Abstract # TP104

Exploratory study of NQO1 expression in advanced solid tumors

Yuan Tian[1], Fabiola Cecchi[1], Wei-li Liao[1], Eunkyung An[1], David E. Gerber[2], David A. Boothman[2], William Hoos[3], Todd Hembrough[1]

[1] Nantomics, Rockville, MD; [2] University of Texas Southwestern Medical Center, Dallas, TX; [3] NQ Oncology, INC., Chapel Hill, NC

Overview

- This study investigated the correlation between NAD(P)H: quinone oxidoreductase 1 (NQO1) protein expression and outcome after ARQ 761 (NQO1 inhibitor) treatment in cancer patients
- NQO1 was absolutely quantitated with mass spectrometric selected reaction monitoring (SRM) in tumor cells microdissected from FFPE tumor tissues
- Patients with higher expression of NQO1 (>1000 amol/µg) had longer durations of stable disease than patients with lower NQO1 levels (<500 amol/µg)

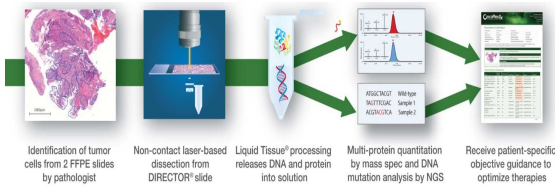
Introduction

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a flavoenzyme that catalyzes 2-electron reduction of quinones to hydroquinones. Expression of NQO1 is elevated in multiple solid tumor types compared to normal tissues. ARQ 761 (ArQule) (beta-lapachone) is an NQO1 inhibitor in Phase 1 clinical trials. Its interaction with NQO1 generates cytotoxic superoxide leading to tumor-specific cell death in NQO1-overexpressing tumors. Patients with such tumors have been reported to respond favorably to ARQ 761 after experiencing disease progression during treatment with chemotherapy, radiochemotherapy, and/or other targeted therapy. We hypothesized that the absolute amount of NQO1 expression may be useful for predicting clinical response to ARQ 761. We developed a quantitative, mass spectrometry-based, multiplexed assay to measure absolute amounts of NQO1 in tumor tissues. We investigated the correlation between NQO1 expression level and clinical outcome after treatment with ARQ 761.

Methods

We developed a selected reaction monitoring (SRM) assay for NQO1 using trypsin digestion mapping of recombinant NQO1 protein. Heavy labeled peptides were synthesized as internal standard. After pre-clinical validation in multiple cell lines, the assay was run on formalin-fixed, paraffin-embedded (FFPE) sections of various types of advanced tumors biopsied from 6 heavily pretreated patients who were enrolled in a phase 1 trial of ARQ 761. FFPE tumor sections were laser microdissected, solubilized, enzymatically digested, and subjected to quantitative proteomic analysis. Expression levels of NQO1 and other target proteins were measured using the multiplexed SRM assay.

Figure 1. Liquid tissue- selected reaction monitoring (LT-SRM) in FFPE tissue[1,2]



Results

Figure 2. Performance of NQO1 SRM assay. Thirteen points were spiked in complex background with a constant amount (5fmol) of heavy synthetic peptide and varying amounts (from 0 to 25000 amol) of light synthetic peptide to build the standard curve.

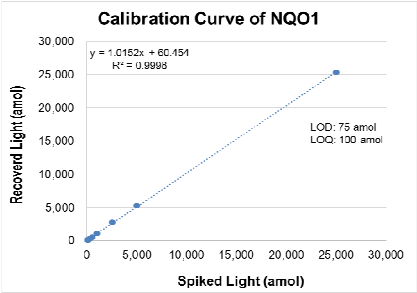


Figure 3. NQO1 protein expression in 14 cell lines is not perfectly correlated with mRNA level. mRNA levels from Broad-Novartis Cancer Cell Line Encyclopedia[3]

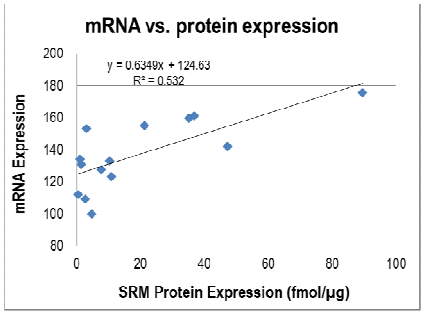


Table 1. Treatment history patients prior to ARQ 761 treatment

Patient ID	Disease	Line of therapy	Regimen	Reason for discontinuation
E0571	Squamous cell carcinoma, lung tumor	1	Carboplatin	Disease progression
		2	Docetaxel	Disease progression
		3	Erlotinib	Disease progression
E0572	Bladder cancer	1	Cisplatin/Gemcitabine	Disease progression
		2	MVAC (methotrexate/vinblastine/doxorubicin/cisplatin)	Disease progression
		3	Paclitaxel	Disease progression
E0573	Lung adenocarcinoma	1	Cisplatin/Vinorelbine	Complicated by tinnitus
		2	Carboplatin/Vinorelbine	
		3	IMC-3G3 clinical trial (carboplatin/paclitaxel + IMC-3G3)	
		4	SBRT + erlotinib clinical trial	Complications
		5	Phase 1 clinical trail of antibody-drug conjugate targeting	Disease progression
E0576	Metastatic pancreatic carcinoma, liver	1	Adriamycin/Cytoxan	Adjuvant
		2	Cemcitabine	Disease progression
		3	Folfirinox	Disease progression
E0577	Small cell carcinoma,lung	1	Carboplatin + Etoposide	Disease progression
		2	Cisplatin + Irinotecan	Disease progression
		3	Taxotere	Disease progression
E0578	Small cell carcinoma, lung	1	Cisplatin + Etoposide	Disease progression
		2	Topotecan	Disease progression
		3	Paclitaxel	

Table 2. ARQ 761 treatment and clinical outcome

Arm A=weekly, Arm B=every other week, Arm C=2 of 3 weeks

Patient ID	Sex at birth	Race & Ethnicity	Age at Enrollment	Assigned dose level (mg/m^2)	Regimen	Doses received	RECIST: best response	RECIST: target lesion baseline (cm)	RECIST: target lesion cycle 2 (cm)	RECIST: target lesion cycle 4 (cm)
E0571	M	NH/W	54	390	Arm A (1hr)	14	SD	12.5	13.8	10.2 --> 9.7
E0572	M	NH/W	56	195	Arm A (2 hr)	16	SD	8.8	7.4	10
E0573	M	NH/W	71	450	Arm C (2 hr)	16	SD	4.9	5.3	5.5
E0576	F	NH/W	57	195	Arm A (2 hr)	13	SD	3.6	3.5	3.7
E0577	F	NH/W	37	390	Arm B (2 hr)	4	PD	5.2	8.2	n/a
E0578	F	NH/W	49	390	Arm C (2 hr)	2	PD	5.6	7.0	n/a

Figure 4. Correlation of NQO1 expression with ARQ 761 outcome. Analysis of FFPE tumor tissues (n=6) yielded NQO1 expression levels ranging from 148 to 5813 amol/µg. Patients (n=4) with NQO1 expression levels >1000 amol/µg had a longer duration of stable disease (4 cycles of ARQ 761 treatment) than patients (n=2) with levels <500 amol/µg (2 cycles of ARQ 761 treatment).

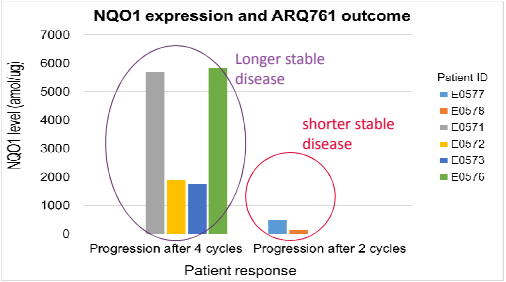
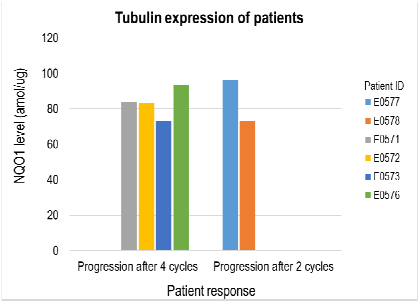


Figure 5. Quality control: Tubulin expression is the same in patients stratified by treatment outcome



Conclusions

- The SRM assay quantitated NQO1 in tumor cells microdissected from FFPE tissue (*lower* limit of detection= 75 amol; lower limit of quantification= 100 amol).
- In this pilot study (n=6), patients with NQO1 expression >1000 amol/µg had better responses to ARQ 761 treatment than patients with lower expression levels.
- Data from a larger group of patients will be necessary to confirm the utility of the NQO1 SRM assay in predicting ARQ 761 treatment outcomes. Our data strongly supports the need to set a prognostic cutoff of NQO1 expression in tumor cells for ARQ 761 treatment.

References

1. Hembrough, T., Thyparambil, S., Liao, W. L., Darfler, M. M., Abdo, J., Bengali, K. M., ... & Burrows, J. (2013). Application of selected reaction monitoring for multiplex quantification of clinically validated biomarkers in formalin-fixed, paraffin-embedded tumor tissue. The Journal of Molecular Diagnostics, 15(4), 454-465.
2. Hembrough, T., Liao, W. L., Hartley, C. P., Ma, P. C., Velcheti, V., Lanigan, C., ... & Burrows, J. (2016). Quantification of Anaplastic Lymphoma Kinase Protein Expression in Non–Small Cell Lung Cancer Tissues from Patients Treated with Crizotinib. Clinical Chemistry, 62(1), 252-261.
3. Broad-Novartis Cancer Cell Line Encyclopedia (http://www.broadinstitute.org/ccle/home)

ASMS, June 5-9, 2016, San Antonio, TX, US