EMBO
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Enhanced cytotoxicity of PARP inhibition in Mantle Cell Lymphoma harboring mutations in both ATM and p53

Chris T. Williamson, Eiji Kubota, Jeffrey D. Hamill, Alexander Klimowicz, Ruiqiong Ye, Huong Muzik, Michelle Dean, Li Ren Tu, David Gilley, Anthony M. Magliocco, Bruce C. McKay, D. Gwyn Bebb and Susan P. Lees-Miller

Corresponding author: Susan Lees-Miller, University of Calgary

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 14 September 2011

Thank you for the submission of your manuscript "Enhanced efficacy of PARP inhibition in Mantle Cell Lymphoma harboring ATM and p53 mutations" to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript. You will see that they find the topic of your manuscript potentially interesting. However, they also raise significant concerns on the study, which should be addressed in a major revision of the manuscript.

In particular, reviewer #1 highlights the need to assess potential side effects on normal proliferating cells and tissues. In addition, this reviewer notes that the effect of anthracyclines should be investigated. Reviewer #3 notes that the it should be clarified whether p53 status affects the effect of PARPi on ATM-proficient cells.

Given the balance of these evaluations, we feel that we can consider a revision of your manuscript if you can convincingly address the issues that have been raised within the space and time constraints outlined below.

Revised manuscripts should be submitted within three months of a request for revision. They will otherwise be treated as new submissions, unless arranged otherwise with the editor.

I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #1:

This is an interesting and potentially very significant translational paper focusing on the factors that can determine sensitivity of tumors to PARP inhibitors. The authors extend the findings in the field that tumors with combined p53 and ATM defects are very sensitive to genotoxic treatments other than PARPi, and show here that this is also the case for PARP inhibitors, on a model of mantle cell lymphoma cell lines with different combination of ATM, p53 and DNA-PK status. The experiments are mostly well controlled and the conclusions adequate. On the other hand, some issues/results are open to alternative explanations and one important control is missing to assess the idea of using the ATM inhibitors concomitantly with PARP inhibitors in vivo.

specific points.

1. The suggestion to use combined ATM and PARP inhibitors needs some validation in terms of potential side effects on normal proliferating cells and tissues. One control would be to perform the mouse in vivo experiments with MCL xenografts, using ATM-proficient, p53-proficient versus deficient cell lines, and threat the mice with both ATM and PARP inhibitors - apart from the effects on tumor growth, impact in proliferating tissues such as intestine should be examined (enhanced endogenous damage, cell death...) to see whether such treatment is feasible or too toxic.

2. Another control to the point (1) above would be to compare responses of MCL cell lines versus stimulated normal lymphocytes or at leat diploid fibroblasts grown in culture, and treated with ATM and PARP inhibitors individually and combined. The points 1 + 2 are important, since otherwise the major message from this study is too speculative.

3. The effects of p53 activation are monitored only by changes in expression of checkpoint proteins such as p21. This should be complemented by key pro-apoptotic targets of p53, such as PUMA and Noxa, which direct the p53 response towards apoptosis and therefore may contribute to the outcome of the treatments that affect p53.

4. The interesting 'discrepancy' between the outcome of DNA-PK inhibition in ATM-deficient tumors in response PARP inhibitor (loss of sensitivity, this study) versus anthracyclines (gain of sensitivity, previous studies) could have an alternative explanation, namely that undefined genetic changes in the different cancer cell lines, rather than the different type of DNA lesions, may explain the opposite biological outcomes. To solve this puzzle, the authors should also treat the models of MCL with epirubicine or doxorubicine, for example, to see whether inhibition of DNA-PK would sensitize the ATM-deficient MCL cells. This would help to strengthen the message of the paper and help to interpret the data within the context of the field.

Referee #2:

This is an excellent and very well written manuscript that clearly addresses the efficacy of PARP inhibitor on MCL cells carrying ATM and p53 mutations. The authors show that such tumors are more sensitive to olaparib if both ATM and p53 are deficient. Further, the inappropriate recruitment of the NHEJ pathway into repair of double-strand breaks by DNA-PK phosphorylation and stabilization of p53 and the inhibition of DNA-PK reduce the toxicity of the PARP inhibitor in ATM-deficient cells.

These studies have novel and direct translational and biological implications and shed light on why PARP inhibition adjunctive to chemotherapy failed a recently completed Phase III study and did not generate the anticipated survival gains.

Minor comments:

Page 16, para 2; First two sentences treat data as singular when it should be plural.

Figure 4B. It is unclear why the phosphorylated p53 levels appear to merely reflect the level of stabilized native p53. Perhaps additional data could be added. Figure 5A suggests a similar result for the irradiated Granta-519 cells.

Figure 4C. The authors should recheck whether the PARP-inhibitor treated Granta-519 cells are really significantly different from the untreated. The raw data do not look like they should be significant.

Referee #3:

The manuscript describes work that furthers our understanding of the cytotoxic activity of PARP inhibitors against mantle cell lymphoma. The authors have previously shown that ATM deficient MCL cells are sensitive to PARP inhibitors. Here they demonstrate that co-deficiency of p53 augments this sensitivity, and go on to show that p53 defective tumour cells are sensitive to a combination of ATM and PARP inhibitors. This may have more widespread therapeutic potential.

They then investigate the more complicated role of DNA-PK in this relationship and show that, while DNA-PK appears to activate p53 in response to PARP inhibition (in the absence of ATM), inhibition of DNA-PK actually protects ATM defective cells from the cytotoxic effects of PARP inhibition. Quoting recent publications, they attribute this to the cytotoxic effect of attempts by NHEJ to execute repair of replication-associated DNA breaks in the absence of functional HR.

The key data is convincing and the paper is well written and mostly very clear.

Recommendations prior to publication:

Introduction:
P6 line 13 the sentence starting 'In addition, PARP inhibition....' needs to be clarified, with regard to what is dependent on DNA-PK activity

It would be helpful to indicate how frequently ATM and p53 are co-mutated in MCL at this stage of the manuscript.
It is also important to cover the proposed role for NHEJ in exacerbating the toxicity of PARP inhibitors in HR defective cells. Without this background the later observations relating to DNA-PK appear to be novel and unexpected, whereas in fact they are predicted by the work of Patel et al.

Figure 1B: survival data for UPN2 +/- DN-p53 should be shown to confirm the p53 dependence of the difference in sensitivity to olaparib. This is particularly important because the difference in effect of pifthirin between the two cell lines is small (Fig 1C). These data and the data in Figure 4B are compatible with there being residual p53 activity in UPN2.

Does p53 status affect the cytotoxicity of PARP inhibitors in ATM proficient cells? The authors should clarify this - indeed Figure 6 shows that olaparib has modest activity against ATM proficient cells and that this is independent of p53 status.

Discussion:
Mostly very clear.
P15 line 10 this sentence should be rewritten to clarify the meaning

P16 lines 15 and 17 - it is misleading to describe DNA-PK mediated activation of p53 as a 'backup pathway'. The authors should distinguish more clearly between DNA repair pathways and cell cycle checkpoint pathways.

P17 final paragraph - the role of PARP in DNA repair is different in the context of DNA damaging agents. The work described in this manuscript relates only to continuous exposure to PARP inhibitors in the absence of exogenous DNA damage. This should be clarified.

1st Revision - Authors' Response 06 January 2012

*Response to Reviewers comments - Responses are indicated by ** and in italics below*

Referee #1:

This is an interesting and potentially very significant translational paper focusing on the factors that can determine sensitivity of tumors to PARP inhibitors. The authors extend the findings in the field that tumors with combined p53 and ATM defects are very sensitive to genotoxic treatments other than PARPi, and show here that this is also the case for PARP inhibitors, on a model of mantle cell lymphoma cell lines with different combination of ATM, p53 and DNA-PK status. The experiments are mostly well controlled and the conclusions adequate. On the other hand, some issues/results are open to alternative explanations and one important control is missing to assess the idea of using the ATM inhibitors concomitantly with PARP inhibitors in vivo.

specific points.

1. The suggestion to use combined ATM and PARP inhibitors needs some validation in terms of potential side effects on normal proliferating cells and tissues. One control would be to perform the mouse in vivo experiments with MCL xenografts, using ATM-proficient, p53-proficient versus deficient cell lines, and threat the mice with both ATM and PARP inhibitors - apart from the effects on tumor growth, impact in proliferating tissues such as intestine should be examined (enhanced endogenous damage, cell death...) to see whether such treatment is feasible or too toxic.

*** The reviewer raises an important point. However, given the time restriction for resubmission, we were unable to perform additional animal experiments. We have however addressed the reviewer's concerns in our response to point 2, below.*

2. Another control to the point (1) above would be to compare responses of MCL cell lines versus stimulated normal lymphocytes or at leat diploid fibroblasts grown in culture, and treated with ATM and PARP inhibitors individually and combined. The points 1 + 2 are important, since otherwise the major message from this study is too speculative.

*** As suggested by the reviewer, we have examined the effects of combining PARP and ATM inhibitors on untransformed normal human fibroblasts (BJ cells) containing either wild type p53 or in BJ cells in which p53 function has been disrupted by expression of HPV E6/E7 (new Figure 3B). The results show that inhibition of ATM and PARP is more cytotoxic in normal human fibroblasts with disruption of p53 than in those with wild type p53, thus confirming our observations in MCL cell lines.*

In addition, we have stressed that additional in vivo experiments will be required before it can be determined whether this approach will have clinical potential. The following section has been added to page 12:

"However, we caution that potential concerns with this approach could be the induction of a synthetic lethal response in non-malignant, p53-proficient cells and enhanced cytotoxicity caused by combining both inhibitors, and further studies in animal models will be required to confirm the utility of this approach in an in vivo setting".

3. The effects of p53 activation are monitored only by changes in expression of checkpoint proteins such as p21. This should be complemented by key pro-apoptotic targets of p53, such as PUMA and Noxa, which direct the p53 response towards apoptosis and therefore may contribute to the outcome of the treatments that affect p53.

*** The reviewer raises a valid point. Accordingly, we have used quantitative RT-PCR to examine the levels of several p53-responsive genes including p21, Puma and NOXA in Granta-519 and UPN2 cells. We have also examined the protein levels of p21, GADD45α, Puma and NOXA by western blot. This new data demonstrates that olaparib induces p21 mRNA and protein expression in Granta-519 cells, but less so in UPN2 cells. Interestingly, despite a clear apoptotic response in both cell lines, no induction of Puma or NOXA mRNA was detected in either cell line, indicating that olaparib induces p53-independent apoptosis. This new data is presented in Figure 4 of the revised manuscript and Supplementary Figures 7A and 7B. The new results are described on pages 9-10.*

4. The interesting 'discrepancy' between the outcome of DNA-PK inhibition in ATM-deficient tumors in response PARP inhibitor (loss of sensitivity, this study) versus anthracyclines (gain of sensitivity, previous studies) could have an alternative explanation, namely that undefined genetic changes in the different cancer cell lines, rather than the different type of DNA lesions, may explain the opposite biological outcomes. To solve this puzzle, the authors should also treat the models of MCL with epirubicine or doxorubicine, for example, to see whether inhibition of DNA-PK would sensitize the ATM-deficient MCL cells. This would help to strengthen the message of the paper and help to interpret the data within the context of the field.

*** To address the reviewer's concerns, we have treated the MCL cell lines with doxorubicin in combination with the DNA-PK inhibitor NU7441 or vehicle control. This new data (shown in Supplementary Figure 9) demonstrates that, unlike combining DNA-PK inhibition with PARP inhibitors, addition of DNA-PK inhibitors did not decrease the toxicity of doxorubicin in any of the MCL cell line examined. The following text has been added to page 11 (results):*

"In contrast, inactivation of DNA-PK has been reported to increase sensitivity to doxorubicin in ATM-depleted p53 positive cells (Jiang et al, 2009). We therefore asked whether or not inhibition of DNA-PK would protect the MCL cell lines from doxorubicin cytotoxicity. In MCL, NU7441 caused a modest increase in sensitivity to doxorubicin, regardless of ATM or p53 status (Supplementary Figure 9)".

In addition, we suggest that this 'discrepancy' is caused by the differences in the type of DNA damage induced by doxorubicin (DNA double strand breaks) and PARP inhibitors (DNA single strand breaks), as discussed in the discussion section (pages 13-14).

Referee #2:

This is an excellent and very well written manuscript that clearly addresses the efficacy of PARP inhibitor on MCL cells carrying ATM and p53 mutations. The authors show that such tumors are more sensitive to olaparib if both ATM and p53 are deficient. Further, the inappropriate recruitment of the NHEJ pathway into repair of double-strand breaks by DNA-PK phosphorylation and stabilization of p53 and the inhibition of DNA-PK reduce the toxicity of the PARP inhibitor in ATM-deficient cells.

These studies have novel and direct translational and biological implications and shed light on why PARP inhibition adjunctive to chemotherapy failed a recently completed Phase III study and did not generate the anticipated survival gains.

** We thank the reviewer for these encouraging comments!*

Minor comments:

Page 16, para 2; First two sentences treat data as singular when it should be plural.

** These sentences have been corrected*

Figure 4B. It is unclear why the phosphorylated p53 levels appear to merely reflect the level of stabilized native p53. Perhaps additional data could be added. Figure 5A suggests a similar result for the irradiated Granta-519 cells.

** The observation is consistent with studies discussed in a recent review on the effects of phosphorylation of p53 function (Maclaine and Hupp (2011), Cell Cycle, which is now cited in the text) as well as our own previous published work, as stress induced phosphorylation of p53 leads to its dissociation form the E3 ubiquitin ligase MDM2. This dissociation blocks the endogenous ubiquition and degradation of p53, leading to its stabilization.*

Figure 4C. The authors should recheck whether the PARP-inhibitor treated Granta-519 cells are really significantly different from the untreated. The raw data do not look like they should be significant.

** We have rechecked the statistics on the above data and have confirmed that, while modest, there is a statistically significant difference between p21 levels in DMSO treated animals and those treated with olaparib. The raw data is provided for the reviewer at the end of this document.*

Referee #3:

The manuscript describes work that furthers our understanding of the cytotoxic activity of PARP inhibitors against mantle cell lymphoma. The authors have previously shown that ATM deficient MCL cells are sensitive to PARP inhibitors. Here they demonstrate that co-deficiency of p53 augments this sensitivity, and go on to show that p53 defective tumour cells are sensitive to a combination of ATM and PARP inhibitors. This may have more widespread therapeutic potential.

They then investigate the more complicated role of DNA-PK in this relationship and show that, while DNA-PK appears to activate p53 in response to PARP inhibition (in the absence of ATM), inhibition of DNA-PK actually protects ATM defective cells from the cytotoxic effects of PARP inhibition. Quoting recent publications, they attribute this to the cytotoxic effect of attempts by NHEJ to execute repair of replication-associated DNA breaks in the absence of functional HR.

The key data is convincing and the paper is well written and mostly very clear.

** Thank you!*

Recommendations prior to publication:

Introduction:

P6 line 13 the sentence starting 'In addition, PARP inhibition....' needs to be clarified, with regard to what is dependent on DNA-PK activity

*** This section has been rewritten.*

It would be helpful to indicate how frequently ATM and p53 are co-mutated in MCL at this stage of the manuscript. It is also important to cover the proposed role for NHEJ in exacerbating the toxicity of PARP inhibitors in HR defective cells. Without this background the later observations relating to DNA-PK appear to be novel and unexpected, whereas in fact they are predicted by the work of Patel et al.

*** The Introduction has been rewritten to take into account both of these points.*

Figure 1B: survival data for UPN2 +/- DN-p53 should be shown to confirm the p53 dependence of the difference in sensitivity to olaparib. This is particularly important because the difference in effect of pifthirin between the two cell lines is small (Fig 1C). These data and the data in Figure 4B are compatible with there being residual p53 activity in UPN2.

*** We have transfected UPN2 cells with either dominant-negative-p53 or wild-type p53 and examined their effects on the toxicity of olaparib. This new data, which is provided in Figure 1C, shows that transfection of dominant-negative p53 into UPN2 cells had no effect on olaparib sensitivity, however, transfection of wild-type p53 modestly (but in a statistically significant manner) reduced the toxicity of olaparib, consistent with the model presented in the manuscript. We thank the reviewer for suggesting this experiment, the results of which further strengthen our conclusions.*

Does p53 status affect the cytotoxicity of PARP inhibitors in ATM proficient cells? The authors should clarify this - indeed Figure 6 shows that olaparib has modest activity against ATM proficient cells and that this is independent of p53 status.

*** Our data indicates that deficiency of p53 does not have a significant effect on the sensitivity of ATM-proficient MCL cells to olaparib. Both JVM-2 (p53 wild-type) and HBL-2 (p53 mutant) are wild-type for ATM, however both cell lines display a similar level of olaparib sensitivity both in vitro and in vivo (Figure 1A and Supplementary Figure 4). Similarly in Figure 3A, while olaparib alone had some cytotoxicity compared to untreated cells but there was no significant difference between Z138 and JVM2 (p53 wild type) and HBL2 and UPN1 (p53 mutant). Similarly olaparib has only a modest effect on JVM2 (p53 proficient, Figure 5C) and on HBL2 (p53 deficient, Figure 5D) and in Figure 3B, olaparib had only a modest effect on both BJ (p53 wild type) and BJ E6/E7 (p53 disrupted). We thank the reviewer for raising this important point and have now strengthened these conclusions in the revised manuscript by adding the following sentence to the Discussion (page 11):*

"Here, we show that MCL cell lines and normal human fibroblasts with inactivation or mutation of both ATM and p53 are more sensitive to the PARP inhibitor olaparib than the same cells with inactivation or mutation of ATM alone. In contrast, p53 mutation had little effect on olaparib-induced cytotoxicity in ATM-proficient cells".

Discussion:

Mostly very clear.

P15 line 10 this sentence should be rewritten to clarify the meaning

** *This section has been rewritten.*

P16 lines 15 and 17 - it is misleading to describe DNA-PK mediated activation of p53 as a 'backup pathway'. The authors should distinguish more clearly between DNA repair pathways and cell cycle checkpoint pathways.

** *This section has been rewritten.*

P17 final paragraph - the role of PARP in DNA repair is different in the context of DNA damaging agents. The work described in this manuscript relates only to continuous exposure to PARP inhibitors in the absence of exogenous DNA damage. This should be clarified.

** *Thank you. This sentence has been removed.*

Additional information for Reviewers:

Summary of calculations on IHC of p21 in xenografts:

	Granta-519		UPN2	
Olaparib Dose	0	50	0	50
AVE p21 IHC score	1.00	1.16	1.00	0.94
SD	0.16	0.26	0.21	0.17
N	24	28	27	21
SEM	0.032587293	0.049393489	0.03959344	0.037308578
Range	0.68 - 1.36	0.78 - 1.74	0.41 - 1.23	0.62 - 1.23
P-valute (T-test 0 vs 50)	*0.012054465*		0.310220926	

Raw data of IHC results for p21 expression in xenografts:

			p21			Norm values
Cell	X	Y	nuc	tumor		
GR 0	9	2	152.8758	178.6482	Pass	1.131918643
GR 0	10	2	108.2849	120.008	Pass	0.801759972
GR 0	9	3	92.24111	110.1772	Pass	0.682968999
GR 0	10	3	175.9999	207.93	Pass	1.303133445
GR 0	9	4	132.6117	173.7429	Pass	0.981879771
GR 0	10	4	131.4813	146.9483	Pass	0.973510096

GR 0	1	5	104.8621	111.8535	Pass	0.776416973
GR 0	10	5	131.1539	151.0825	Pass	0.97108597
GR 0	1	6	132.0255	138.6172	Pass	0.977539446
GR 0	9	6	183.899	207.6345	Pass	1.361619737
GR 0	1	7	131.3056	145.3042	Pass	0.972209183
GR 0	9	7	145.0334	156.9605	Pass	1.073852168
GR 0	10	7	102.2823	111.5208	Pass	0.757315692
GR 0	1	8	134.2558	152.0099	Pass	0.994052969
GR 0	9	8	135.7338	152.8625	Pass	1.004996335
GR 0	10	8	161.2544	190.9685	Pass	1.193955234
GR 0	1	9	141.5827	152.002	Pass	1.048302594
GR 0	10	9	136.8679	156.1371	Pass	1.013393406
GR 0	1	10	114.4722	126.1922	Pass	0.847571802
GR 0	9	10	153.8027	170.5622	Pass	1.13878157
GR 0	10	10	142.4735	152.4418	Pass	1.05489823
GR 0	1	11	135.371	144.9379	Pass	1.002310102
GR 0	1	12	127.5813	140.2298	Pass	0.944633827
GR 0	1	13	133.9747	144.9682	Pass	0.991971657
GR 50	8	2	107.764	146.1118	Pass	0.797903139
GR 50	8	3	109.3191	148.5926	Pass	0.809417366
GR 50	8	4	115.4409	158.0974	Pass	0.854744223
GR 50	8	5	146.857	166.3961	Pass	1.087354415
GR 50	7	6	152.7092	175.1551	Pass	1.130685108
GR 50	8	6	185.8559	206.6029	Pass	1.37610896
GR 50	7	7	127.7526	142.0348	Pass	0.945902161
GR 50	8	7	129.5982	137.1623	Pass	0.9595673
GR 50	6	8	119.0631	165.3138	Pass	0.881563613
GR 50	7	8	179.106	205.496	Pass	1.326131543
GR 50	6	9	129.0518	155.5496	Pass	0.955521661
GR 50	7	9	126.8664	179.797	Pass	0.939340584
GR 50	8	9	141.6183	195.0245	Pass	1.048566182
GR 50	6	10	158.4438	188.355	Pass	1.17314507
GR 50	7	10	139.3392	188.3204	Pass	1.031691335
GR 50	8	10	175.8991	242.7131	Pass	1.302387105
GR 50	6	11	165.4839	191.175	Pass	1.225271178
GR 50	7	11	175.4427	204.319	Pass	1.299007841
GR 50	8	11	145.7399	160.2419	Pass	1.079083215
GR 50	9	11	221.001	251.8157	Pass	1.636329308

GR 50	6	12	171.4411	190.7122	Pass	1.269379308
GR 50	7	12	131.2604	145.0194	Pass	0.971874514
GR 50	8	12	154.0964	167.7621	Pass	1.140956175
GR 50	9	12	231.6208	247.1248	Pass	1.714960129
GR 50	6	13	210.2204	231.2901	Pass	1.556507897
GR 50	7	13	152.3729	167.9877	Pass	1.128195085
GR 50	8	13	147.4406	156.4924	Pass	1.09167549
GR 50	9	13	235.1654	259.7247	Pass	1.741204955
U2 0	13	2	290.8045	403.1226	Pass	0.964724089
U2 0	14	2	255.4755	391.1875	Redacted	0.847522542
U2 0	13	3	238.5689	335.9541	Pass	0.79143605
U2 0	14	3	275.9902	382.7858	Pass	0.91557866
U2 0	13	4	369.8936	383.4177	Pass	1.227096783
U2 0	14	4	268.135	276.6168	Pass	0.88951957
U2 0	13	5	126.3632	174.9939	Pass	0.419201295
U2 0	14	5	314.8762	353.0897	Pass	1.044580312
U2 0	15	5	259.5465	357.9855	Pass	0.861027807
U2 0	13	6	274.0248	289.886	Pass	0.909058579
U2 0	14	6	283.9073	381.4171	Pass	0.941843099
U2 0	15	6	345.086	364.1136	Pass	1.144799262
U2 0	13	7	371.9779	395.7325	Pass	1.234011306
U2 0	14	7	260.6372	371.4087	Pass	0.86464613
U2 0	15	7	214.0795	281.6313	Pass	0.710194136
U2 0	13	8	384.1168	383.086	Pass	1.274281278
U2 0	14	8	302.9417	314.3965	Pass	1.004988422
U2 0	15	8	266.8986	366.5703	Pass	0.885417897
U2 0	14	9	347.8477	355.5339	Pass	1.153961014
U2 0	13	10	278.0489	296.843	Pass	0.922408256
U2 0	14	10	340.5699	351.604	Pass	1.129817409
U2 0	15	10	413.4613	431.7599	Pass	1.371629655
U2 0	14	11	340.0009	354.1303	Pass	1.12792979
U2 0	15	11	346.1588	377.9556	Pass	1.148358203
U2 0	14	12	379.2906	393.4995	Pass	1.258270689
U2 0	15	12	334.4943	354.689	Pass	1.10966202
U2 0	15	13	255.6303	363.1401	Pass	0.84803608
U2 50	11	2	185.7126	218.0753	Pass	0.616088881
U2 50	11	3	314.3507	343.6429	Pass	1.042837001

U2 50	12	4	300.2777	293.6972	Pass	0.996150784
U2 50	11	6	304.5228	320.3783	Pass	1.010233614
U2 50	12	6	256.9825	274.3994	Pass	0.852521912
U2 50	11	7	338.3548	349.3185	Pass	1.122468965
U2 50	12	7	338.206	354.4594	Pass	1.121975332
U2 50	11	8	297.6499	281.5694	Pass	0.987433237
U2 50	12	8	371.2266	396.6263	Pass	1.231518919
U2 50	11	9	212.1721	220.2955	Pass	0.703866467
U2 50	12	9	221.9155	300.1741	Pass	0.736189532
U2 50	11	10	251.3768	342.6041	Pass	0.833925384
U2 50	12	10	254.2082	351.4001	Pass	0.843318361
U2 50	11	11	232.8862	308.1993	Pass	0.77258408
U2 50	12	11	238.8028	312.313	Pass	0.792211997
U2 50	13	11	301.2566	299.6811	Pass	0.999398218
U2 50	11	12	325.7916	335.0558	Pass	1.080791407
U2 50	12	12	292.3225	306.319	Pass	0.969759951
U2 50	13	12	319.021	327.7248	Pass	1.058330403
U2 50	11	13	244.3613	362.3424	Pass	0.810651942
U2 50	13	13	367.0907	385.1448	Pass	1.217798353

2nd Editorial Decision 30 January 2012

Thank you for the submission of your revised manuscript "Enhanced cytotoxicity of PARP inhibition in Mantle Cell Lymphoma harboring mutations in both ATM and p53" to EMBO Molecular Medicine. We have now received the report from the reviewer who was asked to re-review your manuscript.

You will be glad to see that the reviewer is supportive and we can proceed with official acceptance of your manuscript pending the minor changes detailed below:

- Please provide a Table of Contents on the first page of the Supplementary Material.

- Please see below for information regarding EMBO Molecular Medicine guidelines for statistical analysis of data and provide the actual p value for each test.

- Please adjust the format of The Paper Explained according to our journal style. You can find examples in previously published EMBO Molecular Medicine papers.

I look forward to seeing a revised version of your manuscript as soon as possible.

Statistical analysis
The description of all reported data that includes statistical testing must state the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the actual P value for each test (not merely 'significant' or 'P < 0.05').

Yours sincerely,

Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #3:

I have reviewed the resubmission and am happy for it to be published. All comments addressed adequately.

2nd Revision - Authors' Response 05 February 2012

As requested, we have:

1) added p values for each of Figures 1A,B,C,D, 2A, C, D, Figure 3A,B, Figure 4D, Figure 5A,B,C,D to the Figure legends (pages 26-29),

2) provided a table of contents on the first page of the Supplementary Information, and

3) re-cast “The paper explained” to include three sections, “The Problem”, “Results” and Impact”.

On behalf of all authors, my sincere thanks for your interest in our work