Glucocorticoid receptor (GR) expression in circulating tumor cells (CTCs) prognosticates poor overall survival (OS) for metastatic castration-resistant prostate cancer (mCRPC) patients (pts) treated with androgen receptor signaling inhibitors (ARSi)

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Methods

We developed an assay as part of the Epic Sciences platform to assess GR protein expression on individual circulating tumor cells (CTCs).

We sought to determine if the presence of CTCs with upregulated GR protein prior to initiation of either Abi or Enza portends more aggressive disease with greater ability to resist AR signaling inhibition, as assayed by tumor cells in circulation.

The cohort was selected based on very high overall survival (OS) for metastatic castration-resistant prostate cancer (mCRPC) patients (pts) treated with androgen receptor signaling inhibitors (ARSi) such as enzalutamide (Enza) and abiraterone acetate (Abi).

4) CTC candidates detected by a multi-parametric digital pathology algorithm

5) Human reader confirmation of CTCs & quantitation of biomarker expression

Combining ARSi such as Enza (38). The presence of any CTCs was compared to the presence of GR(+) CTCs, both prognostic markers as univariates.

Conclusions

• GR upregulation in mCRPC is an alternate mechanism of resistance to androgen receptor signaling inhibitors (ARSi) such as enzalutamide (Enza) and abiraterone acetate (Abi).

• Pre-clinical studies implicate GR as a potential therapeutic target.

• We developed an assay as part of the Epic Sciences platform to assess GR protein expression on individual circulating tumor cells (CTCs).

• We sought to determine if the presence of CTCs with upregulated GR protein prior to initiation of either Abi or Enza represented an aggressive disease subset.

• 54 mCRPC pt blood samples were collected prior to starting Abi (36) or Enza (38).

• The cohort was selected based on very high overall survival (OS) for metastatic castration-resistant prostate cancer (mCRPC) patients (pts) treated with androgen receptor signaling inhibitors (ARSi) such as enzalutamide (Enza) and abiraterone acetate (Abi).

• Blood samples were plated on microscope slides and every nucleated object imaged, with CTCs detected by a combination of cytokeratin (CK) expression, intact nucleus, lack of CD45 (blood lineage) staining, and malignant morphology.

• Schematic of Epic CTC Platform CTC enumeration, morphology, & biomarker workflow:

1) Nucleated cells from blood sample placed onto slides and stained in a 4-BCD immunoperoxidase

2) Slides stained with cytokeratin (CK), CD45, DAPI, GR

3) Slides scanned

• Monoclonal antibody (clone D6H2L) specific to the glucocorticoid receptor (GR) C-terminal domain was evaluated on single cell line cells spiked into healthy donor blood and processed as pt samples.

• LnCaP (GR negative) and DU-145 (GR positive) prostate cancer cell lines were utilized.

• GR positivity was defined as mean fluorescent intensity (MFI) of 77.7, the 95th percentile of LnCaP GR expression.

• Due to co-expression of GR on WBCs, only CK(+) CTCs could be assessed for GR expression.

• GR expression in CTCs By Patient Sample and Line of Therapy

• GR Protein Expression Across Lines of Therapy

• GR+ CK+ CTC(s) Prognosticate Worse Outcome on ARSi Than for Patients Without GR+, CK+ CTC(s)

Univariate Analysis

• The overall survival of pts with GR(+) CTCs (gold curve) prior to ARSi was compared to pts with either only GR(-) CTCs or no CTCs (blue curve).

Multivariate Analysis

• The cohort contains pts with 0 CTCs, only GR(-) CTCs, and patients who have GR(+) CTCs.

• The presence of any CTCs was compared to the presence of GR(+) CTCs, both prognostic markers as univariates.

Survival Analyses:

(Urine pts n = 53, Samples n = 54)

POSITIVE (≥77.7) GR Expression: 1273.03

NEGATIVE (<77.7) GR Expression: 33.00

Presence of (any) CTCs 1.58 (0.31 – 7.90) 0.57

Presence of GR(+) CTCs 4.16 (1.23 – 14.0) 0.02