TP53\textsuperscript{dis} | 69 | mut, 61 | UM | y |
| UPN12                      | TP53\textsuperscript{dis} | neg | mut, 36 | M | n |
| UPN13                      | TP53\textsuperscript{dis} | neg | mut, 50 | M | n |
| UPN14                      | TP53\textsuperscript{dis} | 24 | mut, 37 | UM | y |
| UPN15                      | TP53\textsuperscript{dis} | monosomy 17 | mut, 50 | UM | y |
| UPN16                      | TP53\textsuperscript{dis} | 96 | mut, 67 | nd | n |
| UPN17                      | TP53\textsuperscript{dis} | neg | mut, 50 | UM | y |
| UPN18                      | TP53\textsuperscript{dis} | 53 | mut, 31 | UM | n |
| UPN19                      | TP53\textsuperscript{dis} | 20 | mut, 92 | UM | y |
| UPN20                      | TP53\textsuperscript{dis} | 88 | nd | UM | n |
| UPN21                      | TP53\textsuperscript{dis} | 21 | mut, 25 | UM | n |
| UPN22                      | TP53\textsuperscript{dis} | 46 | mut, 51 | UM | y |
| UPN23                      | TP53\textsuperscript{dis} | 70 | nd | nd | y |
| UPN24                      | TP53\textsuperscript{dis} | neg | mut, 34 | M | y |
| UPN25                      | TP53\textsuperscript{dis} | 12 | mut, 12 | UM | y |
| UPN26                      | TP53\textsuperscript{dis} | neg | mut, nd | M | y |
| UPN27                      | TP53\textsuperscript{dis} | 82 | mut, nd | M | n |
| UPN28                      | TP53\textsuperscript{dis} | 80 | mut, 63 | UM | y |
| UPN29                      | TP53\textsuperscript{dis} | 68 | mut, 75 | UM | y |
| UPN30                      | TP53\textsuperscript{dis} | nd | mut, 73 | nd | n |
| UPN31                      | TP53\textsuperscript{dis} | 69 | mut, 64 | UM | n |
| UPN32                      | TP53\textsuperscript{dis} | neg | mut, nd | UM | y |
| UPN  | TP53<sup>dis</sup> | Number | Mut Type | Expression | UV | nd  |
|------|--------------------|--------|----------|------------|----|-----|
| UPN33 | TP53<sup>dis</sup> | 43     | mut, 25  | M          | nd | n   |
| UPN34 | TP53<sup>dis</sup> | neg    | mut, nd  | UM         | n  |     |
| UPN35 | TP53<sup>dis</sup> | 91     | wt       | M          | n  |     |
| UPN36 | TP53<sup>dis</sup> | 59     | wt       | nd         | n  |     |
| UPN37 | TP53<sup>dis</sup> | 50     | wt       | UM         | y  |     |
| UPN38 | TP53<sup>dis</sup> | 50     | wt       | UM         | y  |     |
| UPN39 | TP53<sup>dis</sup> | 46     | wt       | M          | y  |     |
| UPN40 | TP53<sup>dis</sup> | 45     | wt       | M          | n  |     |
| UPN41 | TP53<sup>wt</sup>  | neg    | wt       | M          | y  |     |
| UPN42 | TP53<sup>wt</sup>  | neg    | wt       | M          | n  |     |
| UPN43 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN44 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN45 | TP53<sup>wt</sup>  | neg    | wt       | M          | y  |     |
| UPN46 | TP53<sup>wt</sup>  | neg    | wt       | M          | y  |     |
| UPN47 | TP53<sup>wt</sup>  | neg    | wt       | M          | y  |     |
| UPN48 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN49 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN50 | TP53<sup>wt</sup>  | neg    | wt       | M          | n  |     |
| UPN51 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN52 | TP53<sup>wt</sup>  | neg    | wt       | UM         | y  |     |
| UPN53 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN54 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN55 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN56 | TP53<sup>wt</sup>  | neg    | wt       | M          | n  |     |
| UPN57 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN58 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN59 | TP53<sup>wt</sup>  | neg    | wt       | M          | n  |     |
| UPN60 | TP53<sup>wt</sup>  | neg    | wt       | UM         | y  |     |
| UPN61 | TP53<sup>wt</sup>  | neg    | wt       | M          | n  |     |
| UPN62 | TP53<sup>wt</sup>  | neg    | wt       | M          | n  |     |
| UPN63 | TP53<sup>wt</sup>  | neg    | wt       | M          | y  |     |
| UPN64 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN65 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN66 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN67 | TP53<sup>wt</sup>  | neg    | wt       | M          | n  |     |
| UPN68 | TP53<sup>wt</sup>  | neg    | wt       | M          | n  |     |
| UPN69 | TP53<sup>wt</sup>  | neg    | wt       | UM         | n  |     |
| UPN70 | TP53<sup>wt</sup>  | neg    | wt       | nd         | n  |     |
| UPN | TP53<sup>wt</sup> | neg | wt | M | y |
|-----|-----------------|-----|----|---|---|
| UPN71 | TP53<sup>wt</sup> | neg | wt | M | y |
| UPN72 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN73 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN74 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN75 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN76 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN77 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN78 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN79 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN80 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN81 | TP53<sup>wt</sup> | neg | wt | M | y |
| UPN82 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN83 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN84 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN85 | TP53<sup>wt</sup> | neg | wt | nd | n |
| UPN86 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN87 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN88 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN89 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN90 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN91 | TP53<sup>wt</sup> | neg | wt | nd | n |
| UPN92 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN93 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN94 | TP53<sup>wt</sup> | neg | wt | nd | n |
| UPN95 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN96 | TP53<sup>wt</sup> | neg | wt | M | n |
| UPN97 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN98 | TP53<sup>wt</sup> | neg | wt | UM | y |
| UPN99 | TP53<sup>wt</sup> | neg | wt | UM | n |
| UPN100 | TP53<sup>wt</sup> | neg | wt | UM | y |
| UPN101 | TP53<sup>wt</sup> | neg | wt | nd | n |
| UPN102 | TP53<sup>wt</sup> | neg | wt | M | n |

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| Del(17)p     | chromosome 17p13 deletion |
| neg          | negative, <10% del(17p) |
| TP53<sup>mut</sup> | TP53 gene mutation |
| mut          | presence of TP53 gene mutation |
| wt           | wild type, absence of TP53 gene mutation |
| nd           | not determined |
| IGHV         | Immunoglobulin heavy chian variable region genes |
|   |   |
|---|---|
| M | IGHV mutated |
| UM | IGHV unmutated |
Figure S1. The association between HIF-1α expression and the *TP53* status is not influenced by the IGHV mutational status. The expression of HIF-1α was measured in *TP53*wt and *TP53*dis CLL cells, accounting for the IGHV mutational status. *TP53*wt CLL included 8 IGHV M and 9 IGHV UM samples, and *TP53*dis CLL included 5 IGHV M and 8 IGHV UM samples. A representative blot is shown, together with Unique Patients Number (UPN) and cumulative band intensity data obtained from the analysis of the four subsets (i.e. *TP53*wt/IGHV M, *TP53*wt/IGHV UM, *TP53*dis/IGHV M and *TP53*dis/IGHV UM CLL patients). Box plots represent median values and 25%-75% percentiles, whiskers represent minimum and maximum values of band intensity for each group. Vertical lines have been inserted to indicate repositioned gel lanes. **** p<0.0001, *** p<0.001, ** p<0.01.
Supplemental Figure 2

Figure S2. The expression of pVHL is not influenced by IGHV mutational status.

The expression of pVHL was measured in TP53<sup>wt</sup> and TP53<sup>dis</sup> CLL cells, accounting for the IGHV mutational status. TP53<sup>wt</sup> CLL included 9 IGHV M and 6 IGHV UM samples, and TP53<sup>dis</sup> CLL included 6 IGHV M and 6 IGHV UM samples. A representative blot is shown, together with UPN and cumulative band intensity data obtained from the analysis of the four subsets. Box plots represent median value and 25%-75% percentiles, whiskers represent minimum and maximum values of band intensity for each group. Vertical lines have been inserted to indicate repositioned gel lanes. **** p<0.0001, ** p<0.01.
Supplemental Figure 3

A

|     | 0 | 0.001 | 0.01 | 0.1 | 1 | 10 | PD ($\mu$M) |
|-----|---|-------|------|-----|---|----|-------------|
| HIF-1α |   |       |      |     |   |    |             |
| GAPDH |   |       |      |     |   |    |             |
| HIF-1α |   |       |      |     |   |    |             |
| GAPDH |   |       |      |     |   |    |             |

B

|     | 0 | 0.001 | 0.01 | 0.1 | 1 | 10 | LY ($\mu$M) |
|-----|---|-------|------|-----|---|----|-------------|
| HIF-1α |   |       |      |     |   |    |             |
| GAPDH |   |       |      |     |   |    |             |
| HIF-1α |   |       |      |     |   |    |             |
| GAPDH |   |       |      |     |   |    |             |

C

|     | 0 | 0.001 | 0.01 | 0.1 | 10 | Y276 ($\mu$M) |
|-----|---|-------|------|-----|----|---------------|
| HIF-1α |   |       |      |     |    |               |
| GAPDH |   |       |      |     |    |               |
| HIF-1α |   |       |      |     |    |               |
| GAPDH |   |       |      |     |    |               |
**Figure S3.** Increasing concentrations of ERK1-2, PI3K or RHOA kinase inhibitors determine a progressive reduction of HIF-1α levels. Primary CLL cells were cultured for 48 hours in the presence of increasing concentration of PD98059 (PD, 0.01 µM, 0.1 µM, 1 µM or 10 µM) (A), LY294002 (LY, 0.01 µM, 0.1 µM, 1 µM or 10 µM) (B) or Y27632 (Y276 0.01 µM, 0.1 µM, 1 µM or 10 µM) (C). WB analyses of HIF-1α protein expression for 1 *TP53*wt and 1 *TP53*dis representative CLL patient, together with UPN and the corresponding cumulative band intensity data, are shown. ERK1-2, AKT and RHOA kinase activities were evaluated by an immunomeditated assay and are shown by bar graphs on the right.
Supplemental Figure 4

Figure S4. Viability of $HIF-1\alpha^{\text{high}}$ and $HIF-1\alpha^{\text{low}}$ CLL cells. Representative flow cytometry analysis (relative UPN indicated) of AnnV/PI expression on $HIF-1\alpha^{\text{high}}$ and $HIF-1\alpha^{\text{low}}$ CLL cells after 48-hour culture in medium. CLL cells isolated from $HIF-1\alpha^{\text{high}}$ samples had a significantly higher viability than CLL cells isolated $HIF-1\alpha^{\text{low}}$ samples.
Supplemental Figure 5

![Flow Cytometry Analysis](image)

Figure S5. Viability of TP53\textsuperscript{wt} and TP53\textsuperscript{dis} CLL cells exposed to BAY87-2243. Representative flow cytometry analysis (relative UPN indicated) of AnnV/PI expression on TP53\textsuperscript{wt} and TP53\textsuperscript{dis} CLL cells exposed to 1 µM BAY87-2243 (BAY) or left untreated for 48 hours. BAY87-2243 determined a direct cytotoxic effect toward leukemic cells isolated from both patient subsets.
Supplemental Figure 6

**HYPOXIA**

**TP53\(^{\text{wt}}\)**  
CTRL  
PI  
58%  
BAY (1 µM)  
34%  
UPN41  
AnnV

**TP53\(^{\text{dis}}\)**  
CTRL  
56%  
BAY (1 µM)  
31%  
UPN6  
AnnV

Figure S6. Viability of **TP53\(^{\text{wt}}\) and TP53\(^{\text{dis}}\)** CLL cells exposed to BAY87-2243 under hypoxia. Representative flow cytometry analysis (relative UPN indicated) of AnnV/PI expression on **TP53\(^{\text{wt}}\)** and **TP53\(^{\text{dis}}\)** CLL cells exposed to 1 µM BAY87-2243 (BAY) or left untreated for 48 hours, under hypoxic conditions. BAY87-2243 exerted a cytotoxic effect also when **TP53\(^{\text{dis}}\)** and **TP53\(^{\text{wt}}\)** CLL cells were cultured in conditions of hypoxia.
Supplemental Figure 7

Figure S7. Viability of TP53<sup>wt</sup> and TP53<sup>dis</sup> CLL cells exposed to BAY87-2243 in presence of SC. Representative flow cytometry analysis (relative UPN indicated) of AnnV/PI expression on TP53<sup>wt</sup> and TP53<sup>dis</sup> CLL cells exposed to 1 µM BAY87-2243 (BAY) or left untreated for 48 hours, in the presence or in the absence of the murine SC line M2-10B4. BAY87-2243 exerted a cytotoxic effect also when TP53<sup>dis</sup> and TP53<sup>wt</sup> CLL cells were co-cultured with SC.
Figure S8. Viability of $TP53^{\text{wt}}$ and $TP53^{\text{dis}}$ CLL cells exposed to BAY87-2243 + fludarabine. Representative flow cytometry analysis (relative UPN indicated) of AnnV/PI expression on $TP53^{\text{dis}}$ and $TP53^{\text{wt}}$ CLL cells exposed for 48 hours to 1 μM BAY87-2243 (BAY) and/or 10 μM F-ara-A. The combination BAY87-2243 + F-ara-A determined a significant decrease in the viability of $TP53^{\text{wt}}$ and $TP53^{\text{dis}}$ CLL cells, compared to each compound used as single agent and to untreated controls.
Supplemental Figure 9

| F-ara-A (μM) | 0.01 | 0.1 | 1   | 10  |
|--------------|------|-----|-----|-----|
| BAY (μM)     |      |     |     |     |
| 0.01         | 1.58 | 6.9 | 0.28| 1.1 |
| 0.1          | 0.44 | 0.58| 0.29| 1.21|
| 0.5          | 0.67 | 1.74| 0.24| 0.92|
| 1            | 1.53 | 0.73| 0.38| 0.62|

| Combination Index |
|-------------------|

**Figure S9. CI of BAY87-2243 + fludarabine combinations.** Figure showing combination indexes (CI) relative to 48-hour treatment with BAY87-2243 (BAY) and F-ara-A, used at different concentrations in TP53\textsuperscript{wt} and TP53\textsuperscript{dis} CLL cells. CI<1, highlighted in bold, indicate synergistic combinations.
Figure S10. BAY87-2243 + fludarabine combination exerts a significant cytotoxic effect on \( TP53^{\text{dis}} \) and \( TP53^{\text{wt}} \) CLL cultured under hypoxia or in the presence of SC. Normalized cell viability of \( TP53^{\text{dis}} \) and \( TP53^{\text{wt}} \) CLL cells exposed for 48 hours to 1 \( \mu \text{M} \) BAY87-2243 (BAY) and/or 10 \( \mu \text{M} \) F-ara-A, under normoxia and hypoxia, or in co-culture with SC. The combination BAY87-2243 + F-ara-A (striped pattern) determined a significant decrease in the viability of \( TP53^{\text{mt}} \) and \( TP53^{\text{dis}} \) CLL cells, compared to each compound used as single agent, in condition of hypoxia (A, B) or in the presence of SC (C, D). In panels A and C, box plots represent median values and 25%-75% percentiles, whiskers represent minimum and maximum values for each group, together with all the points. In panels B and D, representative flow cytometry analysis (relative UPN indicated) of
AnnV/PI expression on $TP53^{\text{dis}}$ and $TP53^{\text{wt}}$ CLL cells exposed for 48 hours to 1 μM BAY87-2243 and/or 10 μM F-ara-A in the presence of SC.
Supplemental Figure 11

Figure S11. Viability of $TP53^{wt}$ and $TP53^{dis}$ CLL cells exposed to BAY87-2243 + ibrutinib. Representative flow cytometry analysis (relative UPN indicated) of AnnV/PI expression on $TP53^{dis}$ and $TP53^{wt}$ CLL cells exposed for 48 hours to 1 μM BAY87-2243 (BAY) and/or 10 μM ibrutinib. The combination BAY87-3342 + ibrutinib determined a significant decrease in the viability of $TP53^{wt}$ and $TP53^{dis}$ CLL cells, compared to each compound used as single agent and to untreated controls.
Supplemental Figure 12

Supplemental references

1. Ricca I, Roci A, Drandi D, et al. Telomere length identifies two different prognostic subgroups among VH-unmutated B-cell chronic lymphocytic leukemia patients. Leukemia 2007;21(4):697–705.

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