Talazoparib Is a Potent Radiosensitizer in Small Cell Lung Cancer Cell Lines and Xenografts

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Abstract

Purpose: Small cell lung cancer (SCLC) is an aggressive malignancy with a critical need for novel therapies. Our goal was to determine whether PARP inhibition could sensitize SCLC cells to ionizing radiation (IR) and if so, to determine the contribution of PARP trapping to radiosensitization.

Experimental Design: Short-term viability assays and clonogenic survival assays (CSA) were used to assess radiosensitization in 6 SCLC cell lines. Doses of veliparib and talazoparib with equivalent enzymatic inhibitory activity but differing PARP trapping activity were identified and compared in CSAs. Talazoparib, IR, and their combination were tested in three patient-derived xenograft (PDX) models.

Results: Talazoparib radiosensitized 5 of 6 SCLC cell lines in short-term viability assays and confirmed in 3 of 3 cell lines by CSAs. Concentrations of 200 nmol/L talazoparib and 1,600 nmol/L veliparib similarly inhibited PAR polymerization; however, talazoparib exhibited greater PARP trapping activity that was associated with superior radiosensitization. This observation further correlated with an increased number of double-stranded DNA breaks induced by talazoparib as compared with veliparib. Finally, a dose of 0.2 mg/kg talazoparib in vivo caused tumor growth inhibition in combination with IR but not as a single agent in 3 SCLC PDX models.

Conclusions: PARP inhibition effectively sensitizes SCLC cell lines and PDXs to IR, and PARP trapping activity enhances this effect. PARP inhibitors, especially those with high PARP trapping activity, may provide a powerful tool to improve the efficacy of radiotherapy in SCLC.

Introduction

Small cell lung cancer (SCLC) represents 13% to 15% of all lung cancers, and is the sixth most common cause of cancer-related mortality worldwide (1, 2). The prognosis associated with SCLC has remained poor with little improvement over the last few decades; 5-year survival of patients with extensive stage SCLC (ES-SCLC) is a dismal 1% to 5% (3, 4). Most patients experience tumor recurrence after first-line treatment, which consists of chemotherapy with a combination of cisplatin and etoposide as well as radiotherapy in selected patients, particularly those with limited stage SCLC (LS-SCLC; ref. 5). In the setting of these dire results, there is a drastic need for novel therapies to complement and enhance current treatments and improve tumor control in both limited stage and extensive stage SCLC.

One promising molecular target in SCLC has been PARP1. After a landmark analysis identified PARP1 as highly expressed in SCLC and an effective target for PARP inhibitors in SCLC cell lines, PARP inhibitors have now been tested in several clinical trials (4, 6–8). Preliminary results from one trial of patients with relapsed and refractory SCLC reported an objective response rate of 39% for the combination of veliparib and temozolomide compared with 14% with temozolomide alone (9). The combination of PARP inhibition and other DNA-damaging therapies such as platinum agents, etoposide, or temozolomide have proven to be particularly effective in preclinical studies of SCLC (10–12).

The combination of radiation and PARP inhibition may also be an effective way of improving local tumor control. PARP inhibitors have been shown to radiosensitize multiple tumor histologies, including breast, prostate, colorectal, and head and neck squamous cell carcinomas (13–17). Given the integration of irradiation in SCLC management, the use of PARP inhibition to radiosensitize SCLC tumors is a particularly attractive approach. We sought to determine the efficacy of PARP inhibitors as radiosensitizers in SCLC cell lines and patient-derived xenograft (PDX) models. We also examined how the different mechanisms of action of PARP inhibitors contribute to the enhancement of therapeutic irradiation.
Translational Relevance
Small cell lung cancer (SCLC) carries a poor prognosis, and finding regimens to improve patient outcome has been challenging. However, PARP has shown promise as a therapeutic target in SCLC. Our findings suggest that PARP inhibition may be an effective strategy to sensitize SCLC to therapeutic radiation. We also found that talazoparib is a more potent radiosensitizer than veliparib at equivalent levels of enzymatic inhibition, suggesting that the enhanced PARP trapping property of talazoparib may contribute to radiosensitization. On the basis of this preclinical work, combining PARP inhibition with radiation may be a promising strategy to explore in clinical trials.

Materials and Methods

Cell lines and reagents
SCLC cell lines were purchased from ATCC. Cell lines were maintained as recommended. All cell lines were verified by short tandem repeat fingerprinting (DDC Medical) and tested negative for Mycoplasma within 6 months of use. Talazoparib (BMN-673) and veliparib (ABT-888) were aliquoted and stored at –20°C after preparation in DMSO. All in vitro experiments were performed in 1% (v/v) DMSO. For in vivo dosing, talazoparib was administered with 10% dimethylacetamide (Sigma #270555) and 5% Kolli-

Cell radiosensitization assays
Short-term viability assays. Cell lines were plated in 96-well plates 2 days prior to irradiation and treated with drug or DMSO control 1 day prior to irradiation. Cells were exposed to single doses of radiation at a dose rate of 162–164 cGy/minute with a Csium-137 source using the Mark I Irradiator (J.L. Shepherd & Associates). After 5 days of growth, cell viability was assessed with Cesium-137 source using the Mark I Irradiator (J.L. Shepherd & Associates). After 5 days of growth, cell viability was assessed with

Clonogenic survival assays. Cell plating numbers for all clonogenic survival assays (CSA) were optimized such that 50–200 colonies were formed at each dose of radiation and drug. For NCI-H446, cells were plated 2 days prior to irradiation and drug, and administered 24 hours prior to irradiation. Radiation was administered as above. After 1 hour incubation at 37°C, cells were washed with PBS, detached with trypsin, and counted. NCI-H446 cells were plated at preoptimized cell densities in fresh media and allowed to grow for 14 days before fixation and 1% crystal violet staining (Thermo Fisher Scientific) and a compatible plate reader.

PARP trapping
To evaluate the degree of PARP trapping by PARP inhibitors, cells were treated with a PARP inhibitor and 0.01% methyl-

γH2AX staining
NCI-H446 cells were plated 24 hours prior to the addition of PARP inhibitor and 48 hours prior to irradiation with 6 Gy. One hour after irradiation, slides were left in drugged media (contin-

PDX and in vivo tumor growth delay
The Institutional Animal Care and Use Committee approved all animal protocols for this work. All experiments were performed in female NSG mice (NOD.Cg-Pkdcsdc1 Il2rgtm1Wji/SzJ; The Jackson Laboratory) that were 6 to 8 weeks old at time of PDX right flank implantation. PDXs were isolated and passaged as described previously (18, 19). Tumor volumes were calculated from manual caliper measurements using the formula volume = (a × b × c)/2. Mice were randomized at a tumor volume of approximately 150 mm³ and talazoparib was administered daily on Monday to Friday, for
20 total doses. The mice were anesthetized with ketamine and xylazine injection and tumors were irradiated in daily 2 Gy fractions with an X-ray irradiator (XRAD 320, Precision X-Ray) on days 2 to 5 after randomization. Radiation was administered 3 hours after drug dosing. Custom lead cutouts were used to reduce dose to normal mouse tissue. Mouse weights and tumor volumes were measured twice weekly until mouse euthanasia at a tumor size of 1,000 to 1,500 mm³.

Statistical analysis

Clonogenic survival values or growth inhibition values were normalized to nonirradiated controls. Survival curves were fitted using the linear–quadratic model $S = \exp(\alpha D + \beta D^2)$, where $D =$ radiation dose. At each dose of drug, the dose modification factor (DMF), defined as the ratio of radiation dose needed to achieve an equivalent level of survival with radiation alone compared with that for radiation plus drug, was calculated at 37% survival or growth inhibition. Differences between curves were calculated using the extra sum-of-squares $F$ test. Comparisons of in vivo PAR polymerization were made using ANOVA and Tukey multiple comparison analysis. For in vivo tumor growth delay, time to reach 1,000 mm³ was compared using Kaplan–Meier analysis and the log-rank test. All analysis was performed using GraphPad Prism 7.0a (GraphPad Software, Inc.) and RStudio (RStudio, Inc.).

Results

Talazoparib is a radiosensitizer in SCLC cell lines

We sought to characterize talazoparib as a radiosensitizer in SCLC cell lines. When exposed to talazoparib or radiation alone, cell lines showed varying responses (Table 1; Fig. 1). The EC₅₀ for talazoparib ranged from 4.35 nmol/L in the most sensitive cell line (NCI-H146) to 670 nmol/L in the most resistant cell line (NCI-H69). Likewise, the radiation dose to achieve 50% growth inhibition ranged from 3.6 Gy in NCI-H146 to 20.0 Gy in H82 (Fig. 1B).

In 5 of 6 cell lines tested with short-term viability assays, the combination of 6 days of continuous talazoparib exposure and ionizing radiation (IR) on the second day of drug treatment led to a greater than additive effect compared with radiation or drug alone (Fig. 1C). Radiosensitization was observed at a concentration of 0.2 nmol/L in one cell line (NCI-H146; dose modification factor (DMF) 1.56; Table 1). 2 nmol/L in two cell lines (NCI-H446, DMF 1.86; and NCI-H526, DMF 1.34), and 20 nmol/L in four cell lines (DMFs 1.61–2.88). In the remaining cell line, NCI-H69, talazoparib had an additive effect without increased radiosensitization.

We further performed CSA on three of the cell lines above (Fig. 1D). Two cell lines were radiosensitized at a concentration of 20 nmol/L (NCI-H446, DMF 1.40; and NCI-H82, DMF 2.13), and a concentration of 200 nmol/L increased radiosensitization in NCI-H446 to a DMF of 2.20. Statistically significant radiosensitization was noted in NCI-H526 at 20 nmol/L talazoparib and 6 Gy of IR ($P = 0.01$), but not at lower IR doses.

Increasing efficacy of radiosensitization corresponds to increased PARP trapping and enzymatic inhibition

Recently, PARP inhibitors have been found to work by two distinct mechanisms of action: enzymatic inhibition and PARP trapping. PARP inhibitors prevent PARP1 from polymerizing PAR, which is normally induced by radiation (20, 21). In addition, PARP inhibitors trap PARP enzymes at sites of DNA damage, leading to double-stranded DNA breaks and cytotoxicity greater than that caused by enzymatic inhibition alone (22). We tested three concentrations of talazoparib (2, 20, and 200 nmol/L) in NCI-H446. At concentrations that were radiosensitizing by CSA (20 and 200 nmol/L), we observed a reduction of PAR levels and induction of chromatin-bound PARP1, representing enzymatic inhibition and PARP trapping, respectively (Fig. 2). As expected, a dose-dependent effect was observed in which higher concentrations of talazoparib corresponded to greater reductions in PAR (Fig. 2A and B) and greater PARP trapping (Fig. 2C), which corresponded to increasing degrees of radiosensitization (Fig. 1D).

PARP trapping contributes to PARP inhibitor radiosensitization

We next compared two different PARP inhibitors at concentrations, which had equivalent enzymatic inhibition but different PARP trapping potency. Veliparib is an effective inhibitor of PARP enzymatic activity; however, it induces only modest PARP trapping compared with talazoparib, a potent enzymatic inhibitor with superior PARP trapping activity among the clinically investigated PARP inhibitors (21–23). In NCI-H446 cells, we found that 200 nmol/L of talazoparib produced similar levels of enzymatic inhibition as 1,600 nmol/L of veliparib (Fig. 3A and B). This ratio was consistent with results previously reported in cell-free inhibitory assays (24). As expected, this talazoparib concentration was associated with a greater degree of PARP trapping than veliparib (Fig. 3C). Despite inhibition of its enzymatic activity, veliparib did not have a radiosensitizing effect at 1,600 nmol/L, while in contrast 200 nmol/L of talazoparib was sufficient to induced potent radiosensitization (DMF 3.3; Fig. 3D).

Table 1. Dose modification factors and EC₅₀ after treatment with talazoparib. EC₅₀₉₆ of talazoparib for multiple cell lines are quantified by short-term viability assays. The dose of radiation required for 37% proliferation (D₃₇) is listed, in addition to the dose modification factors at 37% proliferation (DMF) for various concentrations of talazoparib.

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*Statistically significant dose enhancement.

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Figure 1.
Talazoparib sensitizes SCLC cell lines to radiation. A, Short-term viability assays show variability in sensitivity to single-agent talazoparib across six different SCLC cell lines. Cell proliferation at each drug concentration is displayed as percentage of cell proliferation with 1% (v/v) DMSO control. Three replicates were performed for each assay. B, The SCLC cell lines tested also showed varying sensitivity to radiation alone, with cell proliferation expressed as a percentage of nonirradiated control. C, Short-term viability assays comparing cell proliferation at selected concentrations of talazoparib in combination with radiation showed statistically significant radiosensitization in 5 of 6 cell lines tested. Values are normalized to nonirradiated control. For dose-modification factors at the concentrations tested, see Table 1. D, Clonogenic survival assays of 3 SCLC cell lines demonstrate statistically significant sensitization to radiation at concentrations of 20 nmol/L talazoparib in NCI-H446 and NCI-H82 and 200 nmol/L talazoparib in NCI-H446. Sensitization of NCI-H526 with 20 nmol/L talazoparib was seen at 6 Gy, but survival curves were not significantly different by the extra sum-of-squares F test. Surviving fractions are normalized to no radiation control at each drug concentration. All error bars represent SD.
Talazoparib contributes to PARP trapping and enzymatic inhibition. A, Western blot analysis of tumor PAR with increasing concentrations of talazoparib with or without radiation demonstrates a dose-dependent reduction in PAR polymerization. Relative lane intensities normalized to actin control are reported below. B, Western blot analyses performed on nuclear soluble and nuclear chromatin-bound fractions show a dose-dependent increase in chromatin-bound PARP1 (PARP polymerization at 6 ($P = 0.02$) and 18 hours ($P = 0.007$) and trends toward significance at 3 ($P = 0.13$) and 24 hours ($P = 0.08$). There were no statistically significant differences between treatment time points (Fig. 5A).

We next assessed the efficacy of combined PARP inhibition and irradiation in two chemo-naïve PDX models. Mice were treated with daily talazoparib and radiation 3 hours after drug gavage in 2 Gy fractions on days 2 to 5. In both the SCRX-Lu149 and JHU-LX44 PDX models, 0.2 mg/kg talazoparib alone did not significantly inhibit tumor growth (Fig. 5B and C). However, the combination of talazoparib and radiation led to significantly greater tumor growth inhibition ($P = 0.03$ for SCRX-Lu149; $P = 0.01$ for JHU-LX44).

We further tested the efficacy of combined PARP inhibition and radiation in a PDX model of acquired chemoresistance (Fig. 5D). Chemoresistant SCRX-Lu149-R tumors had been previously derived from PDXs that had acquired resistance to cisplatin and etoposide after a total of at least 18 weekly cycles of chemotherapy while retaining sensitivity to IR (25). The combination of talazoparib and radiation was again significantly more effective than radiation alone ($P < 0.05$).

To evaluate the in vivo tolerability of treatment, mouse weights were monitored throughout the course of the experiment (Supplementary Fig. S1). Compared with control mice on day 8 of treatment (mean % weight change from baseline ± SE: 3.1% ± 1.2%), groups treated with talazoparib alone (0.1% ± 1.0%) and radiation alone (−1.0% ± 1.1%) gained weight at a decreased rate. The combination of talazoparib and radiation (−5.8% ± 1.3%) led to significantly greater weight loss than either single treatment ($P = 0.003$ for talazoparib and $P = 0.02$ for IR). However, all mean weights recovered to baseline or greater by day 22 (vehicle 12.8% ± 1.6%; talazoparib 9.8% ± 1.6%; IR 5.9% ± 1.4%; and combination 1.8% ± 1.7%).

**Discussion**

In this study, we found that low doses of PARP inhibitor sensitize SCLC cell lines and PDXs to radiation. We further found that PARP trapping may play an important role in radiosensitization of SCLC cells, as talazoparib was a more effective...
Talazoparib is a greater radiosensitizer than enzymatically equivalent concentrations of veliparib. A, Western blot analyses of tumor PAR in NCI-H446 after 1 hour of 200 nmol/L talazoparib or 1.6 μmol/L veliparib show similar levels of inhibition of PAR polymerization in whole-cell lysates. Levels of inhibition remained similar after mock irradiation and 6 Gy radiation. B, Mean and SD of quantified PAR levels after treatment with 200 nmol/L talazoparib or 1.6 μmol/L veliparib, performed over 3 replicates without irradiation, show no statistical difference between the tested concentrations. Lane intensities, expressed as percentage of DMSO control, were quantified between bands at 100 kDa and 200 kDa and normalized to actin-loading control. C, Talazoparib at 200 nmol/L causes greater PARP trapping (chromatin-bound PARP1) in NCI-H446 cells than veliparib at 1.6 μmol/L. Cotreatment with 0.01% MMS was performed to allow detection of PARP trapping by inducing DNA damage. D, Clonogenic survival assay of NCI-H446 shows significant radiosensitization at 200 nmol/L talazoparib but not at 1.6 μmol/L of veliparib. E, Clonogenic survival assay of NCI-H82 shows significant radiosensitization of NCI-H82 at 20 nmol/L talazoparib but not with concentrations of veliparib up to 800 nmol/L. Error bars represent SD.

Despite advances in irradiation techniques, local failure rates after irradiation for SCLC remain high. In LS-SCLC, where thoracic irradiation is an integral part of concurrent therapy with chemotherapy, 36% to 52% of patients have local failure in the thorax as the first site of failure (26). Furthermore, in a recent study of ES-SCLC after chemotherapy then treated sequentially with thoracic irradiation alone, 44% of patients had a local thoracic failure as a part of first recurrence (27). These high rates of failure highlight the need for novel treatments to improve the efficacy of radiation. In our study, we found that PARP inhibition led to substantial radiosensitization, with DMFs of 1.40 to 2.88 at a concentration of 20 nmol/L. In comparison, similar experiments with 1,000 nmol/L of olaparib have produced DMFs from 1.11 to 1.61 for head and neck squamous cell carcinoma and 1.72 to 2.06 for non-BRCA-mutated breast cancer (16). In irradiated glioma cell lines, 500 nmol/L of the PARP inhibitor KU-0059436 produced DMFs of 1.08 to 1.36 (14). In particularly high DMFs, we observed with SCLC cell lines may suggest SCLC is uniquely sensitive to combination PARP inhibition and irradiation. As a highly mutated tumor with universal functional loss of TP53 and RB1, SCLC may be particularly dependent on intact DNA damage repair pathways to manage the cellular insults of radiation and PARP inhibition (28, 29).

A recent study by Owonikoko and colleagues examined the combination of veliparib with radiotherapy in SCLC. Within this larger study that examined the combination of veliparib and chemotherapy in vitro and in vivo, veliparib, when combined with radiotherapy, had an additive effect in vitro as determined by short-term viability assays in 2 SCLC cell lines (12). Our results are consistent with this study, which showed that veliparib had an additive but not synergistic effect with radiation at the concentrations tested. In this study, in addition to viability assays in a larger
panel of SCLC cell lines, we also assessed clonogenicity by CSAs, an important endpoint in assessing the effects of radiotherapy. We further demonstrated the significant radiosensitizing effect of talazoparib as compared with veliparib and confirmed our observations in vivo. As talazoparib was a more effective radiosensitizer than veliparib, this suggests that PARP trapping contributes to radiosensitization and provides evidence that clinical PARP inhibitors with greater PARP trapping activity may be more effective in combination with radiation than less potent PARP trappers. PARP trapping may play a particularly important role in SCLC, given that trapping is dependent on the presence of PARP1 enzyme, and PARP is highly expressed in SCLC (22, 30). Further work on the generalizability of the role of PARP trapping in radiosensitization for other histologies is necessary.

The potential for increased toxicity with an increase in efficacy is of concern, and PARP trapping activity in particular appears to correlate inversely with maximum tolerated PARP inhibitor dose. PARP inhibition has shown to increase esophageal and skin toxicities when combined with thoracic irradiation in a mouse model, with more severe toxicities associated with talazoparib compared with veliparib (31). In our study, mice treated with the combination of talazoparib and radiation had greater weight loss than treatment alone. However, this group began recovering weight soon after combination therapy was completed, with

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### Figure 4

Talazoparib induces more double-stranded DNA breaks than enzymatically equivalent concentrations of veliparib with and without radiation. **A**, Immunostaining of γH2AX nuclear foci after treatment with PARP inhibitor and radiation shows more foci in cells treated with talazoparib compared with veliparib or DMSO control both with and without 6 Gy IR. Talazoparib was applied for 24 hours prior to IR, fresh media was applied 1 hour after IR, and fixing and staining was performed 24 hours after IR (see pulse dosing below). Slides were scanned with a 40×/0.95NA objective. **B**, Experiment timeline and violin plot of γH2AX foci per nuclei, performed over 2 independent experiments. The number of nuclei analyzed was >3,000 in each group. Cells were either treated with 48 hours of continuous drug and irradiated 24 hours into treatment prior to fixing and staining, or were alternatively changed into fresh media 1 hour after irradiation (pulse dosing). Mean counts of γH2AX nuclei per cell are represented as a dot within each violin. All comparisons among drug treatments and doses of radiation were statistically significant using the Mann–Whitney tests and Bonferroni adjustment for multiple comparisons.
Figure 5.
Talazoparib radiosensitizes SCLC PDX in vivo. A, Mean tumor PAR by ELISA after oral administration of 0.2 mg/kg talazoparib (n = 3 for each time point) shows the kinetics of PAR polymerization inhibition over time. Statistically significant reductions in PAR compared with control are seen at 6 and 18 hours after treatment, but no significant differences were noted among treatment times. Error bars represent SD. B–D, Tumor growth curves and Kaplan–Meier plots of (B) SCRX-Lu149 chemo-naive, (C) JHU-LX44, and (D) SCRX-Lu149-R chemoresistant PDX treated with 20 doses of 0.2 mg/kg talazoparib, 8 Gy radiation in 4 daily fractions (days 2–5), or the combination. A Kaplan-Meier event was defined as tumor volume greater than 1,000 mm$^3$ for both SCRX-Lu149 models and 700 mm$^3$ for JHU-LX44.
baseline weight reestablished within 3 weeks. Of note, doses of talazoparib well below those required for single agent efficacy were sufficient for radiosensitization. Radiosensitization was observed in cell lines at 0.2 to 20 nmol/L of talazoparib, frequently at least 10-fold below the single agent EC50, and the radiosensitizing in vivo dose of 0.2 mg/kg is at least 33% reduced from doses that are safe and effective as a single agent (10, 24). With a low dose of PARP inhibitor for radiosensitization, systemic toxicities are less likely, and local radiosensitization of normal tissues may be of greater concern. The high DMF of PARP inhibitors in SCLC may allow for a substantial window between efficacious doses of radiation and unacceptable local toxicity; however, future study and clinical corroboration is warranted.

Finally, we noted substantial differences among SCLC cell lines in sensitivity to talazoparib, radiation, and their combination. For instance, concentrations necessary for radiosensitization varied from under 0.2 nmol/L to 20 nmol/L talazoparib, and in one cell line, NCI-H69, we did not observe radiosensitization at any concentration. Recently, several proteins have been explored as predictors of PARP inhibitor sensitivity, including SLFN11, which we and others have reported (10, 32, 33). In this study, cell lines were radiosensitized irrespective of SLFN11 expression, and PDXs both high (chemo-naïve SCRX-Lu149) and low (IHU-LX44 and chemoresistant SCRX-Lu149) in SLFN11 were radiosensitized by talazoparib, broadening the patient population that may benefit from this combination. Furthermore, the effect of other proteins that correlate with PARP inhibitor sensitivity, such as ATM, E-cadherin, and DNA-PKcs, remains unclear in the combination with radiation (12, 33). The mechanisms of resistance and sensitivity to the combination of radiation and PARP inhibitor will be important to identify in future investigation.

In summary, low doses of PARP inhibitor, especially those with greater PARP trapping activity, are sufficient to radiosensitize SCLC cell lines and PDXs. We are currently planning a phase I clinical trial to investigate the safety and feasibility of combining a PARP inhibitor with radiation for patients with SCLC. With the dismal outcomes still seen for patients with SCLC, new treatment approaches and novel molecular therapies are critical to improve tumor control and patient outcomes.

Disclosure of Potential Conflicts of Interest
C.M. Rudin is a consultant/advisory board member for AbbVie, AstraZeneca, Bristol-Myers Squibb, Celgene, and Harpoon Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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References


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