Blocking c-Met–mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors

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Poly (ADP-ribose) polymerase (PARP) inhibitors have emerged as promising therapeutics for many diseases, including cancer, in clinical trials. One PARP inhibitor, olaparib (Lynparza, AstraZeneca), was recently approved by the FDA to treat ovarian cancer with mutations in BRCA genes. BRCA1 and BRCA2 have essential roles in repairing DNA double-strand breaks, and a deficiency of BRCA proteins sensitizes cancer cells to PARP inhibition. Here, we show that the receptor tyrosine kinase c-Met associates with and phosphorylates PARP1 at Tyr907 (PARP1 pTyr907 or pY907). PARP1 pY907 increases PARP1 enzymatic activity and reduces binding to a PARP inhibitor, thereby rendering cancer cells resistant to PARP inhibition. The combination of c-Met and PARP1 inhibitors synergized to suppress the growth of breast cancer cells in vitro and xenograft tumor models, and we observed similar synergistic effects in a lung cancer xenograft tumor model. These results suggest that the abundance of PARP1 pY907 may predict tumor resistance to PARP inhibitors, and that treatment with a combination of c-Met and PARP inhibitors may benefit patients whose tumors show high c-Met expression and who do not respond to PARP inhibition alone.

Increased levels of reactive oxygen species (ROS) in cells can cause oxidative DNA damage that leads to genomic instability and tumor development. ROS-induced DNA damage, such as single-strand breaks (SSBs), recruits PARP1 to lesion sites to orchestrate the DNA-repair process through poly-ADP-ribosylation (PARylation) of PARP1 and its target proteins, including histone proteins. PARylated histones destabilize the chromatin structure, allowing the DNA-repair machinery to access the damaged DNA site. Therefore, in theory, inhibiting PARP1 activity would prevent DNA repair and promote death of tumor cells. The tumor suppressors BRCA1 and BRCA2 have essential roles in repairing DNA damage. Notably, mutations in the BRCA1 and BRCA2 genes have been associated with increased risk of ovarian and breast cancers. In addition, tumor cells that lack functional BRCA1 or BRCA2 have demonstrated sensitivity to PARP1 inhibition in both pre-clinical and clinical studies. PARP inhibitors were therefore initially investigated in clinical trials for both ovarian cancer and in triple-negative breast cancer (TNBC)—which can harbor defective BRCA1 or BRCA2—as well as in other cancer types. Recently, olaparib was approved by the FDA to treat BRCA1 or BRCA2 mutation–carrying ovarian cancer. Although TNBC is an aggressive subtype of breast cancer that is closely related to basal-like breast cancer (BLBC), which initially responds to chemotherapy, a majority of TNBCs eventually develop resistance to chemotherapy. There are no approved targeted therapies to treat TNBC. Whereas encouraging results were reported in one study of olaparib treatment of TNBC patients carrying tumors with BRCA1 or BRCA2 mutations, beneficial effects of olaparib treatment were not observed in another cohort. These discrepant clinical observations raise the important question of how to increase the response rate of TNBC—and that of other cancer types—to PARP inhibitors. To address this question, we investigated the molecular mechanisms that contribute to PARP inhibitor resistance in TNBC.

We first noticed that TNBC showed higher oxidative damage DNA than non-TNBC tissue, as indicated by immunohistochemical (IHC) staining for the DNA damage marker 8-hydroxydeoxyguanosine (8-OHdG) on a human breast cancer tissue microarray (Fig. 1a and Supplementary Table 1) and in human breast cancer cell lines (Fig. 1b,c and Supplementary Fig. 1a) by immunofluorescence staining (1.9-fold difference TNBC versus non-TNBC, 95% confidence interval (CI) = 1.6–2.2) and ELISA assay (2.1-fold difference TNBC versus non-TNBC, 95% CI = 1.8–2.4). Oxidative DNA damage caused by ROS stimulates the activity of PARP1 (refs. 16–20). In accordance with

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this, the abundance of ROS (Fig. 1d and Supplementary Fig. 1b,c), as measured by the marker 2′,7′-dichlorofluorescein (DCF; intensity: 2.6-fold difference TNBC versus non-TNBC, 95% CI = 1.9–3.3; absorbance 1.33-fold difference, 95% CI = 1.3–1.4), and the level of PARP1 activity (Fig. 1e, right), as measured by poly(ADP)-ribose (PAR; 2.7-fold difference difference TNBC versus non-TNBC, 95% CI = 2.3–3.2), were higher in most TNBC cell lines than in non-TNBC cell lines, suggesting a positive association between ROS and PARP1 activity in TNBC.

ROS are also known to activate receptor tyrosine kinases (RTKs)\(^1\), which are druggable targets commonly overexpressed in TNBC\(^22,24\). To investigate the underlying molecular mechanisms regulating PARP1 response under ROS-induced oxidative stress and to identify potential targets, we searched for RTKs that associate with PARP1 upon ROS stimulation. To this end, PARP1-knockdown MDA-MB-231 TNBC cells re-expressing hemagglutinin (HA)-tagged PARP1 were treated with sodium arsenite to induce ROS production, and a human phospho-RTK antibody array analysis was performed on the whole-cell lysates to determine the specific activated PARP1-interacting RTKs by using an HA-specific antibody. The top three candidates—defined according to the ratio of the density of binding after sodium arsenite treatment to that of untreated cells—were ERBB3, HGFR, and FLT3 (Supplementary Table 2). Analysis of The Cancer Genome Atlas (TCGA)’s invasive breast carcinoma cohort (Supplementary Fig. 2a) indicated that only HGFR (encoding c-Met) expression was significantly higher (\(P = 1e–10\)) in TNBC than in non-TNBC tumors (Supplementary Fig. 2b).

c-Met is a proto-oncogene, and c-Met expression correlates with poor survival of patients with TNBC\(^23,25\). We detected higher expression of c-Met in TNBC cell lines than in non-TNBC cell lines, suggesting a positive association between ROS and PARP1 activity in TNBC.

Figure 1 ROS induce the association of c-Met and PARP1. (a–e) Human breast cancer tissue microarray was stained with 8-OHdG–specific antibody. (a) Representative images of 216 non-TNBC and 90 TNBC cases. Scale bar, 100 µm. (b) Human breast cancer cell lines shown in e were stained with 8-OHdG–specific antibody (Supplementary Fig. 1a). Quantification of 8-OHdG is shown. A.U., arbitrary units. (c) Human breast cancer cell lines shown in e were subjected to an ELISA assay to measure 8-OHdG abundance. (d) Human breast cancer cell lines shown in e were incubated with 10 µM 2′,7′-dichlorofluorescein diacetate (DCFDA) for 30 min. Quantification of DCF is shown. (e) Left, western blot showing expression of PAR, PARP1, and tubulin in lysates of the indicated human breast cancer cell lines. Blots are representative of triplicate experiments. Tubulin was used as a loading control. Right, band intensity of PAR normalized to tubulin. (f) Detection of PARP1 and c-Met co-localization (green) in MDA-MB-231 cells treated with H\(_2\)O\(_2\) or sodium arsenite (As), and in those cells not treated (control) by a Duolink endogenous PARP1 and c-Met association detected by immunoprecipitation (IP) and western blotting. Right, input control. Cell fractionation markers, calregulin (cytoplasmic) and lamin B (nuclear) were used as controls. LAR, luminal androgen receptor; As, sodium arsenite; L, long exposure; S, short exposure. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2. OD, optical density. Error bars represent s.d. * \(P < 0.05\), t-test.
interaction between c-Met and PARP1 in both the cytosol and nucleus as shown by a Duolink assay (Fig. 1g). As shown by treatment of MDA-MB-231 cells with the c-Met inhibitor crizotinib, the kinase activity of c-Met was required for the interaction between c-Met and PARP1, which was enhanced by H₂O₂ treatment (Fig. 1h). Nuclear trafficking of RTKs, including EGFR, from the cell surface has been proposed to use a vesicle membrane-associated pathway28–30 that requires the motor protein dynein and the SNARE (soluble NSF attachment protein receptor) protein syntaxin 6 (ref. 31). Nuclear translocation of c-Met in response to H₂O₂ stimulation also required dynein and syntaxin 6 (Supplementary Fig. 3i), suggesting that c-Met might use a similar trafficking route. Together, these findings indicated that oxidative stress induces nuclear transport of c-Met and its interaction with PARP1.

To determine whether c-Met influences tumor response to PARP inhibition, we examined TNBC cell line growth and colony formation in the presence of three different PARP inhibitors: the US Food and Drug Administration (FDA)-approved olaparib (AZD2281), as well as veliparib (ABT-888) and rucaparib (AG014699), which are under evaluation in clinical trials32, shRNA-mediated knockdown of c-Met expression (by targeting MET) rendered MDA-MB-231 cells more sensitive to all three PARP inhibitors, as indicated by decreased cell viability (Fig. 2a and Supplementary Fig. 4a–c). For example, c-Met knockdown cells showed 4.2-fold (shMet-A; 95% CI = 4.0–4.5) or 4.6-fold (shMet-B; 95% CI = 4.4–4.8) growth inhibition when treated with 60 µM ABT-888. Treatment with the c-Met inhibitors crizotinib or foretinib also enhanced MDA-MB-231 sensitivity to the PARP inhibitors (Fig. 2b and Supplementary Fig. 4d,e); anchorage-independent cell growth also decreased when c-Met was knocked down (Supplementary Fig. 4f–h). Consistent with previous findings33, inhibition of c-Met either by shRNAs or small molecules reduced ROS abundance compared to scrambled shRNA or no-treatment control (Supplementary Fig. 4i,j), suggesting that a feed-forward mechanism regulating c-Met activation and ROS may be involved in the response to PARP1-mediated DNA damage and PARP inhibition.

To further investigate the function of c-Met during responses to PARP inhibitors, we re-expressed wild-type (WT) and kinase-dead (KD) mutant c-Met in MDA-MB-231 cells subjected to c-Met shRNA-mediated knockdown (Fig. 2c, right): re-expression of WT but not KD c-Met increased the cell survival (Fig. 2c, left and Supplementary Fig. 4k). Similarly, MCF-7 cells ectopically expressing c-Met had increased cell viability (Fig. 2d and Supplementary Fig. 5a–c), clonogenicity (Fig. 2e and Supplementary Fig. 5d), and anchorage-independent cell growth (Supplementary Fig. 5e,f) in the presence of PARP inhibitors. Of note, the doses used here for the in vitro assays were comparable to those used in previous studies14,34. Together, these results indicated that c-Met activity attenuates response to PARP inhibitors.

Although BRCA1 and/or BRCA2 (BRCA1/2) mutations in and loss of BRCA1/2 are thought to be predictive markers for response

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**Figure 2** c-Met regulates resistance to PARP inhibitors. (a) Left, c-Met–knockdown cells were treated with the indicated concentrations of ABT-888 for 72 h and subjected to a cell viability assay. Right, western blot showing c-Met expression in c-Met–knockdown MDA-MB-231 cells. (b) MDA-MB-231 cells were treated with the indicated concentrations of AG014699 and crizotinib (Cri) or foretinib (Ft) for 8 d and subjected to clonogenic cell survival assay. Quantification of clonogenic cells from three independent experiments is shown. (c) Right, western blot showing c-Met expression in c-Met–knockdown cells at the 3′-UTR (shMet-C). Nuclear trafficking of RTKs, including EGFR, from the cell surface has been proposed to use a vesicle membrane-associated pathway28–30 that requires the motor protein dynein and the SNARE (soluble NSF attachment protein receptor) protein syntaxin 6 (ref. 31). Nuclear translocation of c-Met in response to H₂O₂ stimulation also required dynein and syntaxin 6 (Supplementary Fig. 3i), suggesting that c-Met might use a similar trafficking route. Together, these findings indicated that oxidative stress induces nuclear transport of c-Met and its interaction with PARP1.

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**LETTERS**
to PARP inhibitors in ovarian and breast cancers\(^2,3\), on the basis of the reported objective response, a certain percentage of patients carrying \(BRCA1/2\) mutations do not respond to PARP inhibition\(^{10,15}\). In agreement with these clinical findings, although both the MDA-MB-436 and HCC1937 breast cancer cell lines harbor \(BRCA1\) mutations, MDA-MB-436 cells are sensitive to and HCC1937 cells are resistant to PARP inhibition\(^4\). We speculated that the differences in PARP inhibitor response observed in \(BRCA1\)-mutated TNBC cells may be attributed to different expression of c-Met. Indeed, western blot analysis indicated that HCC1937 cells, which expressed higher levels of c-Met than MDA-MB-436 cells (Fig. 2f, top), were also more resistant to PARP inhibition (Fig. 2f, bottom); in addition, knockdown c-Met rendered HCC1937 cells more sensitive to PARP inhibition (Fig. 2g and Supplementary Fig. 6a,b). For example, when treated with 38 \(\mu\)M ABT-888, c-Met knockdown cells showed twofold (shMet-A; 95% CI = 1.5–2.5) or 1.9-fold (shMet-B; 95% CI = 1.3–2.5) growth inhibition. In contrast, increasing the ectopic expression of WT but not KD mutant c-Met in MDA-MB-436 cells attenuated the effects of PARP inhibition on cell viability (Fig. 2h and Supplementary Fig. 6c,d). Knockdown or ectopic expression of c-Met had no effect on the abundance of BRCA1 and BRCA2 proteins (Supplementary Fig. 6e,f).

To further investigate the relationship between BRCA1, BRCA2 and c-Met, we knocked down BRCA1 and BRCA2 expression in a pair of WT-BRCA1 and WT-BRCA2 cell lines with high c-Met (MDA-MB-231) and low c-Met (MDA-MB-157) expression (Fig. 2i) and treated them with PARP inhibitors. Knocking down BRCA1 or BRCA2 sensitized only MDA-MB-157 cells expressing low levels of c-Met (Fig. 2j and Supplementary Fig. 6g–l). Collectively, these results suggest that enhanced expression of c-Met renders cells resistant to PARP inhibitors in the context of BRCA1 or BRCA2 inactivation, thereby providing a potential molecular explanation for discrepant clinical results involving PARP inhibitor response.

To address whether c-Met activates PARP1, we exposed MDA-MB-231 cells expressing control shRNA or c-Met–specific shRNA to \(H_2O_2\), and then subjected them to a comet assay to evaluate the extent of DNA damage. c-Met–knockdown cells had higher tail intensity, which is indicative of increased oxidative DNA damage, than control cells (Supplementary Fig. 7a). Knockdown of c-Met also reduced DNA repair activity, as measured by oxidative DNA damage (Supplementary Fig. 7b). Consistent with the shRNA results, inhibition of c-Met by foretinib increased the sensitivity of cells to PARP inhibitor–induced DNA damage, as indicated by enhanced \(\gamma\)-H2AX foci formation, an indicator of DNA damage (Fig. 3a). DNA repair also required the kinase activity of c-Met, as expression of WT but not KD c-Met in MCF-7 cells reduced \(H_2O_2\)-induced DNA damage; this was restored by pre-treatment with a c-Met inhibitor (Supplementary Fig. 7c–e). Ectopic expression of c-Met in MCF-7 cells decreased ABT-888–induced \(\gamma\)-H2AX foci formation, as compared to the vector control (Supplementary Fig. 7f). MDA-MB-231 cells expressing c-Met–specific shRNA had higher \(\gamma\)-H2AX foci formation than those with vector control after ABT-888 treatment (Fig. 3b, top, left); re-expression of WT c-Met but not re-expression of vector control (Fig. 3b, bottom, left), KD c-Met, or WT c-Met plus pre-treatment with the c-Met inhibitor crizotinib restored this (Fig. 3b, top, right). These findings together suggest that c-Met kinase activity enhances the DNA repair function of PARP1.

Given that c-Met and PARP1 physically associate \(in\) \(vivo\) (Fig. 1f,g and Supplementary Fig. 3a–f), we speculated that c-Met could phosphorylate PARP1 under oxidative stress. Indeed, in HEK293T cells expressing FLAG-tagged c-Met and V5-tagged PARP1, \(H_2O_2\) induced PARP1 tyrosine phosphorylation (Fig. 3c). The software program NetworKIN (V2.0)\(^{35}\) predicted that Tyr907 (Y907), which is located on the H-Y-E motif in the catalytic domain of PARP1 (ref. 36), is the c-Met phosphorylation site. An \(in\) \(vivo\) kinase assay showed that compared to WT PARP1, phosphorylation, as read out by \(\gamma\)-\(32P\) incorporation, was substantially reduced in the Y907F mutant, but not in PARP1 bearing a mutation at Y986, another Tyr residue in the H-Y-E domain (Supplementary Fig. 8a,b). These results suggest that Y907 is a bona fide c-Met phosphorylation site.

Because Y907 is located within the catalytic domain of PARP1, we next asked whether Y907 phosphorylation affects the function of PARP1. We stably expressed WT, Y907F (non-phosphorylatable) or Y907E (phosphomimetic)–mutant PARP1 in PARP1–knockdown MDA-MB-231 cells (Fig. 3d, left) and measured \(H_2O_2\)-induced DNA damage by comet assay. PARP1–knockdown cells had more DNA damage than control cells (Fig. 3d, center and right). Re-expression of WT PARP1 reduced DNA damage compared to the Y907F mutant, and cells expressing the Y907E mutant had the least amount of DNA damage (Fig. 3d, right). To determine whether phosphorylation of PARP1 at Y907 affects its activity, we compared the PARylation (PAR) levels in MDA-MB-231 cells expressing WT and mutant PARP1. Cells expressing WT PARP1 had increased PAR in response to \(H_2O_2\) (Fig. 3e). Cells expressing the phosphomimetic Y907E mutant had higher levels of PAR than the non-phosphorylatable Y907F mutants; however, both mutants were no longer sensitive to \(H_2O_2\) treatment (Fig. 3e). To further investigate the functional importance of PARP1 phosphorylation at Y907, we generated an antibody to specifically detect pY907 (Supplementary Fig. 8c–g). Treatment with either crizotinib or foretinib abolished \(H_2O_2\)-induced phosphorylation of PARP1 at Y907 (Fig. 3f). These results suggest that \(H_2O_2\)-induced WT PARP1 activity requires Y907 phosphorylation.

We then asked whether c-Met–mediated phosphorylation of Y907 of PARP1 affects PARP inhibitor response. MDA-MB-231 cells expressing WT or mutant PARP1 were treated with or without \(H_2O_2\) and/or increasing concentrations of ABT-888, and they were then subjected to a PARP enzyme activity assay to measure the median inhibitory concentration (IC\(_{50}\)) of ABT-888. The activity of the phosphomimetic Y907E mutant was similar to that of WT PARP1 treated with \(H_2O_2\) (higher IC\(_{50}\)), whereas the activity of the non-phosphorylatable Y907F mutant was similar to that of WT PARP1 without \(H_2O_2\) (lower IC\(_{50}\)) (Supplementary Fig. 8h). In addition, we measured the direct binding of WT and mutant (Y907F and Y907E) PARP1 to ABT-888 by an \(in\) \(vivo\) isotothermal titration calorimetry (ITC) assay (Supplementary Fig. 8i). The results indicated a higher \(K_d\) value for the PARP1 Y907E mutant than either the WT or the Y907F mutant, suggesting that phosphorylated PARP1 exhibited a lower binding affinity for ABT-888 than the non-phosphorylated form. Together, these results indicate that phosphorylation of PARP1 at Y907 attenuates the inhibitory effect of ABT-888.

Next, in MDA-MB-231 cells expressing PARP1–specific shRNA (i.e., targeting PARP1), we re-expressed WT, Y907F-mutant or Y907E-mutant PARP1 (Supplementary Fig. 8j) and subjected them to control or c-Met–specific shRNA knockdown and/or ABT-888 treatment. We then evaluated the extent of DNA damage by \(\gamma\)-H2AX foci formation (Fig. 3g). Knocking down c-Met sensitized cells to ABT-888–induced DNA damage in cells expressing WT PARP1, but it did not affect DNA repair in cells expressing Y907F-mutant or Y907E-mutant PARP1. We observed similar results in clonogenic cell survival (Fig. 3h) and cell viability assays (Supplementary Fig. 8k,l), and by using the inhibitor AG014699 instead of ABT-888.
To evaluate the clinical relevance of our findings, we validated the antibody specific to pY907-PARP1 in formalin-fixed paraffin-embedded (FFPE) tumor tissues obtained from breast cancer patients (Supplementary Fig. 9a). Next, we used this antibody to measure pY907-PARP1 abundance in a human breast cancer tissue microarray by IHC staining; we observed a positive correlation between

**Figure 3** c-Met mediates PARP1 functions through phosphorylation of PARP1 at Y907. (a) MDA-MB-231 cells were treated with ABT-888 (50 μM), foretinib (1 μM), or the combination (ABT/Fr) for 18 h. Representative images and quantification of γ-H2AX (green) from three independent experiments are shown. Scale bars, 20 μm. (b) c-Met– (shMet-C) or control-knockdown MDA-MB-231 cells as well as c-Met–knockdown MDA-MB-231 cells re-expressing vector control (shMet-C/Vector), WT (shMet-C/c-Met) or KD mutants (shMet-C/KD) were treated with the indicated drugs for 18 h. Representative images and quantification of γ-H2AX (green) from three independent experiments are shown. Scale bar, 20 μm. (c) HEX293T cells were transfected with V5-PARP1 and FLAG–c-Met expression plasmids, and the cells were treated with 10 mM H2O2 for 15 min. PARP1 was immunoprecipitated with V5-specific antibody, followed by western blotting with 4G10 (phosphor-tyrosine–specific antibody). (d) Left, western blot showing expression of PARP1 and tubulin in PARP1-knockdown (shRNA targeting the 3′-UTR) MDA-MB-231 cells and PARP1-knockdown MDA-MB-231 cells re-expressing WT PARP1 or the Y907 mutant. Center, DNA damage as measured by comet assay with pre-incubation with formanidopyrimidine DNA glycosylase (Fpg) in PARP1-WT–, PARP1-Y907E–, or PARP1-Y907F– expressing MDA-MB-231 stable cells treated with 20 mM H2O2 for 30 min. Right, quantification of intensity of damaged DNA from three independent experiments. Scale bar, 100 μm. (e) Western blot showing poly-ADP ribosylation as indicated by PAR in the MDA-MB-231 stable cells described in d treated with or without 20 mM H2O2 for 30 min. (f) MDA-MB-231 cells were treated with or without 20 mM H2O2 for 30 min or H2O2 plus 2 μM crizotinib (Cri) or 1 μM foretinib (Fr) pre-treatment for 1 h. Cell lysates were subjected to western blot analysis using the indicated antibodies. (g) Re-expression of WT or mutant PARP1 in PARP1-knockdown MDA-MB-231 cells with or without c-Met knockdown. Cells were treated with 50 μM ABT-888 for 18 h. γ-H2AX (green) was detected by immunofluorescence confocal microscopy. Scale bars, 20 μm. Representative images and quantification of three independent experiments are shown. (h) Stable cells (described in g) were treated with the indicated concentrations of AG014699 and subjected to clonogenic formation assay for 8 d. Representative images and quantification of clonogenic cells from three independent experiments are shown. Scale bar, 10 mm. Error bars represent s.d. *P < 0.05, t-test. n.s., not significant. ABT, ABT-888. shRNA knockdown was performed with multiple target-specific shRNAs as indicated by the letters A, B and C.
pY907-PARP1 and c-Met expression in both TNBC (Fig. 4a and Supplementary Table 3) and non-TNBC tumors (Supplementary Fig. 9b). High ROS (8-OHdG) also correlated with high pY907-PARP1 abundance (Supplementary Fig. 9c). These results suggest that intracellular ROS may induce phosphorylation of PARP1 at Y907 in a c-Met–dependent manner.

Next, we examined the effects of combining c-Met inhibitors (foretinib and crizotinib) and PARP inhibitors (ABT-888 and AG014699). Both the ABT-888–foretinib and AG014699–crizotinib combinations demonstrated synergistic cell growth inhibition in MDA-MB-231 and HCC1937 TNBC cells (Fig. 4b), but not in MCF10A mammary epithelial cells (Supplementary Fig. 10a). The combined treatment of AG014699 and crizotinib also synergized to suppress clonogenicity (Supplementary Fig. 10b,c) and anchorage-independent growth (Fig. 4c and Supplementary Fig. 10d). We observed similar inhibitory effects on clonogenic cell survival for the ABT-888–foretinib combination (Supplementary Fig. 10e). Synergistic inhibition of c-Met and PARP1 was also observed in another breast cancer cell line, BT549 (Supplementary Fig. 10f). H2O2-induced phosphorylation of Y907-PARP1 was abolished by c-Met inhibition (Supplementary Fig. 10g). In addition to human breast cancer cell lines, we evaluated the effect of the combination treatment in two mouse mammary tumor cell lines derived from a transgenic mouse model of TNBC that expressed constitutively active human c-Met37. Combined treatment with c-Met and PARP inhibitors synergistically inhibited mouse tumor cell growth (Supplementary Fig. 10h,i). Also, pY907-PARP1 was stimulated by H2O2 and abolished by c-Met inhibition in these mouse cell lines (Supplementary Fig. 10j).

We also evaluated the effect of combining PARP and c-Met inhibitors in vivo in established TNBC xenograft models. In MDA-MB-231 xenograft tumor models, combination treatment (AG014699–crizotinib and ABT-888–foretinib) substantially reduced tumor growth compared to either inhibitor alone (Fig. 4d,e and Supplementary Fig. 11a,b). The AG014699–crizotinib combination also inhibited growth of mouse mammary tumor cells (A1034) in a syngeneic FVB mouse model and in an HCC1937 TNBC xenograft tumor model (Supplementary Fig. 11c,d). Increased apoptosis (as measured by TUNEL staining), reduced cell proliferation (as measured by Ki67 staining) and greater DNA damage (as measured by γ-H2AX staining) were observed in MDA-MB-231 xenograft tumor tissues harvested from mice within 24 h after the last treatment (Fig. 4f, Supplementary Fig. 11e). In addition, the overall health of the mice was not adversely affected by either the AG014699–crizotinib or ABT-888–foretinib combination, as compared to no treatment or single treatment (as indicated by clinical chemistry analysis and body weight; Supplementary Fig. 11f–l).

Because PARP inhibitors have been used in clinical trials for multiples cancer types, including lung cancer, we also tested a non-TNBC cell line (MCF-7 with ectopic expression of c-Met) and two lung cancer cell lines (H1993 and A549) in vitro and in vivo. Synergistic inhibition of cell growth was observed in the MCF7
with ectopic expression of WT c-Met (MCF-7/c-Met) and H1993 cells (high c-Met expression) but not in MCF7 with vector control (MCF7/control). MCF7 with ectopic expression of KD c-Met (MCF7/c-Met KD), or A549 (low c-Met expression) cells (Fig. 4g and Supplementary Fig. 12a,b). c-Met inhibitor treatment abolished H2O2-induced y907-PARP1 in both MCF-7/c-Met and in H1993 cells (Supplementary Fig. 12c,d). Furthermore, the combined treatment of AG014699 and crizotinib demonstrated significant anti-tumor activity in MCF/c-Met breast cancer and H1993 lung cancer xenograft tumor models (Fig. 4h,i).

Taken together, our study revealed that c-Met—phosphorylated PARP1 at Y907 leads to PARP inhibitor resistance (Supplementary Fig. 12e) and identified c-Met as an important regulator of PARP inhibitor response, suggesting that pY907-PARP1 may be a useful marker with which to stratify patients for PARP inhibitor treatment alone or in combination with a c-Met inhibitor. Of note, many studies have found aberrant c-Met activation and increased expression of c-Met in TNBC tumors. Notably, we observed positive correlation between c-Met and pY907-PARP expression in TNBC patient samples (Fig. 4a). On the basis of our findings, one-third (24/77 in Fig. 4a and Supplementary Table 3) of TNBC patients who are positive for pY907-PARP1 and c-Met would probably be resistant to PARP inhibitor alone, and they could benefit from the combined therapy of c-Met and PARP inhibition using pY907-PARP1 and c-Met as biomarkers.

It should be mentioned that the combined inhibition of EGFR and PARP induces synthetic lethality in TNBC. However, the underlying mechanism of this combination is not yet clear, and given that EGFR was also identified from our phospho-RTK antibody array analysis, it is conceivable that EGFR may induce resistance to PARP inhibitors through a similar mechanism in a subpopulation of patients who do not respond to PARP inhibition. It is important to further investigate the relationship between PARP1 and c-Met and between PARP1 and phosphatases that can dephosphorylate pY907 or other functionally important phosphorylation sites of PARP1. Moreover, investigating whether other protein kinases also regulate PARP inhibitor response may reveal a new perspective on the development of combination therapy strategies and benefit a broader population of patients.

PARP inhibitors are being used in clinical trials for many cancer types in addition to TNBC. c-Met is a proto-oncogene that is overexpressed in multiple cancer types. Although we initiated our study using TNBC samples for historical (original synthetic lethality) and clinical (no effective target therapy for TNBC in the clinic) reasons, we also demonstrated that the combined treatment of c-Met and PARP inhibitors effectively reduced tumor growth in MCF-7/c-Met (Fig. 4h) and c-Met–expressing H1993 NSCLC xenograft tumor models (Fig. 4i). These results raise the interesting possibility that cancer patients with tumors that overexpress c-Met may benefit from this combination therapy regardless of the cancer type. Thus, it may be worthwhile to systematically test whether the combined inhibition of both PARP and c-Met also exhibits synergistic therapeutic effects in other types of tumors.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.D. designed and performed the experiments, analyzed data and wrote the manuscript; H.Y., Y.W., Y.-H.H., W.-C.L., W.-H.Y., P.G.L., G.R.L., W.-C., C.-H.C. and M.-C.K. performed experiments and analyzed data; H.-L.W. generated the antibody; M.-C. Hsu, C.-T.C., K.N. and Y.S. performed experiments; Y.W., W.-C., C.-L.L. and Y.-C.C. provided patient tissue samples; H.Y. and J.L.H. provided scientific input and wrote the manuscript; M.P. provided transgenic mouse cell lines; G.N.H. provided scientific and clinical input. P.J. provided scientific input. M.-C. Hung supervised the entire project, designed the experiments, analyzed data and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Chemicals and antibodies. Hydrogen peroxide (#216763), cycloheximide (#C4859) and sodium arsenite solution (#35000) were obtained from Sigma-Aldrich (St. Louis, MO). Antibodies detecting tubulin (#T5168), flag (#F53165) and actin (#A2066) were also from Sigma-Aldrich (St. Louis, MO). Antibodies detecting γ-H2AX (#05-636) and phosphotyrosine (#05-321, 4G10) were from EMD Millipore (Billerica, MA). Antibodies detecting GST fusion protein (#sc-53909), HA-tag (#sc-805) and PARP1 (#sc-7150) for western blot were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against PARP1 (#9532) for IP and for detecting c-Met (#8198) and phosphorylated c-Met (#3077) were from Cell Signaling Technology (Danvers, MA). Antibody against 8-OHdG was obtained from Genox Corporation (Baltimore, MD).

All fluorescence-labeled secondary antibodies were obtained from Invitrogen (Carlsbad, CA). The mouse phospho-y907-PARP1 antibody was generated against a phosphorylated synthetic peptide (ADMVSKSAN-Yp-CHTSQGD) at China Medical University, Center of Molecular Medicine. The horseradish peroxidase (HRP)-conjugated secondary antibodies for western blot were obtained from eBioscience (San Diego, CA); All primary antibodies were used according to the manufacturer’s datasheet, or diluted to 1:1000 for western blot or 1:100 for IP and western blotting. For secondary antibody, a 1:500 dilution was used. c-Met kinase inhibitors crizotinib (#C-79900) and foretinib (#F-4185) were from LC Laboratories (Woburn, MA). PARP inhibitors ABT-888 (Veliparib, #CT-A888) and AG014699 (Rucaparib, #CT-AG01) were from ChemieTek (Indianapolis, IN); AZD2281 (Olaparib, #S1060) was from Selleck Chemicals (Houston, TX).

Cell culture. All cell lines were obtained from American Type Culture Collection (ATCC) and maintained in Dulbecco’s modified Eagle’s medium (DMEM)/F12 or RPMI-1640 supplemented with 10% FBS and antibiotics. A1034 and A1471 mouse cell lines were previously described37. Cell lines were obtained from ATCC and were used according to the manufacturer’s datasheet, or diluted to 1:1000 for western blot or 1:100 for IP and western blotting. For secondary antibody, a 1:500 dilution was used. c-Met kinase inhibitors crizotinib (#C-79900) and foretinib (#F-4185) were from LC Laboratories (Woburn, MA). PARP inhibitors ABT-888 (Veliparib, #CT-A888) and AG014699 (Rucaparib, #CT-AG01) were from ChemieTek (Indianapolis, IN); AZD2281 (Olaparib, #S1060) was from Selleck Chemicals (Houston, TX).

Plasmids and transfection. For stable knockdown of c-Met or PARP1 and c-Met or PARP1 overexpression studies, breast cancer cells were transfected with pgPiZ shRNA (control) vector (Thermo Fisher Scientific, Rockford, IL) or pLKO.1 shRNA vector Sigma-Aldrich (St. Louis, MO) and pCDH-neo vector (System Biosciences, Mountain View, CA). shRNA sequences used in knockdown experiments are as follows (5’ to 3’): MET (CCATCCGAGATGTCATCT; GCATTAACGACAGGTATC; GCATTAACGACAGGTATC; TGTGGTTATGTCGATAAA; CTTTCG AAGGGCTGCTGATGA; PARP1 (TGGGAAGGTGTTAAGCCTAT); BRCAl (TTCATTTGCTTATATGCGC; TTAAATGCTAATCTGTG; TTCCGA TAAATGCTG; RBCAl (TGGGTTCAGCAGATTCCATG; TCTTATAGACAGCTAAAG; TATATAAGTACCTTGGGC). *Targeting the 3’-UTR.

8-OHdG ELISA assay. Total DNA was purified from breast cancer cells by using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). 8-OHdG levels were measured by 8-OHdG ELISA assay. Total DNA was purified from breast cancer cells by using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). 8-OHdG levels were measured by 8-OHdG ELISA assay.

ROS detection. Cells were seeded in the 12- or 96-well plates. After overnight growth, cells were incubated with 10 µM 2′,7′-dichlorofluorescin diacetate (DCFDA) in PBS for 1 h. Cells were washed and the media replaced with PBS. 2′,7′-dichlorofluorescein (DCF) was measured under a Zeiss microscope with a 20X objective. The Axiovision software was used for data analysis.

Cellular fractionation. Cytosolic and nuclear fractions were prepared as described previously31. The abundance of cytoplasmic to nuclear proteins was quantified by using the Cell Line Integrated Molecular Authentication database.

Hierarchical clustering and display. Clustering of ERBB3, MET and FLT3 gene expression with TNBC signature genes (ERBB2, ESR1, and PGR) from The Cancer Genome Atlas database was analyzed using the Cluster and TreeView41 program, as previously described31. Briefly, for any set of target receptor tyrosine kinases, an upper-diagonal similarity matrix was computed by using average-linkage clustering. This algorithm was determined by computing a dendrogram as described42. The heat map was represented graphically by coloring each cell on the basis of the measured fluorescence ratio. Log ratios of 0 (a ratio of 1.0 indicates that the genes are unchanged) were colored in black, positive log ratios were colored in red, and negative log ratios were colored in green.

Immunoprecipitation and immunoblotting. The immunoprecipitation, sodium dodecyl sulfate–PAGE (SDS-PAGE) and western blot analyses were performed as described previously31. The GS-800 Calibrated Densitometer (Bio-Rad Laboratories, Hercules, CA) was used to quantify the protein band density.

Confocal microscopy analysis of γ-H2AX foci. For fixed cells, confocal microscopy was performed as described previously31. Briefly, cells grown on chamber slides (Labtek, Scotts Valley, CA) were treated as described in the text. After washing with ice-cold PBS, cells were fixed, permeabilized, and incubated with γ-H2AX antibodies and fluorescence-labeled secondary antibodies. Immunostained cells were examined using Zeiss LSM 710 laser-scanning microscope (Carl Zeiss, Thornwood, NY) with a 63X/1.4 objective. The ZEN and AxioVision (Carl Zeiss) and ImageJ software programs (NIH, Bethesda, MD) were used for data analysis.

Fluorescence microscopy analysis of xenograft tumor tissues. Frozen MDA-MB-231 xenograft tumor tissues were stained with 3,3-diaminobenzidine (DAB) (Vector Laboratories, Burlingame, CA) and imaged using a Zeiss Axioskop 2 image capture system. TUNEL assay of MDA-MB-231 xenograft tumor tissues. TUNEL assay of xenograft tumor tissues was performed with the DeadEnd Fluorometric TUNEL Detection System Kit (Cat # G2320, Promega, Madison, WI) following the manufacturer’s protocol. Briefly, the frozen tissue sections were incubated with 3′-deoxyadenosine 5′-triphosphate (3′-dATP) using terminal deoxynucleotidyl transferase (TdT). Fragmented DNA was detected by streptavidin–conjugated fluorescence using Zeiss Axioplan 2 microscope. The AxioVision (Carl Zeiss) was used for data analysis.
is 5:1 after cellular fractionation. The same amount of cytoplasmic or nuclear lysates was used for IP and western blotting, resulting in more proteins in the nuclear fraction.

**Cell viability assay.** Cells (1,500) were seeded in a 96-well plate and treated with the indicated inhibitors for 72 h. Then cells were incubated in fresh medium with 100 μM resazurin for 1 h. Cell viability was measured by fluorescence plate readers at spectra of 560 nm excitation/590 nm emission. Survival curves were expressed as mean ± s.d. relative to DMSO-treated control from three independent experiments.

**Clonogenic cell survival assay.** Cells were plated into 12- or 24-well plates. After overnight incubation, cells were treated with inhibitors followed by 8 d of incubation. The colonies were fixed and stained with 0.5% crystal violet, washed, dried and imaged. Crystal violet was dissolved from colonies by methanol and measured at 540 nm. Based on the absorbance at 540 nm, survival curves were expressed as a percentage ± s.d. relative to DMSO-treated control from three independent experiments.

**Soft agar anchorage-independent cell growth assay.** The base layer of cell growth matrix containing DMEM/F12 medium, 10% FBS, and 0.5% agar was paved in 6-well plates (1.5 ml/well). After solidification of the base layer, the top layer (1.5 ml/well) containing DMEM/F12 medium, 10% FBS, and 0.35% agarose, and cells were plated. Culture medium (1 ml) was added to each well and changed every 3 d. After 4 weeks of culture, colonies were stained by 0.05% crystal violet. Colonies were counted by ImageJ software. Survival curves were expressed as mean ± s.d. relative to DMSO-treated control from three independent experiments.

**Synergy quantification of drug combination.** Cell growth was measured by cell viability, clonogenic cell survival, or soft agar anchorage-independent cell growth assay. Synergistic effects were determined by the Chou–Talalay method to calculate the combination index (CI)43.

**Patient tissue samples and immunohistochemical staining.** A human breast cancer tissue microarray was obtained from Pantomics (Richmond, CA). Human tumor tissue specimens were obtained from patients undergoing surgical resection of breast cancer as primary treatment at either the University of Texas MD Anderson Cancer Center or Mackay Memorial Hospital (Taipei, Taiwan) between 1995 and 2009 under the guidelines approved by the Institutional Review Board at MD Anderson, and written informed consent was obtained from patients in all cases at the time of enrollment24. The tissue microarray (#BRC2281, #BRC1021; Pantomics, Richmond, CA) was incubated with primary antibodies specific to 8-OhdG, c-Met or pY907-PARP1, detected with biotin-conjugated secondary antibody and avidin–peroxidase, and visualized by aminoethyl carbazole chromogen. Images were analyzed by ACIS (Dako North America, Carpinteria, CA). To validate the specificity of pY907-PARP1 antibody in IHC, we performed a peptide competition assay by staining human breast tumor samples with pY907-PARP1 antibody blocked with mock peptide, pY907-PARP1 peptide, non-pY907-PARP1 peptide, or another phosphotyrosine peptide, pY986-PARP1. Patient tumor samples were deparaffinized and rehydrated. Antigen retrieval was carried out by heating in 0.1 M sodium citrate buffer (pH 6.0) using a microwave oven. To block endogenous peroxidase activity, the sections were treated with 1% hydrogen peroxide in methanol for 30 min. After 1 h preincubation in 10% normal serum to prevent nonspecific staining, the samples were incubated with primary antibodies at 4 °C overnight. The sections were then treated with biotinylated secondary antibody, followed by incubations with avidin–biotin peroxidase complex solution for 1 h at room temperature. Color was developed with the 3-amino-9-ethylcarbazole solution. Counterstaining was carried out using Mayer’s hematoxylin.

**Comet assay.** A comet assay was performed as described previously44. Briefly, cells were treated with H2O2 for 10 min to induce DNA damage or with H2O2 and hydroxyurea/cytosine-β-arabinofuranoside (Hu/AraC) to induce DNA damage and allow DNA damage to accumulate to evaluate the extent of DNA repair. Trypsinized cells were washed with PBS and mixed with 1% low-melting-point agarose (LMPA). LMPA-mixed cells were placed onto slides pre-coated with 1% LMPA and incubated on ice until the agarose layer solidified. A third 0.5% LMPA layer was then placed over the second layer. Prepared slides were washed three times in water for 5 min and incubated with formaldehyde (37 °C for 1 h) to digest oxidative damage DNA and induce comet tails, which were imaged by fluorescence microscopy and analyzed by using ImageJ software. The mean ± s.d. of DNA intensity in the tail from 20 cells in each treatment group was calculated.

**In vitro kinase assay.** Recombinant glutathione S-transferase (GST)–WT PARP1 (Ala374–Tyr1014 of human PARP1) and mutants (GST–Y907F and GST–Y986E) were expressed by induction of isopropyl β-D-1-thiogalactopyranoside (IPTG) and purified with glutathione agarose beads. After cold-PBS washing three times, beads were suspended with 500 μl 1× kinase buffer, with 50 μl saved for western blotting with GST. The beads were spun down and 100 μM ATP, 0.5 μg human recombinant active c-Met protein and 50 μCi [32P]-ATP were added in 50 μl kinase buffer at 30 °C for 15–30 min. The kinase reaction was stopped by heating at 100 °C for 5 min in SDS loading dye. The samples were subjected to two identical SDS-PAGE assays. One was used for Coomassie blue staining of GST fusion PARP1 protein. The second gel was dried and used to detect phosphorylation of substrate by autoradiography.

**PARP enzyme activity assay.** PARP1 enzyme activity was measured by using a commercial assay kit (Cat# 17-10149) from EMD Millipore according to the manufacturer's protocol, with the exception that cell lysates containing WT PARP1 or PARP Y907 mutants were used in place of the PARP1 protein included with the kit. Total lysate (500 ng) was added to each reaction. The dose course of PARP inhibitor ABT-888 was from 0.01 to 1,000 μM. PARP enzyme activity of WT and mutants was determined after incubation with the substrate by using a plate reader.

**Isothermal titration calorimetry.** ITC experiments were carried out using either a TA nanoITC instrument or an Auto ITC system (TA Instruments) at 25 °C. Titrations of 55–110 μM ABT-888 into the sample cell containing a 5–10 μM solution of either GST–WT PARP1, GST–Y907F PARP1, or GST–Y907E PARP1, dissolved in TIC buffer (19.8 mM HEPES, 148.5 mM NaCl, 0.495 mM TCEP and 1% (vol/vol) DMSO at pH 7.5). The heat of dilution control experiments was measured independently by titrating the same ABT-888 solution into the same buffer and subtracted from the observed heat measured for the titration of compound into proteins. Experimental data were fitted using the independent binding site model in NanoAnalyze v3.4.0 software (TA instruments).

**Mouse xenograft models and toxicity studies.** All animal procedures were conducted under the approval of the Institutional Animal Care and Use Committee (IACUC) at The University of Texas MD Anderson Cancer Center (protocol number 10-14-07231). MDA-MB-231 (0.5 × 106), HCC1937 (2 × 106) or MCF-7 (5 × 106) cells were injected into the mammary fat pads of female nude (Swiss Nu/Nu) mice of 6–8 weeks of age (Department of Experimental Radiation Oncology Breeding Core, The University of Texas MD Anderson Cancer Center). A1034 (0.5 × 106) cells were injected into the mammary fat pads of female nude (Swiss Nu/Nu) mice of 6–8 weeks of age (Department of Experimental Radiation Oncology Breeding Core, The University of Texas MD Anderson Cancer Center). A1034 (0.5 × 106) cells were injected into the mammary fat pads of female nude (Swiss Nu/Nu) mice of 6–8 weeks of age. When the tumor volume reached ~50 mm3, crizotinib (5 mg/kg) and foretinib (5 mg/kg), AG014699 (25 mg/kg) and ABT-888 (25 mg/kg), dissolved in aqueous 50 mM sodium acetate, pH 4, were administered to mice five times per week as single agents or in combination for the number of days specified in the figure legends. Tumor was measured at the indicated time points, and tumor volume was calculated by the formula π/6 × length × width2. For MDA-MB-231 and A1034 xenograft mouse models, mice were imaged before and after treatment
using the IVIS Imaging System to assess tumor growth. Mice were injected with 100 µl of d-luciferin (Xenogen; 15 mg/ml in PBS). After 10 min, mice were anesthetized with a mixture of oxygen and isoflurane (Inhalation Anesthesia System; Matrix Medical, Orchard Park, NY) and imaged using the IVIS Imaging System. Imaging parameters were maintained across experiments for comparative analyses. Tumors were not allowed to grow larger than allowed by the animal welfare protocol.

Tumor samples were collected after final treatment and analyzed by immunofluorescence staining. For toxicity assessment, mice were weighed before and after treatment (on day 21 for AG014699 and crizotinib, and on day 16 for ABT-888 and foretinib. Blood samples were collected from the orbital sinus using a microhematocrit tube after each treatment and subjected to biochemical analysis for liver marker enzymes alanine transaminase (ALT) and aspartate transaminase (AST) and kidney marker by-products creatinine and blood urea nitrogen to evaluate treatment toxicity by COSBA INTERGRA 400 plus (Roche Diagnostics, Rotkreuz, Switzerland) at The Department of Veterinary Medicine and Surgery, The University of Texas MD Anderson Cancer Center. All in vivo experiments were conducted with 10 mice for each treatment and control group. No statistical methods were used to predetermine sample size.

Statistical analyses. Unless otherwise noted, each sample was assayed in triplicate. For in vitro analyses, each experiment was repeated at least three times. All error bars represent s.d. Student’s t test was used to compare two groups of independent samples. Repeated-measures ANOVA analysis was used to evaluate the statistical significance of dose curve response. Correlations were analyzed using the Pearson chi-square test. A P value of <0.05 was considered statistically significant. No statistical methods were used to determine sample size.

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Corrigendum: Adjuvant-dependent innate and adaptive immune signatures of risk of SIVmac251 acquisition

Monica Vaccari, Shari N Gordon, Slim Fourati, Luca Schifanella, Namal P M Liyanage, Mark Cameron, Brandon F Keele, Xiaoqing Shen, Georgia D Tomaras, Erik Billings, Mangala Rao, Amy W Chung, Karen G Dowell, Chris Bailey-Kellogg, Eric P Brown, Margaret E Ackerman, Diego A Vargas-Inchaustegui, Stephen Whitney, Melvin N Doster, Nicolo Binello, Poonam Pegu, David C Montefiori, Kathryn Foulds, David S Quinn, Mitzi Donaldson, Frank Liang, Karin Loré, Mario Roederer, Richard A Koup, Adrian McDermott, Zhong-Min Ma, Christopher J Miller, Tran B Phan, Donald N Forthal, Matthew Blackburn, Francesca Caccuci, Massimiliano Bissa, Guido Ferrari, Vaniambadi Kalyanaraman, Maria G Ferrari, DeVon Thompson, Marjorie Robert-Guroff, Silvia Ratto-Kim, Jerome H Kim, Nelson L Michael, Sanjay Phogat, Susan W Barnett, Jim Tartaglia, David Venzon, Donald M Stablein, Galit Alter, Rafick-Pierre Sekaly & Genoveffa Franchini

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In the version of this article initially published online, an affiliation for Luca Schifanella was omitted and there was an error in the description of the phenotypic analyses of plasmablasts in the Online Methods. The error has been corrected for the print, PDF and HTML versions of this article.

Corrigendum: Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi

Yang Li, Marije Oosting, Patrick Deelen, Isis Ricaño-Ponce, Sanne Smeekens, Martin Jaeger, Vasiliki Matzaraki, Morris A Swertz, Ramnik J Xavier, Lude Franke, Cisca Wijmenga, Leo A B Joosten, Vinod Kumar & Mihai G Netea

Nat. Med.; doi:10.1038/nm.4139; corrected online 28 July 2016

In the version of this article initially published online, the url in the Online database section of the Methods was incorrect. The original version included the url http://www.bbmri.nl/molgenis/500FG. The correct url is http://hfgp.bbmri.nl/. The error has been corrected for the print, PDF and HTML versions of this article.

Corrigendum: Blocking c-Met–mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors

Yi Du, Hirohito Yamaguchi, Yongkun Wei, Jennifer L Hsu, Hung-Ling Wang, Yi-Hsin Hsu, Wan-Chi Lin, Wen-Hsuan Yu, Paul G Leonard, Gilbert R Lee IV, Mei-Kuang Chen, Katsuya Nakai, Ming-Chuan Hsu, Chun-Te Chen, Ye Sun, Yun Wu, Wei-Chao Chang, Wen-Chien Huang, Chien-Liang Liu, Yuan-Ching Chang, Chung-Hsuan Chen, Morag Park, Philip Jones, Gabriel N Hortobagyi & Mien-Chie Hung

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In the version of this article initially published, the concentrations of H2O2 were incorrectly labeled as micromolar (μM) instead of millimolar (mM) in the legends of Figures 1h, 3c–f and Supplementary Figures 3, 8f–h, 10g,j and 12c,d. The error has been corrected in the HTML and PDF versions of the article.

Corrigendum: High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma

Carsten Linnemann, Marit M van Buuren, Laura Bies, Els M E Verdegaal, Remko Schotte, Jorg J A Calis, Sam Behjati, Arno Velds, Henk Hilkmann, Dris el Atmioui, Marten Visser, Michael R Stratton, John B A G Haanen, Hergen Spits, Sjoerd H van der Burg & Ton N M Schumacher

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In the version of this article initially published, the article did not mention some restrictions on the availability of reagents. The following text has been added to the HTML and PDF versions of the paper: “The retroviral vectors containing BCL-6 and BCL-XL have been generated by a for-profit company, AIMM Therapeutics, which makes the plasmids available. Obtaining the plasmids requires an MTA (http://www.aimmtherapeutics.com/partnering/academic-collaboration/) that includes financial obligations.” The error has been corrected in the HTML and PDF versions of the article.