Abstract# 312805: Circulating Tumor Cells (CTCs) With Small-Cell Like Features are Prevalent in Metastatic Castration Resistant Prostate Cancer (mCRPC) and Show Selective Pharmacodynamic Reductions in Patients Treated With Platinum Therapy but not an ARSi or Taxane

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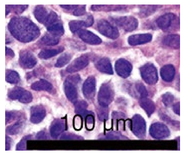
BACKGROUND

- The increasing availability and earlier use of life prolonging drugs targeting the androgen receptor signaling axis (ARSI) has resulted in an increase in the frequency of late stage tumors with “small cell/neuroendocrine like (NESC like or NESCl) features” similar to small-cell lung cancer (SCLC).
- Pathologic criteria to diagnose the prostate cancer “entity” are not definitive.
- Clinical trial eligibility criteria/requirements include a range of clinical, histologic and/or biologic measures but are not consistent, limiting the ability to relate outcomes between studies.
- We hypothesize that an analytically valid assay for a rigorously defined “small-cell CTC” phenotype might serve as a unifying biomarker for the presence of NESC-like tumors in an individual for use in clinical trials.

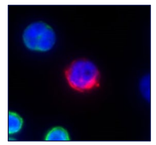
METHODS

Strategy: Apply the Reported Pathologic Defined Criteria for Small-Cell Neuroendocrine Cancer(s) to Circulating Tumor Cells (CTCs) from Prostate Cancer Patients To Detect the Presence of Neuroendocrine/Small-Cell (NESC) Like Disease (ICD-O 8041/3)



H&E stain from SCLC patient tissue biopsy[2].

Single-cell features for small cell carcinoma according to WHO[1,*]:
1. Small cells with scant cytoplasm
2. High nuclear-to-cytoplasmic ratio
3. Absent or inconspicuous nucleoli
4. Salt-and-pepper chromatin



CTC in a liquid biopsy

Hypothesis: The features that defined small-cell carcinoma of the lung can be used to reliable and reproducibly detect NESC like CTCs for use as a non-invasive real-time diagnostic

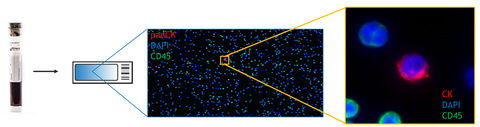
[1] Travis WD, Burke AP, Marx A, Nicholson AG. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press 2015.
[2]Dorantes-Heredia, R., Ruiz-Morales, J.M. & Cano-Garcia, F. Histopathological transformation to small-cell lung carcinoma in non-small-cell lung carcinoma tumors. *Translational Lung Cancer Research* 5, 401-412 (2016).
*Note Same diagnostic code is used for SCLC and prostate small-cell neuroendocrine (ICD-O 8041/3)

Translating Single-Cell WHO Small-Cell Morphologic Criteria to an Image Based CTC Detection Algorithm Using the EPIC Platform

I - High Throughput Enrichment-Free Cell Imaging (EPIC Sciences Platform)

II - CTC Detection & Verification

III - Apply WHO Neuroendocrine / Small-Cell criteria (ICD-O 8041/3)



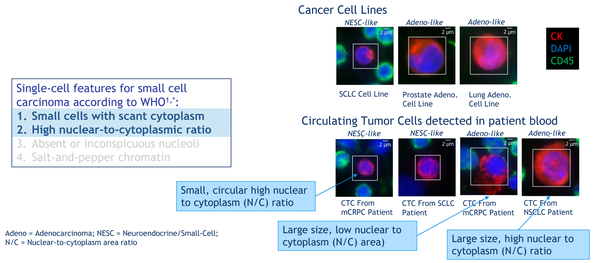
Single-cell Features for Small-Cell Carcinoma According to WHO[1,*]:
1. Small cells with scant cytoplasm
2. High nuclear-to-cytoplasmic ratio
3. Absent or inconspicuous nucleoli
4. Salt-and-pepper chromatin

- *Limits human interpretation bias: reproducible, computationally defined*
- *Compatible with regulatory requirements*

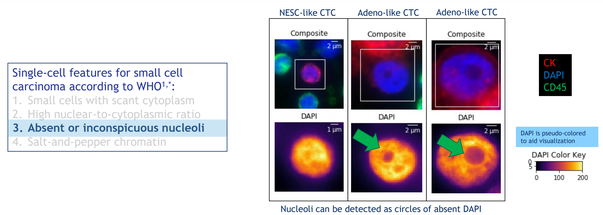
Methods: Nucleated cells from a blood sample are deposited onto glass slides and bio-banked at -80-C. Upon analysis, slides are stained with cytokeratin (CK), CD45, & DAPI , and scanned. CTC candidates are detected by a multi-parametric digital pathology algorithm followed by human reader confirmation of CTCs and quantification of biomarker expression. CTCs are segmented within the DAPI, and AR channels and single cell morphology features are extracted.
References: Werner et al. J Circulating Biomarkers 2015; Scher et al. Cancer Research 2017

Defining Neuroendocrine Small-Cell (NESC) Features in CTCs

Features #1 & 2: Small CTCs With High N/C Ratios Are Observed In SCLC, mCRPC, And Cell Line Controls

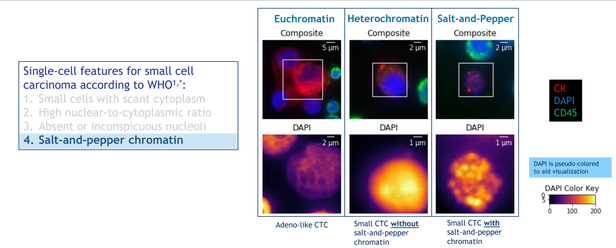


Feature #3: The Presence or Absence of Nucleoli Readily Determined From DAPI Staining That Are Expected to be Absent in Small-Cell Neuroendocrine

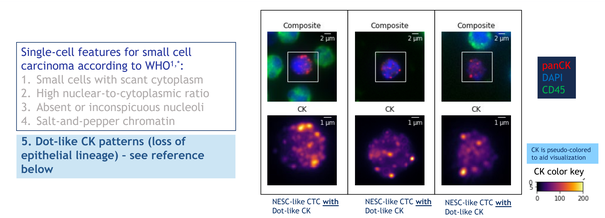


Reference: Pontvianne, F., *et al.* Identification of Nucleolus-Associated Chromatin Domains Reveals a Role for the Nucleolus in 3D Organization of the A. thaliana Genome. *Cell Rep* **16**, 1574–1587 (2016).

Feature #4: Salt-and-Pepper Chromatin are Observed In Small CTCs with High N/C Ratios



Feature #5: (Not Accessible By H/E): Dot-like Cytokeratin Staining Patterns Are Often Observed In Small Cell CTCs

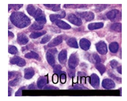


Reference: Thunnissen, E.; et al. The Use of Immunohistochemistry Improves the Diagnosis of Small Cell Lung Cancer and Its Differential Diagnosis. An International Reproducibility Study in a Demanding Set of Cases. Journal of Thoracic Oncology, Volume 12, Issue 2, 334-346 (2017).

Proposed Features to Define Neuroendocrine/Small-Cell (NESC) like CTC phenotypes

Current Single-cell features for small cell carcinoma according to WHO[1,*]:
1. Small cells with scant cytoplasm
2. High nuclear-to-cytoplasmic ratio
3. Absence of inconspicuous nucleoli
4. Salt-and-pepper or granular chromatin

Proposed CTC neuroendocrine/small-cell features:
1. Small Cell Size (6-10X magnification)
2. High N/C ratio (6-10X magnification)
3. Nucleoli Absence (20-40X magnification)
4. Salt & Pepper Chromatin (20-40X magnification)
5. Dot-like CK speckling (20-40X magnification)



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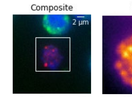


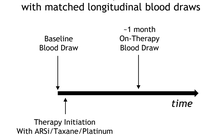
Small-Cell CTC from mCRPC patient blood sample with 5/5 features

Note: This single CTC also has PTEN, P53 copy-loss from low-pass sequencing

[1] Travis WD, Burke AP, Marx A, Nicholson AG. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press 2015.
[2]Dorantes-Heredia, R., Ruiz-Morales, J.M. & Cano-Garcia, F. Histopathological transformation to small-cell lung carcinoma in non-small-cell lung carcinoma tumors. *Translational Lung Cancer Research* 5, 401-412 (2016).
*Note Same diagnostic code is used for SCLC and prostate small-cell neuroendocrine (ICD-O 8041/3)

Assessing Longitudinal Changes in Neuroendocrine/Small-Cell - Like in CTCs From Metastatic Castration Resistant Prostate Cancer (mCRPC) patients Treated With ARSIs, Taxanes, and Platinum

Cohort: 233 mCRPC patients at MSK-CC with matched longitudinal blood draws



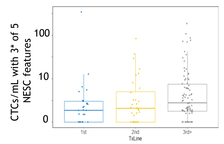
Patient Characteristics	
Total N	N=233
1st Line	54 (23.2%)
2nd Line	63 (27.0%)
3rd Line	57 (24.5%)
4th+ Line	58 (24.9%)
Note: 1 sample missing treatment line data	
Therapies Started	
Abiraterone or Enzalutamide	111 (47.6%)
Cisplatin or Carboplatin	33 (14.2%)
Docetaxel or Cabazitaxel	89 (38.2%)

All CTCs assessed for Size, N/C, nucleoli

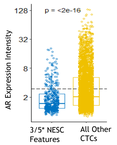
Hypothesis: CTCs with Neuroendocrine/Small-Cell features will only be sensitive to platinum chemotherapy and be resistant to targeted therapy

Neuroendocrine/Small Cell – Like CTC Features Are Prevalent in mCRPC Patients, Have Lower AR Expression Levels, and Decrease After Treatment with Platinum Based Chemotherapy in Preliminary Analyses

NESC-like CTCs are more frequent in the later lines of therapy



NESC-like CTCs have lower AR expression



Post-Therapy Changes in CTC Populations Characterized with **3* of the 5 Defining NESC-Like Features** Differ By Drug Class

Change in Small, circular CTCs with high N/C ratios in Patients With a Post-Treatment Blood Draw by Therapy Class*

	N (%) Decline‡	N (%) Increase‡	Bonferroni adjusted p-value for decrease‡‡
ARSi	29 (57%)	22 (43%)	0.87
Taxane	28 (60%)	19 (40%)	0.276
Platinum	**14 (82%)**	**3 (18%)**	**0.039***

‡Proportion test, % Decline, ARSi vs Platinum, p-value = 0.055
‡‡BL Pos/mL vs OnTx Pos/mL, paired Wilcoxon one-sided, alternative: BL > OnTx, Bonferroni adjusted p-value
Patients with zero CTCs at baseline and on-therapy were excluded

*All CTCs assessed for Size, N/C ratio, & nucleoli absence; Nuclear and CK texture analysis are forthcoming

CONCLUSIONS

- **Single Circulating Tumor Cells (CTCs) that are consistent with the WHO pathologic criteria for a neuroendocrine / small-cell diagnosis can be detected in patients with mCRPC.**
- **The frequency of CTCs with small-cell features were higher in patients who had failed one or more therapies.**
- **Reductions in the number of NESC-like CTCs subtypes characterized by 3 of the 5 features after treatment vary by drug class and suggest the higher sensitivity to a platinum-based regimen.**
- **A rapid, reproducible, and non-invasive method for identifying neuroendocrine / small-cell disease could serve as a unifying biomarker in clinical trials**

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