Single CTC Characterization Identifies Phenotypic and Genomic Heterogeneity as a Mechanism of Resistance to AR Signaling Directed Therapies (AR Tx) in mCRPC Patients

Howard I. Scher 1, Ryon Graf 2, Jessica Louw 3, Adam Jendrisak 2, Ann Johnson 4, Stephanie Green 5, Angel Rodriguez 5, Nicole A. Schreiber 1, Brigitt McLaughlin 1, Lyndsey Dugan 6, Martin Fleisher 1, Jerry Lee 7, Laura Leitz 8, Yipeng Wang 9, Dena Marrinucci 2, Mark Landers 3, Ryan Dittamore 2

Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan-Kettering Cancer Center, New York, NY

2 Epic Sciences, Inc., San Diego, CA

Background

Therapies targeting the androgen receptor (AR) and AR signaling such as Abiraterone Acetate (A) and Enzalutamide (E), and Taxane (T) based chemotherapy, prolong life in castration resistant prostate cancer (CRPC). The optimal sequence to administer them to maximize survival for an individual is unknown. Tumor heterogeneity (diversity) has been proposed as a biomarker of treatment resistance. We studied heterogeneity in CTCs on a cell by cell basis to develop predictive biomarkers of sensitivity for use at decision points in management to better sequence available therapies.

Methods for CTC Detection; Phenotypic, Genomic Characterization, and Heterogeneity Score

(223) blood samples from 51 unique patient (pts) were analyzed with the Epic Sciences platform which included digital pathology of 23 discrete phenotypic cell features inclusive of AR and CK expression, and cellular size and shape measures. 223 single CTCs were characterized, data standardized, and clustered into 15 phenotypic subtypes. Individual cell scores were then analyzed for the frequency and heterogeneity (Shannon Index) of CTC subtypes and monitored for clinical endpoints. A subset of CTCs (n=30) were individually sequenced and analyzed for chromy and CNV to assess genomic heterogeneity.

CTC Heterogeneity is Observed in Patient Samples and Increases by Line of Therapy

Patient Line of Therapy

1st Treatment Decision

2nd Treatment Decision

3rd Treatment Decision

AR-T (Enzalutamide)

AR-E and Taxane (Enzalutamide and Docetaxel)

AR-E alone

n=31

n=31

n=31

22% of CTCs were classified as high heterogeneity phenotype, which tended to increase with each subsequent line of therapy (p=0.00182). The hazard ratio and 95% CI for high heterogeneity phenotype patient samples increasing by line of therapy was HR: 6.4 (2.6 to 15.2) (*p=0.00182). This analysis was performed on a subset of CTC samples (n=30) from patients that had at least two CTCs in a patient sample.

High CTC Phenotypic Heterogeneity Predicts Shorter Survival Times on AR Tx but not Taxane Tx

Phenotype: Shannon Index

CTC Subtypes by Patient Sample and Line of Therapy

Sample Burst by CTC Type: Normal, Low, High

Shannon Index

Swarm Plot by Line of Therapy

CTC Heterogeneity is Associated with Genomic Instability

High Heterogeneity Phenotype Patient Samples

High Heterogeneity Phenotype Patient Samples

CTC Phenotype Heterogeneity Correlates with CTC Genomic Heterogeneity: Genotype to Phenotype Subset Analysis

Patient=31 CTCs=741

1 Cell

2 Cells

7 Cells

5 Cells

8 Cells

A sub-cohort of 31 patient samples with CTCs individually sequenced as per case study above. A "clonal" is a distinct CNV profile observed by at least two CTCs in a patient sample.

Conclusions

• Single-CTC phenotypic and genomic characterizations are feasible and can be used to assess tumor heterogeneity in a patient.
• High phenotypic heterogeneity identifies patients in a cohort with:
  • increased risk of death on Abiraterone & Enzalutamide but not taxane chemotherapy
  • increased likelihood to have genomic heterogeneity (multiple clones)
• CTC clustering identifies a CTC subtype with:
  • Resistance to both AR and Taxane therapy
  • Increased genomic instability (high LSI #)
• A non-invasive liquid biopsy can be used to target metastatic cancer can be used to guide treatment selection. Ongoing validation in progress.

Prevalence of a CTC Subtype (Type K) Predicts Poor Outcome on AR Tx and Taxanes

Example Image of Cell Type K

Cell Type K Key Features

• Large Nucleus
• High Nuclear Entropy
• Prominent Nucleolus

CTC Heterogeneity Predicts Better Survival on Taxane over AR Tx in Multivariate Model

• High Heterogeneity is associated with improved survival in all other clinical covariates significant in the model
• Patient samples with high heterogeneity have a shift reduction in the risk of death on Taxane compared to AR Tx.