Etirinotecan pegol Target-Specific Pharmacodynamic (PD) Biomarkers Measured in Circulating Tumor Cells (CTCs) from Patients in the Phase 3 BEACON Study in Patients with Metastatic Breast Cancer (mBC)

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INTRODUCTION

- Etirinotecan pegol (EP, NKTR-102) is a unique long acting topoisomerase 1 inhibitor designed for prolonged tumor cell exposure.
- In patients, etirinotecan pegol leads to greatly prolonged plasma SN38 exposure compared to irinotecan (elimination half-life 50 days compared to 2 days), yet peak SN38 concentrations are 5- to 10-times less.
- In a Phase 2 trial in patients with metastatic breast cancer whose disease had failed prior taxane-based treatment, etirinotecan pegol administered q14d or q21d demonstrated objective response rate by RECIST of 28.6% in the ITT population.[1] See Table below.

Objective Response Rate of Etirinotecan pegol in Patients With Metastatic Breast Cancer (ITT Population)

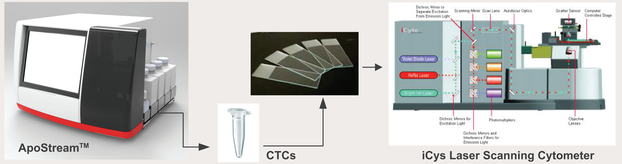
	NKTR-102 q14d (N=35)	NKTR-102 q21d (N=35)	Total (N=70)
Objective Tumor Response Rate (CR+PR), N (%)	10 (28.6%)	10 (28.6%)	20 (28.6%)
Complete Response (CR) Partial Response (PR) Stable Disease (SD) Progressive Disease (PD)	2 (5.7%) 8 (22.9%) 16 (45.7%) 9 (25.7%)	0 10 (28.6%) 15 (42.9%) 10 (28.6%)	2 (2.9%) 18 (25.7%) 31 (44.3%) 19 (27.1%)
Clinical benefit (CR, PR, SD ≥ 6 months)	13 (37.1%)	17(48.6%)	30 (42.9%)

- Enrollment in BEACON, a Phase 3 open-label, randomized, multicenter study of Etirinotecan pegol versus treatment of physician's choice (TPC) in patients with locally recurrent or metastatic breast cancer previously treated with an anthracycline, a taxane, and capecitabine, is currently ongoing. Enrollment is anticipated to complete in Q3/2013.

STUDY DESIGN

BACKGROUND

- Topoisomerase 1 is a nuclear enzyme that plays an essential role in DNA replication, transcription, recombination and repair.
- SN38, the active metabolite of Etirinotecan pegol, stabilizes the DNA-topoisomerase 1 complex subsequently resulting in DNA-double strand breaks.
- Resistance mechanisms described for topoisomerase 1 inhibitors include:
  - Decreased drug-accumulation resulting from over-expression of ATP binding cassette transporters
  - Increased topoisomerase 1 degradation through ubiquitination or sumoylation
  - Increased expression of anti-apoptotic proteins
  - Increased repair of topoisomerase 1 inhibitor induced lesions
- Circulating tumor cells (CTCs) are cancer cells shed from either the primary tumor or its metastases that circulate in the peripheral blood. The number of CTCs or change in number of CTCs upon treatment potentially indicate responders vs. non-responders.
- CTCs are an attractive minimally invasive alternative to tumor biopsies for clinical applications.
- Newer CTC isolation techniques yield increased numbers of isolated CTCs compared to the first generation EpCAM-dependent methods, enabling downstream molecular profiling.
- ApoStream™ CTC isolation is based on dielectrophoresis (DEP) field-flow fractionation (DEP-FFF) technology and is antibody independent.[2] Recovered cells are suitable for multiple diagnostic applications, including protein quantification, FISH analysis, genetic mutation analysis, and gene expression profiling.

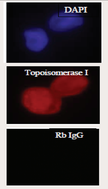
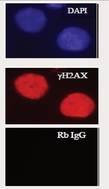
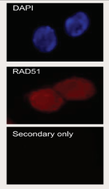
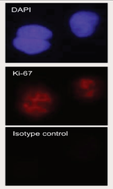
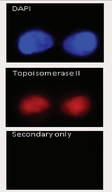
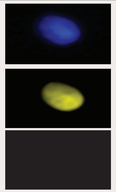


METHODS

Primary and secondary antibodies were obtained from commercial sources.Control (0.1% DMSO) and drug-treated (SN38, 10 uM) tumor cell lines (HCT116, MCF7, A549, SKBr3) and PBMCs from healthy donors were used for biomarker qualification. For each marker, 1-3 different antibodies were tested. Signals of biomarker staining were compared to secondary antibody staining only and isotype controls processed in the same experiment. The antibody with the highest mean fluorescent intensity (MFI) was selected for further development, with preference given to mono- over polyclonal antibodies. Titration was performed to identify the optimal dilution. Specificity was tested in positive and negative biological controls. The optimal antibody for each biomarker was multiplexed in a panel with antibodies against cytokeratin, CD45 and DAPI for phenotypic identification of CTCs. Multiplexed assay performance was tested using a mixture of tumor cells and PBMC. For BEACON pts, serial 7.5 mL whole blood samples were drawn and shipped ambient to ApoCell (Houston, TX) for further processing. Results from baseline (predose) samples are presented. PBMCs were separated and CTCs were isolated using ApoStream™ technology. CTCs were stained for PD markers and analyzed using an iCys laser scanning cytometer equipped with image analysis software. All assays were performed on an iCys laser scanning cytometer (CompuCyte, Westwood, MA) equipped with iCys 3.4.12 image analysis software.

ASSAY DEVELOPMENT AND QUALIFICATION

Relevance of Etirinotecan pegol target-specific PD biomarkers, assay specifications and qualification results are summarized in the Table below. Markers in each staining panel were multiplexed in a panel with antibodies phenotypic for identification of CTCs (cytokeratin, CD45, DAPI).

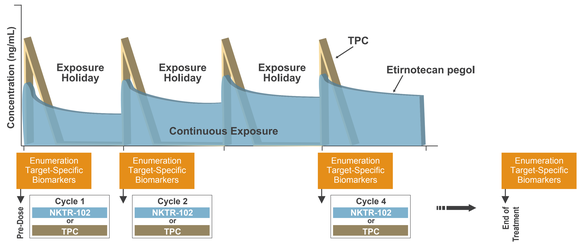
	Staining Panel 1		Staining Panel 2		Staining Panel 3		TUNEL
	Top 1	γ- H2Ax	RAD51	Ki-67	Top2	ABCG2	TdT
Relevance to Etirinotecan pegol Mechanism of Action	• Molecular target • Top 1 levels predicted response to IRN-based treatment in colorectal cancer[3]	• Marker of double-strand DNA damage • Increased γ-H2Ax in CTCs isolated from pts treated with topotecan[4]	• Member of DNA double strand break repair machinery • Increased expression of Rad51 conferred resistance to SN38 in cell lines[5]	• Marker for proliferating cells • Ki-67 index is a prognostic factor and a powerful predictor of higher chemosensitivity in patients with breast cancer[6]	• Cell lines selected for resistance to Top1 and Top 2 inhibitors show upregulation of the alternate topoisomerase[7] • Increased levels of Top 2 have been reported after treatment with Top 1 inhibitors[8]	• Efflux transporter for irinotecan and SN38[9] • Restricts SN38 entry into brain[10]	• Percentage of apoptotic CTCs
Antibody Vendor Catalogue # Clone Antibody Conjugation	Abcam Ab28432 NA Rabbit polyclonal unconjugated	Milipore 16-193 JBW301 Mouse Mab IgG1 Biotin	Abcam AB63801 NA Rabbit polyclonal unconjugated	eBiosciences 51-5699 20Raj1 Mouse-monoclonal Alexa Fluor 647	Epitomics 1826-1 EP1102Y Rabbit monoclonal unconjugated	R&D Systems BAM995 5D3 Mouse Mab IgG1 Biotin	Promega rTdT G3250 NA dUTP-Cy5 (GE-PA55022)
Dilution	1/200	1/200	1/200	1/50	1/200	1/25	NA
Biologic Controls High, MFI (x10³) Low, MFI (x10³)	HCT116, 964 A549: 685	HCT116 (SN38), 487 HCT116 (DMSO), 124	HCT116 (SN38), 948 HCT116 (DMSO), 178	A549, 1071 PBMC, 40	SKBr3, 624 MCF7, 436	A549, 365 PBMCs, 91	DNAse I Treated HCT116 Untreated HCT116
MFI Negative Control (x10³)	7	113	31	NA	62	67	NA
Images (40x Magnification) DAPI Marker Isotype Control							
Staining Panel Qualification							
Reproducibility (n=27) %CV % Positive Cells %CV MFI	1.4 0.2	15.5 15.4	7.9 22	1.7 NA	11 17	23 19	NA NA
Inter-day Variability (n=3) %CV % Positive Cells %CV MFI	2.2 4.4	18 23	11 24	1.7 NA	13 16	20 20	NA NA
Inter-tech Variability (n=3) %CV % Positive Cells %CV MFI	1.8 9.4	11 15	12 15	1.8 NA	6 16	20 24	NA NA

References: 1.Garcia et al., J Clin Oncol 2011, Suppl 27, Abstr 269. 2.Vishal et al., Biomicrofluidics 2012, 6. 3.Braun et al., JCO 2008, 28(16), 2690-2698. 4.Wang et al., Clin Cancer Res. 2010, 16(3), 1073-1084. 5.Wu et al., Molecular Cancer Therapeutics 2011, 10(11), Supplement 1. 6.Fasching et al., BMC Cancer 2011, 11(486), 1-13. 7.Saleem et al., Ann NY Acad. Sci. 2000, 922, 46-65. 8.Rubin et al., Clin Cancer Res 1995, 1(3), 269-276. 9.Nakatomi et al., Biochem Biophys Res Commun 2001, 288, 827-832. 10.Lin et al., Clin. Cancer Res. 2013, 19(8), 2084–95.

PHASE 3 BEACON

CTC Sampling Schedule

The CTC sampling schedule was driven by the sustained concentration-time profile observed with Etirinotecan pegol. The figure shows the relationship between the CTC sampling schedule and the pharmacokinetic profile of Etirinotecan pegol or treatment of physician choice (TPC).

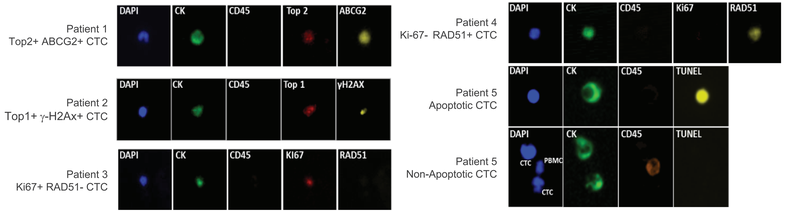


Pre-Dose CTC Results

The following table summarizes results from the initial 167 pre-dose samples obtained through 30-Oct-12. Shown are statistics for successful processing, CTC detection rate, median number of CTCs, and detection rate and range for individual biomarker values.

Successfully Processed	Detectable CTCs	Median # CTCs (Range)	Top 1	γ-H2Ax	RAD51	Ki-67	Top 2	ABCG2	TUNEL
99% of Samples	93% of Samples	217 (7.5-15000)							
% Cells Marker Positive			82	16	53	52	89	31	93
Range of Cells Marker Positive (%)			1-100	1-25	1-100	1-100	1-100	1-100	1-100
Range of Marker MFI (x10³)			85-2420	150-5144	109-1786	NA	127-7252	47-4187	NA

Representative Images of Biomarkers on Pre-Dose CTCs Isolated From BEACON Patients



CONCLUSIONS

- Staining panels for Etirinotecan pegol target-specific pharmacodynamic biomarkers have been successfully developed and qualified.
- CTC collection using ApoStream™ technology was successfully incorporated into the BEACON study.
- CTC substudy patient participation is projected to be over 75%.
- ApoStream™ isolates CTCs in >90% of samples. Median number of CTCs is high.
- Etirinotecan pegol target-specific pharmacodynamic biomarkers can be reliably measured in CTCs isolated from patients participating in BEACON and can be a potential predictive measure of clinical response.

ASCO 2013. Abstract ID: 1087. Session Title: General Poster Session: Breast Cancer - Triple-Negative/Cytotoxics/Local Therapy. Session Time/Location: 01June13, 1-4.45 pm, Hall A