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Etirinotecan Pegol Target-Specific Pharmacodynamic Biomarkers in Circulating Tumor Cells from Patients with Metastatic Breast Cancer in the Phase 3 BEACON Study

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INTRODUCTION

- Etirinotecan pegol (NKTR-102) is a 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 maximal SN38 concentrations are at least 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 (n=70; 35 per treatment regimen) demonstrated objective response rate by RECIST of 29%, with both schedules showing similar overall response rate[10]. See Table below.

Objective Response Rate of Etirinotecan pegol in Patients with Metastatic Breast Cancer

	Total (N=70)
Response Rate Overall Response Rate Prior ATC Subset (n=18)	29% 32%
Progression-Free Survival	4.7 months (5.6 months in q21d)
Overall Survival	10.3 months (13.1 months in q21d)

- Enrollment in BEACON, a Phase 3 open-label, randomized, multicenter study of NKTR-102 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 completed in Q3/2013. Topline results are expected in Q1/2015.

STUDY DESIGN

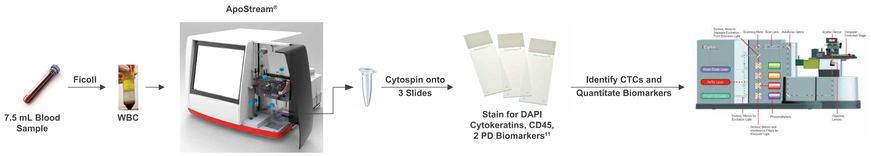
BACKGROUND

- 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.
- 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) and microfluidic technology and is antibody independent.[9]

METHODS

For BEACON patients participating in the CTC substudy, serial 7.5 mL whole blood samples were drawn and shipped ambient to ApoCell (Houston, TX) for further processing as shown in the BEACON CTC Sample Flow Diagram. Peripheral blood mononuclear cells (PBMCs) were harvested using the Ficoll-Paque gradient separation method. An iCys laser scanning cytometer (CompuCyte, Westwood, MA), equipped with iCys 3.4.12 image analysis software was used for quantitation. CTC samples were analyzed for the number of CTCs, the percent of cells staining positive for a given biomarker, and the mean fluorescence intensity, reflecting the normalized intensity of the specific biomarker in the biomarker positive CTCs. Results from the baseline samples will be presented.

BEACON CTC SAMPLE FLOW



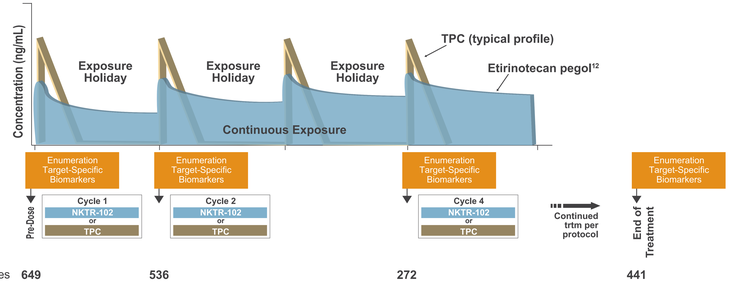
BIOMARKER SELECTION

Relevance of etirinotecan pegol target-specific PD biomarkers and grouping of staining panels

	Staining Panel 1		Staining Panel 2		Staining Panel 3		TUNEL
	Top 1	γH2Ax	RAD51	Ki - 67	Top 2	ABCG2	TdT
Relevance to Etirinotecan pegol Mechanism of Action	• Molecular target • Top 1 levels predicted response to IRN-based treatment in colorectal cancer[1]	• Marker of double-strand DNA damage • Increased γ-H2Ax in CTCs isolated from pts treated with topotecan[2]	• Member of DNA double strand break repair machinery • Increased expression of Rad51 conferred resistance to SN38 in cell lines[3]	• Marker for proliferating cells • Ki-67 index is a prognostic factor and a powerful predictor of higher chemosensitivity in patients with breast cancer[4]	• Cell lines selected for resistance to Top1 and Top 2 inhbitors show upregulation of the alternate topoisomerase[5] • Increased levels of Top 2 have been reported after treatment with Top 1 inhibitors[6]	• Efflux transporter for irinotecan and SN38[7] • Restricts SN38 entry into brain[8]	• Percentage of apoptotic CTCs

BIOMARKER SAMPLING SCHEME AND PATIENT PARTICIPATION

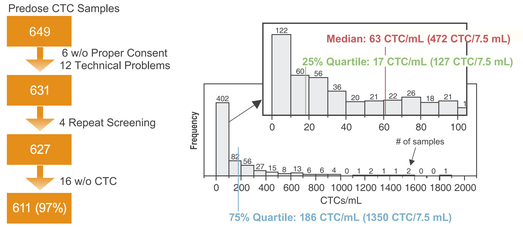
CTC Sampling Schedule
The CTC sampling schedule was driven by the sustained concentration-time profile observed with etirinotecan pegol. The figure shows the relationship between the CTC sampling schedule and the pharmacokinetic profile of etirinotecan pegol or treatment of physician choice (TPC).



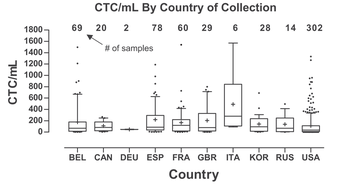
# Samples	649	536	272	441

High Patient Participation
Patient participation was high, with CTCs collected from 80% of BEACON patients, yielding the number of samples for analysis noted above.

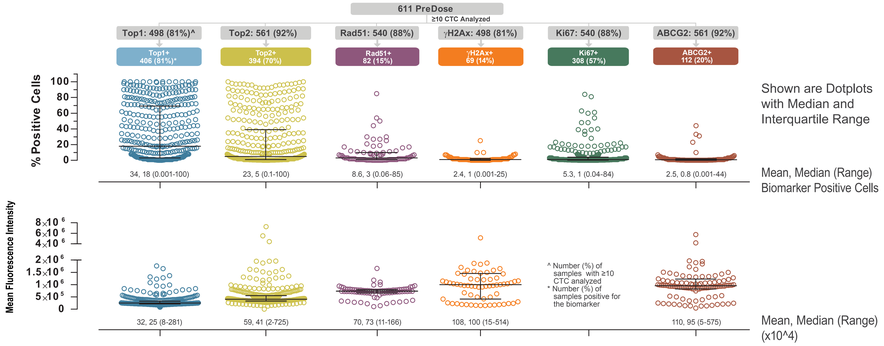
CTCs DETECTED IN 97% OF PATIENT SAMPLES WITH HIGH MEDIAN NUMBER OF CTCs



No difference in CTC yield between countries, supporting conclusion that extended shipment time had no impact on CTC harvest.



EXPRESSION of BIOMARKER SIGNAL in BASELINE SAMPLES



Shown are Dotplots with Median and Interquartile Range

Mean, Median (Range) Biomarker Positive Cells

Mean, Median (Range) (x10^4)

CONCLUSIONS

- Blood sample collection for CTC analysis was successfully incorporated into the BEACON study with 80% patient participation.
- Blood samples were successfully processed, with a low technical failure rate of 2%.
- CTC detection rate using ApoStream® was high (97% of patient; median # of CTCs: 472 CTCs/7.5 mL) and permitted evaluation of biomarkers at baseline and over time.
- Etirinotecan pegol target-specific pharmacodynamic biomarkers can be measured in CTCs isolated from patients participating in BEACON.
- BEACON efficacy and safety results are expected in Q1/2015, which will allow analysis of baseline CTC data and change of CTC data over time with patient outcome (response, PFS, OS).

SABCS 2014. Poster Number: P3-10-03. Date and Time: Thursday, December 11th from 5:00 PM - 7:00 PM. Location: Halls A-B
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References: [1]Braun et al., JCO 2008, 28(16), 2690-2698; [2]Wang et al., Clin. Cancer Res. 2010, 16(3), 1073-1084; [3]Wu et al., Mol. Cancer Ther. 2011, 10(11), Supplement 1; [4]Fasching et al., BMC Cancer 2011, 11(486), 1-13; [5]Saleem et al., Ann. NY Acad. Sci. 2000, 922, 46-65; [6]Rubin et al., Clin. Cancer Res. March 1995, 1(3), 269-276; [7]Nakatomi et al., Biochem. Biophys. Res. Commun 2001, 288, 827-832; [8]Lin et al., Clin. Cancer Res. 2013, 19(8), 2084–95; [9]Vishal et al., Biomicrofluidics 2012, 6. [10]Awada et al., Lancet Oncology 2013, 14(2), 1216–1225. [11]Hoch et al., J. Clin. Oncol. 2013, 31 (suppl; abstr 1087); [12]Jameson, Clin. Cancer Res. 2013, 19, 268-278.