A highly sensitive and specific PARylation assay confirms significant and durable target engagement by AZD5305 in patients

Benedetta Lombardi,[1] Paola Marco-Casanova,[1] Athanasios Karapetsas,[1] Mya Hornett,[1] Edit Lukacs,[1] Ko Sugibayashi,[1] Sabina Cosulich,[1] Adam Dowson,[1] Ganesh Moorthy,[1] Jessica Brown,[1] Timothy A. Yap,[2] Alison M. Schram,[3] Judith Balmaña,[4] Stephen J. Luen,[5] Seock-Ah Im,[6] Adam Sharp,[7] Richard Baird,[8] Elizabeth A. Harrington[9] and Spiros Linardopoulos[1]

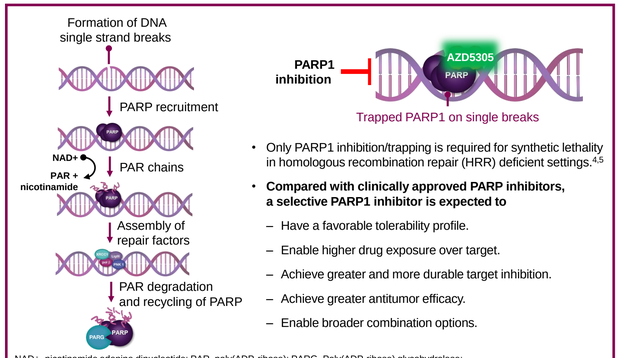
[1]AstraZeneca, Cambridge, UK; [2]University of Texas MD Anderson Cancer Center, Houston, TX, USA; [3]Memorial Sloan Kettering Cancer Center, New York, NY, USA; [4]University Hospital Vall d'Hebron, Barcelona, Spain; [5]Peter MacCallum Cancer Centre, Melbourne, Australia; [6]Cancer Research Institute, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea; [7]The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, UK; [8]Cancer Research UK Cambridge Cancer Centre, Cambridge, UK; [9]AstraZeneca, Macclesfield, UK

CT269

Background

- AZD5305 is a selective poly(ADP-ribose) polymerase 1 (PARP1) inhibitor with improved selectivity compared with clinically approved PARP inhibitors (PARPi).[1]
- AZD5305 is being investigated in the PETRA clinical trial (NCT04644068), a Phase 1/2 study in patients with advanced solid tumors.
- Poly(ADP-ribosyl)ation, also known as PARylation, is catalyzed by PARPs. Measuring the inhibition of PARylation upon treatment is the most direct way to assess pharmacodynamic (PD) activity of a PARPi (**Figure 1**).
- Measurement of PARylation inhibition has been used to determine the PD of PARPi in clinical trials, but the assays used to date have not had optimal selectivity and specificity.[2,3]
- We report a new, highly sensitive and specific PARylation assay to evaluate the inhibition of PARylation by AZD5305 in peripheral blood mononuclear cells (PBMCs) from patients enrolled in the PETRA trial (NCT04644068).

Figure 1. PARylation is a direct biomarker to measure PARP enzyme inhibition



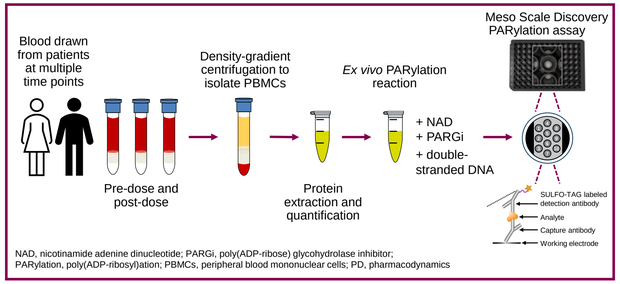
- Only PARP1 inhibition/trapping is required for synthetic lethality in homologous recombination repair (HRR) deficient settings.[4,5]
- **Compared with clinically approved PARP inhibitors, a selective PARP1 inhibitor is expected to**
  - Have a favorable tolerability profile.
  - Enable higher drug exposure over target.
  - Achieve greater and more durable target inhibition.
  - Achieve greater antitumor efficacy.
  - Enable broader combination options.

NAD+, nicotinamide adenine dinucleotide; PAR, poly(ADP-ribose); PARG, Poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase

Methods

- Our aim was to define the AZD5305 PD profile by measuring PARylation inhibition in PBMCs as a surrogate tissue collected at baseline and multiple timepoints during treatment (**Figure 2**).

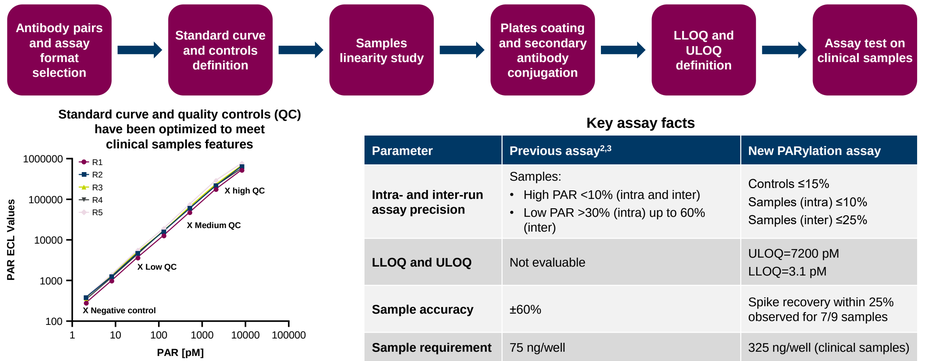
Figure 2. Analysis workflow for PD samples



NAD, nicotinamide adenine dinucleotide; PARGi, poly(ADP-ribose) glycohydrolase inhibitor; PARylation, poly(ADP-ribosyl)ation; PBMCs, peripheral blood mononuclear cells; PD, pharmacodynamics

- Key eligibility criteria for patients enrolled in the PETRA study were age ≥18 years; histologic or cytologic confirmation of ovarian, breast, prostate or pancreatic cancer with homologous recombination repair gene mutations; and ≤1 prior line of therapy with a PARPi-based regimen.
- PBMCs were collected at multiple timepoints following a single dose of AZD5305, at cycle (C) 0 day (D) 1 pre-dose, 1, 4, 24 and 48 hours (h) post-dose, and at C1D1 pre-dose. Collected PBMCs were lysed and subjected to an *ex vivo* PARylation reaction.
- PARylation levels were quantified by a newly developed Meso Scale Discovery assay to determine inhibition levels (**Figure 3**).

Figure 3. Development of the Meso Scale Discovery PARylation assay



Key assay facts

Parameter	Previous assay[2,3]	New PARylation assay
Intra- and inter-run assay precision	Samples: • High PAR <10% (intra and inter) • Low PAR >30% (intra) up to 60% (inter)	Controls ≤15% Samples (intra) ≤10% Samples (inter) ≤25%
LLOQ and ULOQ	Not evaluable	ULOQ=7200 pM LLOQ=3.1 pM
Sample accuracy	±60%	Spike recovery within 25% observed for 7/9 samples
Sample requirement	75 ng/well	325 ng/well (clinical samples)

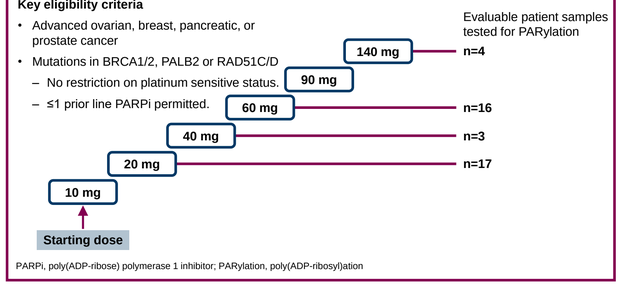
Features of the new PAR assay

- Absolute quantification for PAR.
- Applicable to PBMCs, cell culture and tumor lysates.
- Higher specificity.
- Control over reproducibility.

ECL, electrochemiluminescence; LLOQ, lower limit of quantitation; PAR, poly(ADP-ribose); PARylation, poly(ADP-ribosyl)ation; PBMC, peripheral blood mononuclear cell; QC, quality control; ULOQ, upper limit of quantitation

- **Criteria for sample evaluation were**
  - Baseline PARylation levels ≥100 pM.
  - Protein quantification of the sample within bicinchoninic acid assay linear range (125–2000 µg/mL).
- Based on the above criteria, evaluable PBMC samples were obtained and analyzed from patients who received AZD5305 at 20 mg (n=17), 40 mg (n=3), 60 mg (n=16), and 140 mg (n=4) (**Figure 4**).
  - No evaluable samples were identified from patients who received 10 mg or 90 mg at the time of analysis; however, additional samples have been collected from these patients and will be assessed.
- Based on preclinical studies using AZD5305 in *in vivo* models, maximal efficacy is associated with high and sustained target engagement of ≥90% PARylation inhibition.[1]

Figure 4. Study design



PARPi, poly(ADP-ribose) polymerase 1 inhibitor; PARylation, poly(ADP-ribosyl)ation

Results

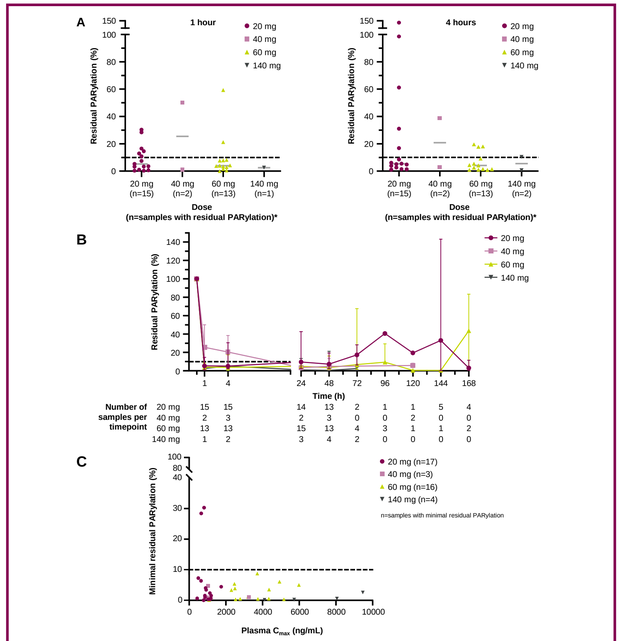
- Based on AZD5305 plasma concentration versus time after a single dose, a dose-proportional increase in exposure (maximum plasma concentration [C_{max}] and area under the curve [AUC]) was observed with increasing doses (10–140 mg once daily), with a quick time to maximum plasma concentration (T_{max}, 0.5–3 h) and a mean terminal elimination half-life of 13.1–16.4 h across cohorts.[6]
- Analysis of the evaluable PBMC samples from patients treated with increasing doses of AZD5305 showed:
  - AZD5305 significantly inhibited PARylation in PBMC, with maximum inhibition occurring at around T_{max} (**Table 1**, **Figure 5A**).
  - An extensive duration of PARylation inhibition occurred after a single dose of AZD5305 (20–140 mg) (**Figure 5B**).
  - There was no significant correlation between C_{max} and minimal residual PARylation (**Figure 5C**).

Table 1. Residual PARylation levels by dose in the PETRA study

	AZD5305 20 mg		AZD5305 60 mg	
	1 hour post-dose	4 hours post-dose	1 hour post-dose	4 hours post-dose
Patients with >90% PARylation inhibition, n (%)	9/15 (60.0)	10/15 (66.7)	11/13 (84.6)	10/13 (76.9)
Median residual PARylation levels (residual PARylation), %	5.3	5.0	4.0	3.7

Only data from dose groups with adequate sample sizes to determine correlation were included.
PARylation, poly(ADP-ribosyl)ation

Figure 5. PARylation inhibition after treatment with AZD5305



*n represents the number of evaluable samples per timepoint according to the criteria for sample evaluation.
C_{max}, maximum plasma concentration; PARylation, poly(ADP-ribosyl)ation

Conclusions

- We have developed and optimized a new, highly sensitive and specific PARylation assay that can be used for direct measurement of PARP inhibition, and utilized in clinical settings.
- This assay has been successfully employed in the PETRA clinical trial to measure the inhibition of PARylation by AZD5305 in PBMCs at various timepoints and doses.
- Significant and durable inhibition of PARylation by AZD5305 in PBMCs was confirmed in the majority of patients tested using the novel PARylation assay.

References

1. Illuzzi G, et al. *Clin Cancer Res*. 2022;28:4724–36.
2. Fong PC, et al. *N Engl J Med*. 2009;361:123–34.
3. Bundred N, et al. *Invest New Drugs*. 2013;31:949–58.
4. Murai J et al. *Cancer Res*. 2012;72:5588–99.
5. Ronson GE, et al. *Nat Commun*. 2018;9:746.
6. Yap TA, et al. AACR 2022. Abstract CT007.

Acknowledgements

We would like to thank the patients in the PETRA study, their families and caregivers. Editorial support for this presentation, under the direction of the authors, was provided by Werner Gerber of Ashfield MedComms (Kimberley, South Africa), an Inizio Company, and was funded by AstraZeneca. This study was funded by AstraZeneca.

Disclosures

Spiros Linardopoulos is employed by, and has stock and stock options in, AstraZeneca. For other authors, please see abstract.

Contact Email: spiros.linardopoulos@astrazeneca.com

Presented at the 2023 American Association for Cancer Research (AACR) Annual Meeting, April 14–19, 2023, Orlando, FL, USA