Spatial Landscape of the Immune Microenvironment in Metastatic Prostate Cancer using GeoMx™ Digital Spatial Profiler

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Summary

Introduction Improved understanding of the biological mechanisms involved in disease progression is paramount for developing new clinical strategies in advanced prostate cancer (PC). The emergence of distinct subtypes of castrate resistant prostate cancer (CRPC) represents the adaption of cancer cells to altered microenvironments and highlights the need for further understanding and characterization of tumor heterogeneity and the drivers of PC growth and resistance to therapy.

Methods A tissue microarray (TMA) comprising 3 tumor cores was constructed from 2 CRPC metastases obtained from 28 patients and included both soft tissue and decalcified bone metastases. The TMA was phenotypically diverse comprising 4 subtypes of CRPC: androgen receptor (AR) active neuroendocrine (NE) negative tumors (AR+/NE-), neuroendocrine tumors (AR-/NE+), amphicrine or mixed tumors (AR+/NE+) and double-negative tumors (AR-/NE-), as defined by RNA-seq and immunohistochemistry. An immunophenotyping and oncology RNA panel for the NanoString GeoMx™ Digital Spatial Profiler (DSP) was used to determine cell phenotypes within and in proximity to tumor margins. We further profiled 27 metastatic CRPC full faced sections from nine patients (3 mets/patient) belonging to multiple genomic subtypes and three phenotypes.

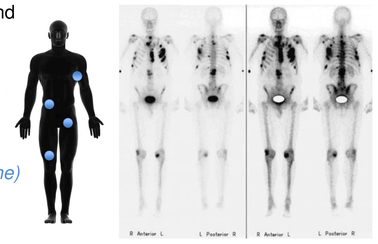
Results Spatial profiling of the TMA correlated strongly with bulk RNA-seq expression across AR, NE and cell cycle progression signature scores. Further, averaged tumor cores per patient clustered strongly within their assigned subtype when assessed by DSP. DSP identified intra-patient heterogeneity between assigned subtypes, while intra-patient heterogeneity within subtypes was also observed. Within multiple samples, differential expression of NE associated genes was identified between three tumor cores from the same tissue site. De-calcified bone samples had a lower fold count than soft tissue analyzed from the same patient but still classified tumor and cellular phenotypes. Notably, a strong intra-patient correlation between soft tissue and bone was observed.

Conclusion GeoMx DSP can provide critical insight into potential genetic drivers of the subtypes of CRPC with minimal sample input and could potentially inform treatment strategies with the overall goal of improving clinical options for men with CRPC.

Introduction

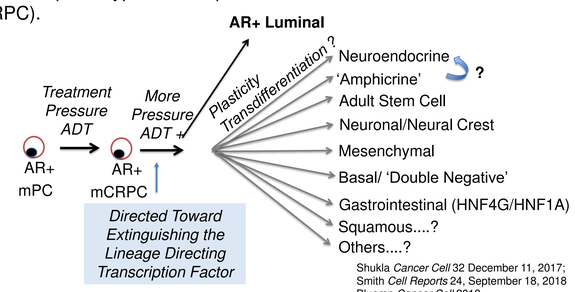
Prostate Carcinoma[2020]: How to Reduce Mortality ?

- Metastatic Prostate Cancer is an incurable and lethal disease, with 30,000 deaths this year
- Better therapies are urgently needed for metastasis
  - ✓ *Identify and understand the drivers*
  - ✓ *Apply specific therapies (Precision Medicine)*
  - ✓ *Anticipate and understand resistance*
  - ✓ *Co-target resistance mechanisms*



Metastatic Castration-Resistant Prostate Cancer (mCRPC) is a Heterogeneous Disease

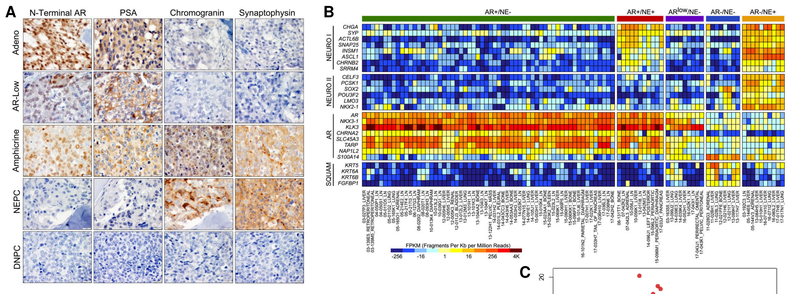
- Widespread and long-term use of first and second line androgen deprivation therapy is changing the molecular and phenotypic landscapes of metastatic castration-resistant prostate cancer (mCRPC).



Directed Toward Extinguishing the Lineage Directing Transcription Factor

Shukla *Cancer Cell* 32 December 11, 2017;
Smith *Cell Reports* 24, September 18, 2018
Bluemn *Cancer Cell* 2018
Aggarwal *J Clin Oncol* 36:2018

- Observations made through University of Washington Rapid Autopsy Program (1998-2018; over 150 rapid autopsies performed) supports a shift in mCRPC towards androgen receptor (AR)-null phenotypes
- Our recent molecular profiling study revealed five mCRPC phenotypic subtypes based on expression of androgen receptor (AR) or neuroendocrine (NE) genes.



(A) Representative immunohistochemical stains for AR, PSA, synaptophysin and chromogranin used to classify metastases from rapid autopsy patients as adenocarcinoma (Adeno), AR-Low, amphicrine, neuroendocrine (NEPC), or double-negative (DNPC). **(B)** RNAseq of prostate epithelial and neuroendocrine-associated genes in patient specimens. Mean-centered ratios of genes are colored according to scale. Results are expressed as mean-centered $\log_2$ FPKM. **(C)** Multidimensional scaling plots of patient CRPC metastases using RNAseq expression profiles of genes governed by AR and REST activity or associated with adenocarcinoma, NEPC or squamous phenotypes.

Labrecque…Nelson, Morrissey *J Clin Investig* 129(10):2019

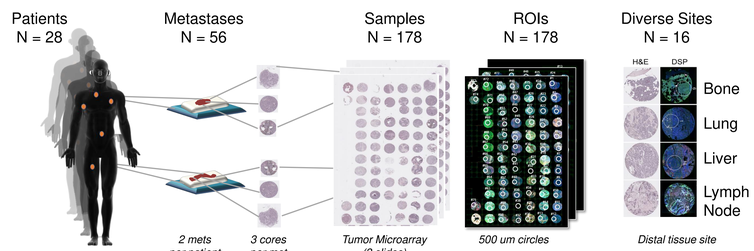
Major Clinical and Research Questions

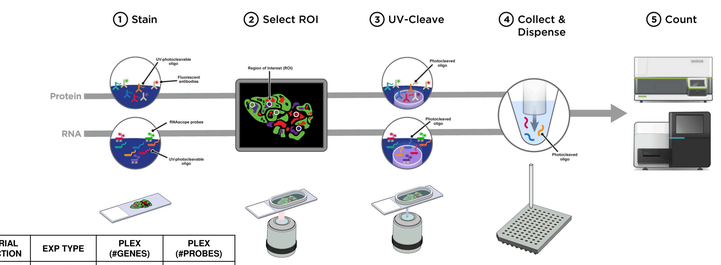
- ✓ *What is the extent of inter- and intra-tumor heterogeneity in metastatic disease ?*
  Could it impact treatment resistance ?
- ✓ *Why is prostate cancer refractory to immune-based therapeutics (ICB) ?*
  Are there other immune targets ?

Results

Molecular Profiling of mCRPC Samples using GeoMx Digital Spatial Profiler (DSP)

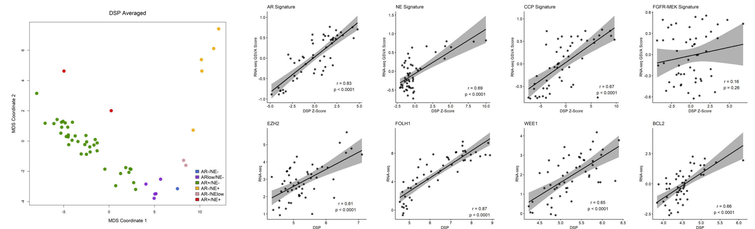
Experimental Design 1: Multi-analyte Analysis using Tumor Microarrays



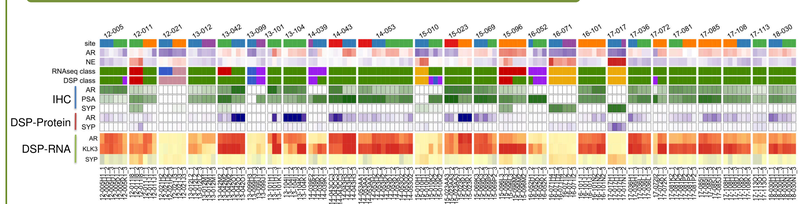


SERIAL SECTION	EXP TYPE	PLEX (#GENES)	PLEX (#PROBES)
1	H & E	-	-
2	DSP RNA	2106	18,121
3	DSP Protein	60 ABs	-

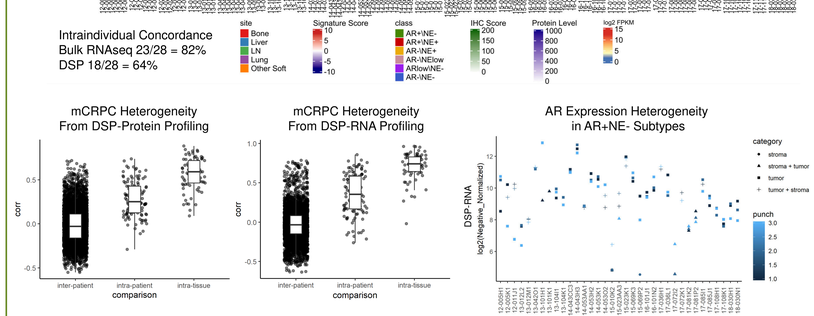
Question 1: Does DSP-RNA Classify mCRPC Phenotypes ?



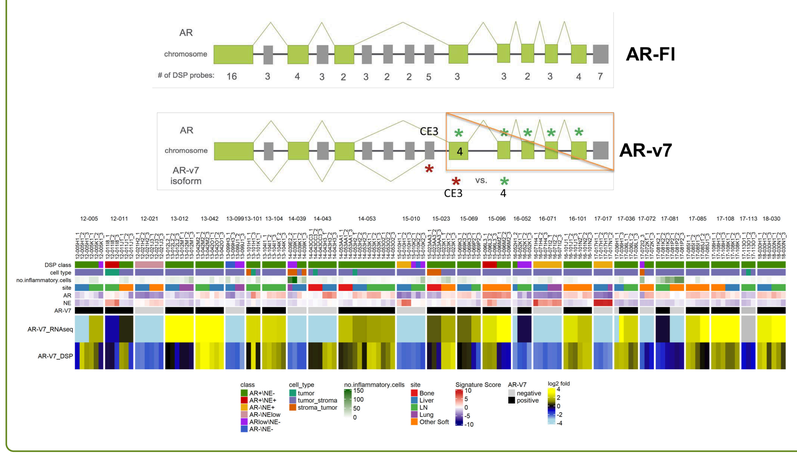
Question 2: What is the Extent of Heterogeneity in mCRPC ?



Intraindividual Concordance
Bulk RNAseq 23/28 = 82%
DSP 18/28 = 64%

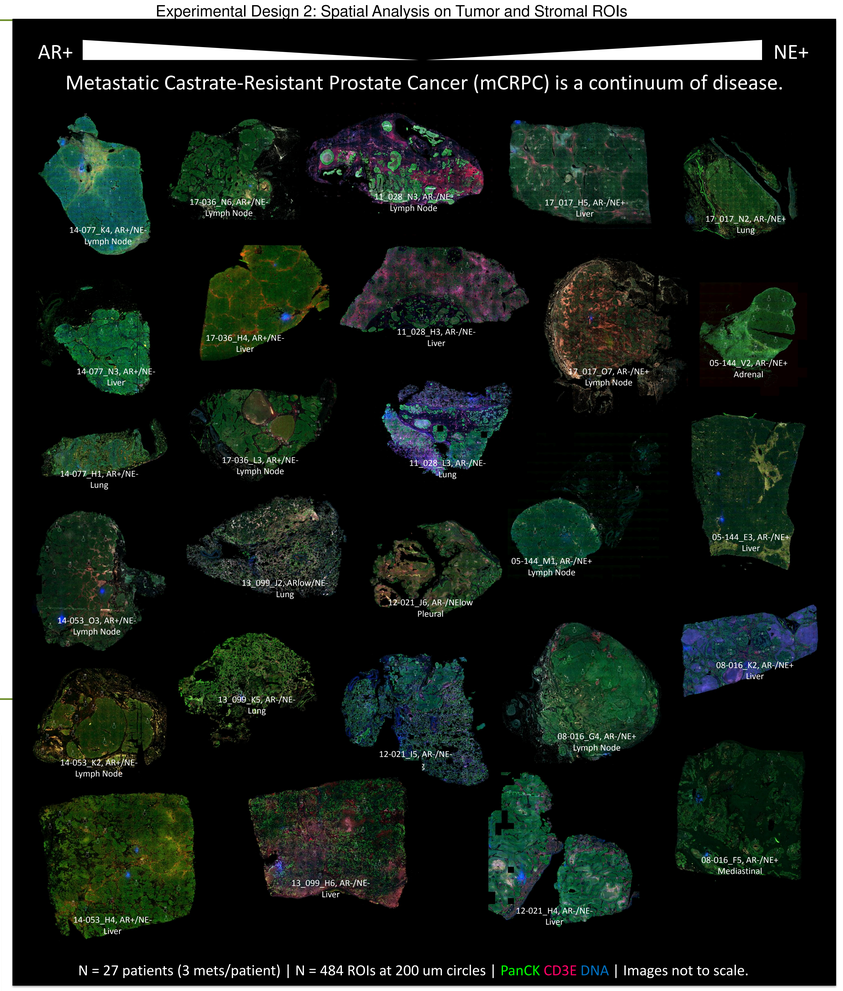


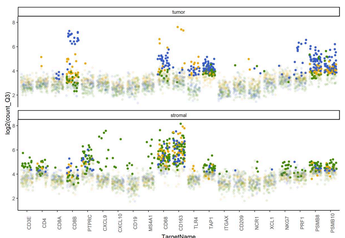
GeoMx DSP Detects Inter- and Intra-patient Heterogeneity in ARv7 Splice Variant



Question 3: What is the Cellular and Molecular Immune Composition of mCRPC ?

Experimental Design 2: Spatial Analysis on Tumor and Stromal ROIs





Tumor ROIs

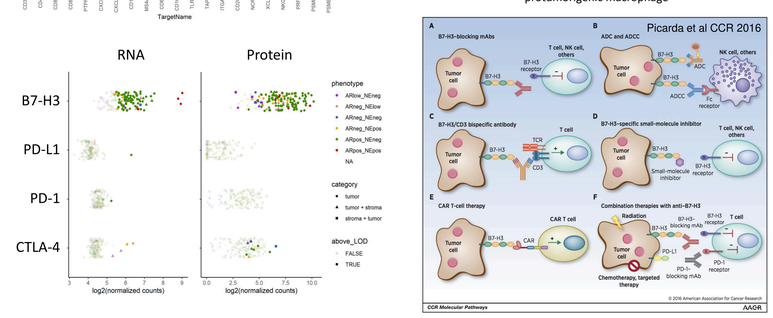
AR-/NE- and AR-/NE+ tumors have a strong intratumoral infiltrate

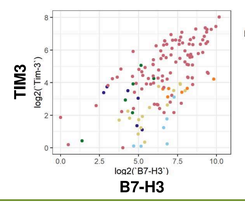
- TAP1 is involved in antigen processing, and may be associated with attraction of T cells within tumor region
- AR-/NE-have high CD8 and PRF1 associated with cytotoxic T cell

AR+/NE- tumors are 'immune cold'

Stromal ROIs

- CD68+ (monocyte / macrophage) are the predominant immune cell population in the TME
- High levels of peritumoral CD163+ cells – marker of protumorigenic macrophage





B7-H3/CD276

- CD28 family member
- T cell inhibitory function
- Expressed on a variety of tumor types
- Associated with poor clinical outcomes
- mAbs toward B7-H3 developed
  - ✓ ADCC
  - ✓ Bispecific
  - ✓ Blocking
  - ✓ CAR T cells

Conclusions

- ✓ CRPC comprises many 'subtypes' with therapeutic implications.
- ✓ DSP-based profiling
  - High correlation with RNA-seq methods – defines PC Subtypes
  - Identifies intra-tumoral heterogeneity
  - Enables detection of splice isoform
  - FFPE source is a major advantage
  - DSP-protein correlates with traditional IHC – high multiplexing
- ✓ As determined by DSP – subtypes can co-exist across and within metastatic tumors from the same individual
- ✓ Paucity of intra- tumoral immune components in metastatic PC
- ✓ Limited PD1 and PD-L1 expression explains lack of treatment efficacy
- ✓ New Immune Checkpoint Blockade targets: B7:H3 with phenotype associations

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