Neuroendocrine Prostate Cancer (NEPC): Are We Selecting For It With Our Current Androgen Annihilation?

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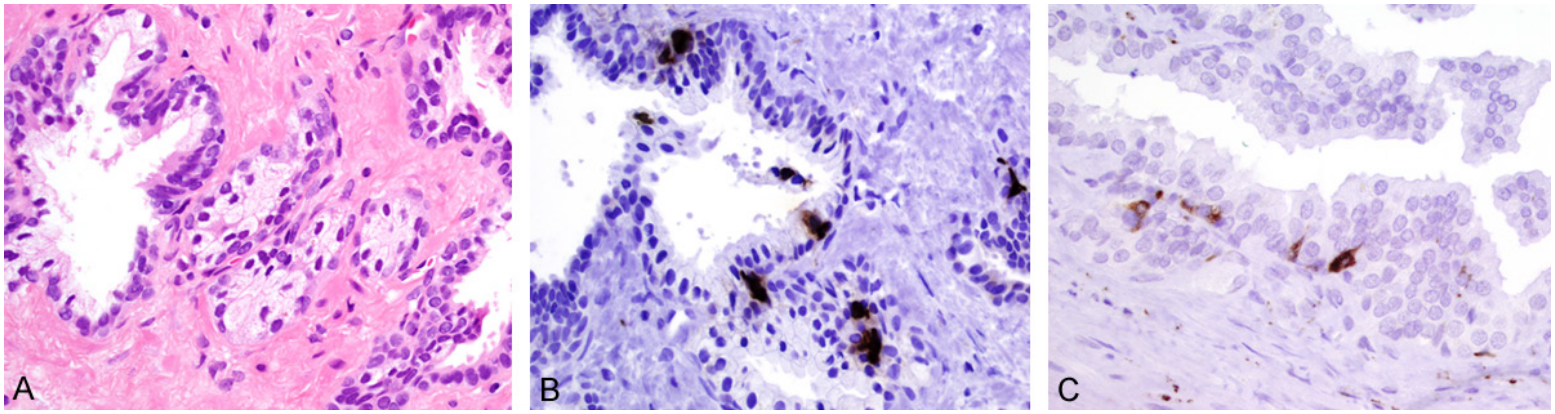
CURRENT OPINION

Androgen annihilation as a new therapeutic paradigm in advanced prostate cancer

Kyle O. Rove and E. David Crawford

- **Cardiovascular disease**
- **Emergence of NEPC**

Neuroendocrine cells are part of normal, benign prostate cells



H&E **CgA** **Synaptophysin**

• NEC are typically situated in the basal cell compartment with dendritic cell processes projecting into the layer of luminal.
•Need for specific staining to identify.
•Secrete trophic neuropeptide (bombesin, calcitonin, serotonin, parathyroid like hormone) and growth factors (VEGF).
•Terminally differentiated cells (no proliferative activity, express anti-apoptotic factors).

Am J Clin Exp Urol 2014;2(4):273-285

Primary ("*de novo*") NEPC

- Very rare (<1%)
- SCCP, large cell NE carcinoma, carcinoid
- frequent visceral and bulky soft-tissue metastases and limited duration of response to both hormonal therapy and cytotoxic chemotherapy.
- Low serum prostate-specific antigen (PSA) level and high serum levels of NE markers (CgA)
- Treatment involves cisplatin or carboplatin in combination with taxanes

NE differentiation in hormone naïve PC

- 5-10% of prostatic adeno-carcinoma contain clusters/aggregates of “NE like” malignant cells (focal NE differentiation).

- Genetic characterization of these cells suggest their linkage to the neighboring adeno-carcinoma cells.

- Unclear prognostic significance.

Secondary development of NEPC

- Common, estimated to represent up to 25% of lethal prostate cancer.
- Trans-differentiation of adeno-carcinoma cells (epithelial plasticity d/t selective pressure?, common progenitor?).
- Resistant to ADT
- May promote Adeno-carcinoma cell tumorginicity through paracrine non AR mediated pathways

Terry and Beltran 2014

NE differentiation is very common in metastatic sites

- Evaluation of an archival set of metastatic site biopsies (MSB) to determine NED expression patterns
- 237 MSB from 187 pts. bone (102), lung (40), liver (40), lymph node (20), bladder (14), soft tissue (11), brain (4), others (4).
- IHC for chromogranin-A and synaptophysin.
- All tumors were adenocarcinomas or poorly differentiated carcinomas
- No small cell carcinoma found, BUT, **50% showed positive NED.**
- NED expression was positive in 41% bone sites, compared to 53% of non-bone sites
- **NED expression was observed in 44% of hormone sensitive cases and 56% of CRPC cases.**

Jimenez et al ASCO meeting 2014

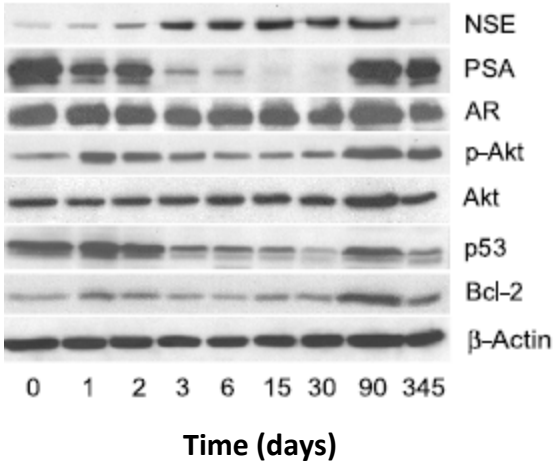
CTCs from patients with NEPC identified in a subset (10.7%)of CRPC patients.

Beltran et al, Clinic Cancer Res 2015

Trans-differentiation from epithelial-like phenotype to a NE-like phenotype as a consequence of treatment induced-selective pressure?

Modeling *in-vitro*

- LNCaP cells
- Androgen depletion induce NE-differentiation
- Restoring androgens suppress NE-differentiation



- Acquisition of an NE phenotype by PCa cells can be induced by chronic exposure to docetaxel

Terry et al, Neoplasia 2013

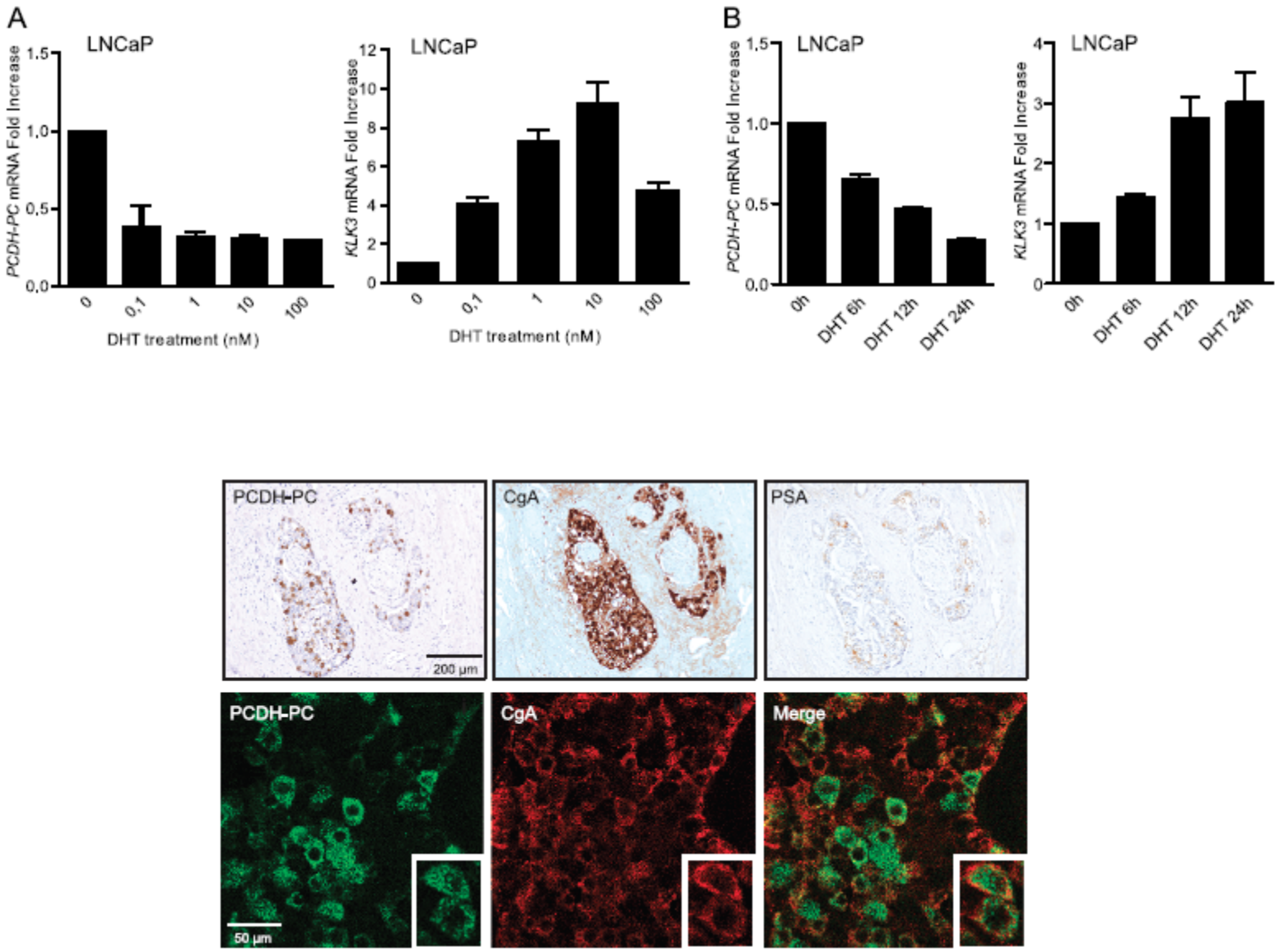
The trans differentiation process from epithelial to neuroendocrine tumor phenotype can be considered a consequence of the selective pressure (ADT)

- NEC lack the AR
- NEC are deficient in cell regulators (P53, RB1)
- NEC over-express cell cycle genes (ex. cyclin D1, AURA Kinase A- **AURKA**)
- Androgen receptors splice variants are associated with up regulation of NE genes (AGR2, **AURKA**, SSTR2)-***Ferrari ASCO 2014 meeting***

Over-expression of *protocadherin-PC* (*PCDH-PC* or *PCDH11Y*) can drive NE trans-differentiation

- **ADT upregulates PCDH-PC**
- PCDH-PC is an anti-apoptotic gene.
- Encodes on the Y-chromosome (Yp11.2)
- PCDH-PC expression reflects early-onset adaptive mechanism following ADT
- PCDH-PC over-expression induces NE phenotype in PC cells and promotes their survival under diverse stress conditions.

Terry et al, Neoplasia 2013



Terry et al, Neoplasia 2013

Is the plasticity induced by the selective pressure reversible?

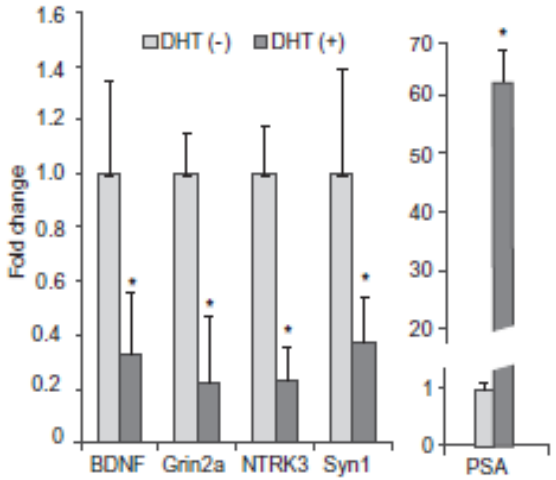
- In patients with *EGFR*-mutant non–small-cell lung cancer who develop small-cell features as a mechanism of resistance to EGFR inhibition, the discontinuation of the EGFR inhibitor results in reversal of the small-cell phenotype.
- It is not known whether clinically such plasticity exists in small-cell/neuroendocrine prostate cancer.

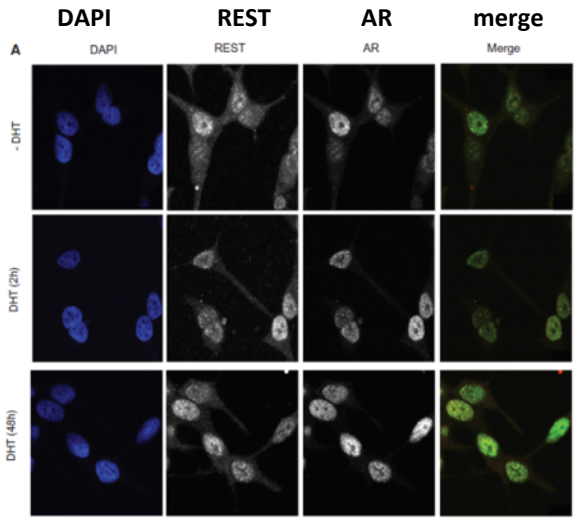
Sequist LV et al. Sci Transl Med. 2011.

Molecular pathways that are involved in NE differentiation of prostate cancer

- RB loss
- MYC over-expression
- PCDH-PC upregulation
- AURKA over-expression
- FoxA2/HIF-1a complex
- Down-regulation of RE1-silencer factor (REST)

Andrgens inhibits REST expression which regulates NE differentiation in LNCaP cells



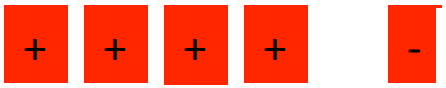


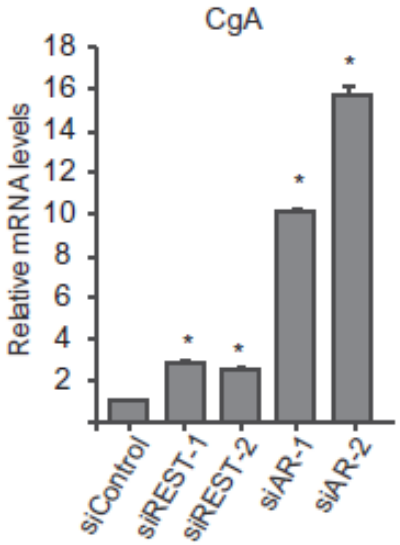
-DHT

+DHT (24hr)

+DHT (48hr)

REST target genes

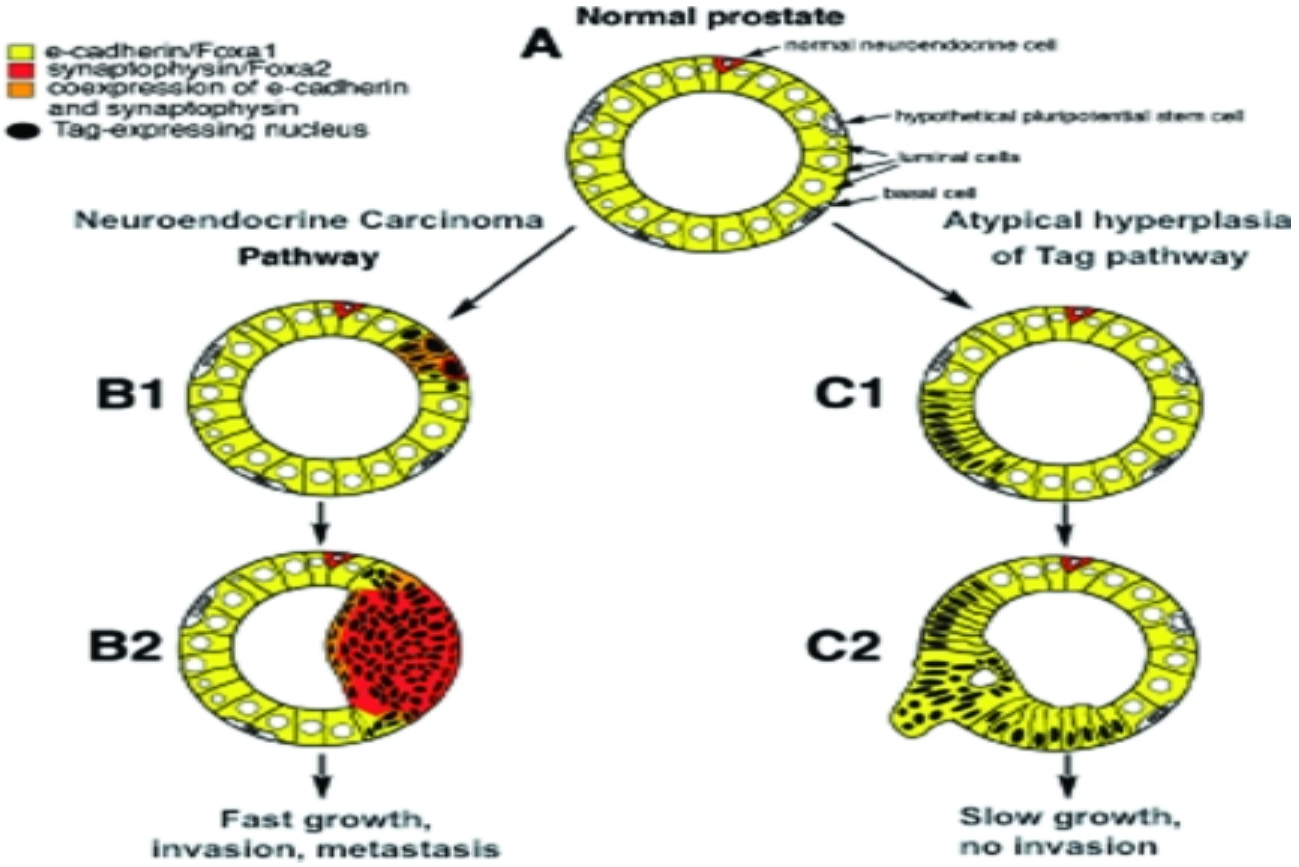




Svensson et al Nucleic Acids Res. 2014

A common progenitor cell?

Experimentally, NEC are not arising from pre-existing committed Epithelial cells but probably develop from bipotential stem/progenitor cells



FVB-TRAMP mice have worse survival than B6-TRAMP mice because of rapid development and progression of NEC-PC arising from bi-potential stem cells that express epithelial (E- cadherin) and NE (synaptophysin) markers and FOX1/FOX2 transcription factors.

Chiaverotti et al The American Journal of Pathology 2008

ERG gene rearrangements are common in prostatic small cell carcinomas

- The occurrence of ERG gene rearrangements was examined by fluorescence in situ hybridization in prostatic, bladder and lung small cell carcinomas.
- Presence of *ERG* rearrangements was found in nearly half of the prostatic small cell carcinomas is a similar rate of rearrangement to that found in prostatic acinar carcinomas.
- No cases of bladder or lung small cell carcinomas showed *ERG* rearrangement.
- A high concordance rate of *ERG* rearrangement between the small cell and acinar components in a given patient was found (83%).

- **These findings support a common origin for acinar prostatic adenocarcinoma and small cell carcinoma of the prostate**

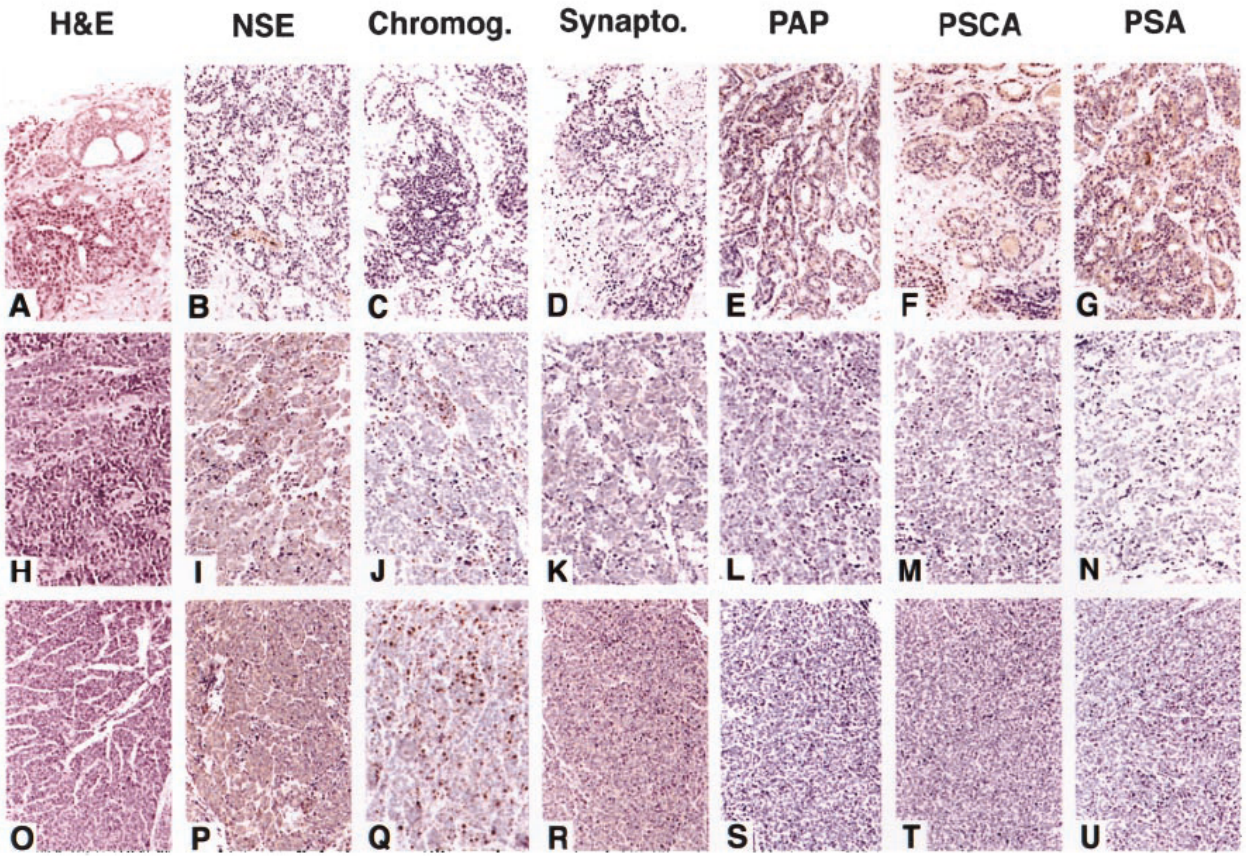
Lotan et al, Mod Pathol 2011

[CANCER RESEARCH 60, 6563–6567, December 1, 2000]

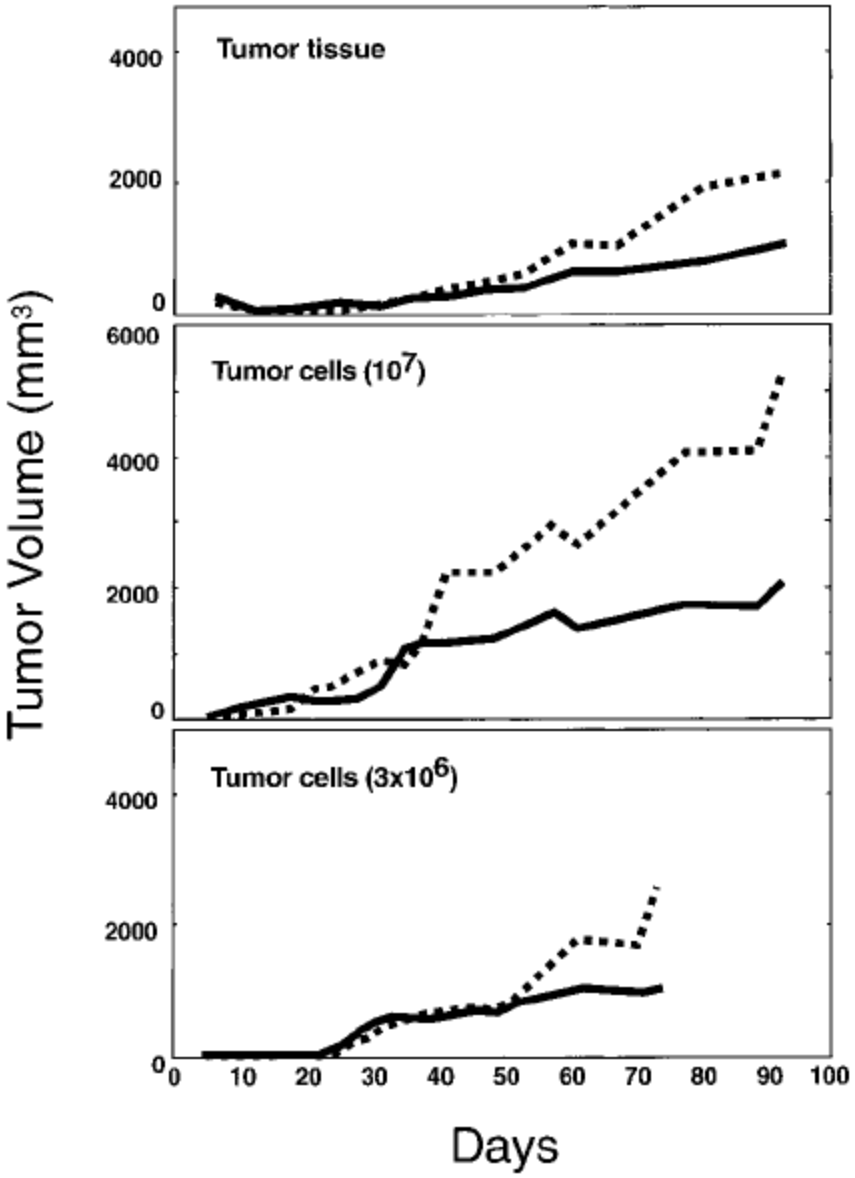
Advances in Brief

WISH-PC2: A Unique Xenograft Model of Human Prostatic Small Cell Carcinoma[1]

Jehonathan H. Pinthus, Tova Waks, Daniel G. Schindler, Alon Harmelin, Jonathan W. Said, Arie Belldegrun, Jacob Ramon, and Zelig Eshhar[2]



Androgen responsive growth of pure SSCP is related to off target growth effects of testosterone



- in the first generation, 20% of the mice into which the tumor pieces were implanted had elevated serum PSA levels

- The WISH-PC2 xenograft grows relatively rapidly and with a high take rate (90–100% of the animals).

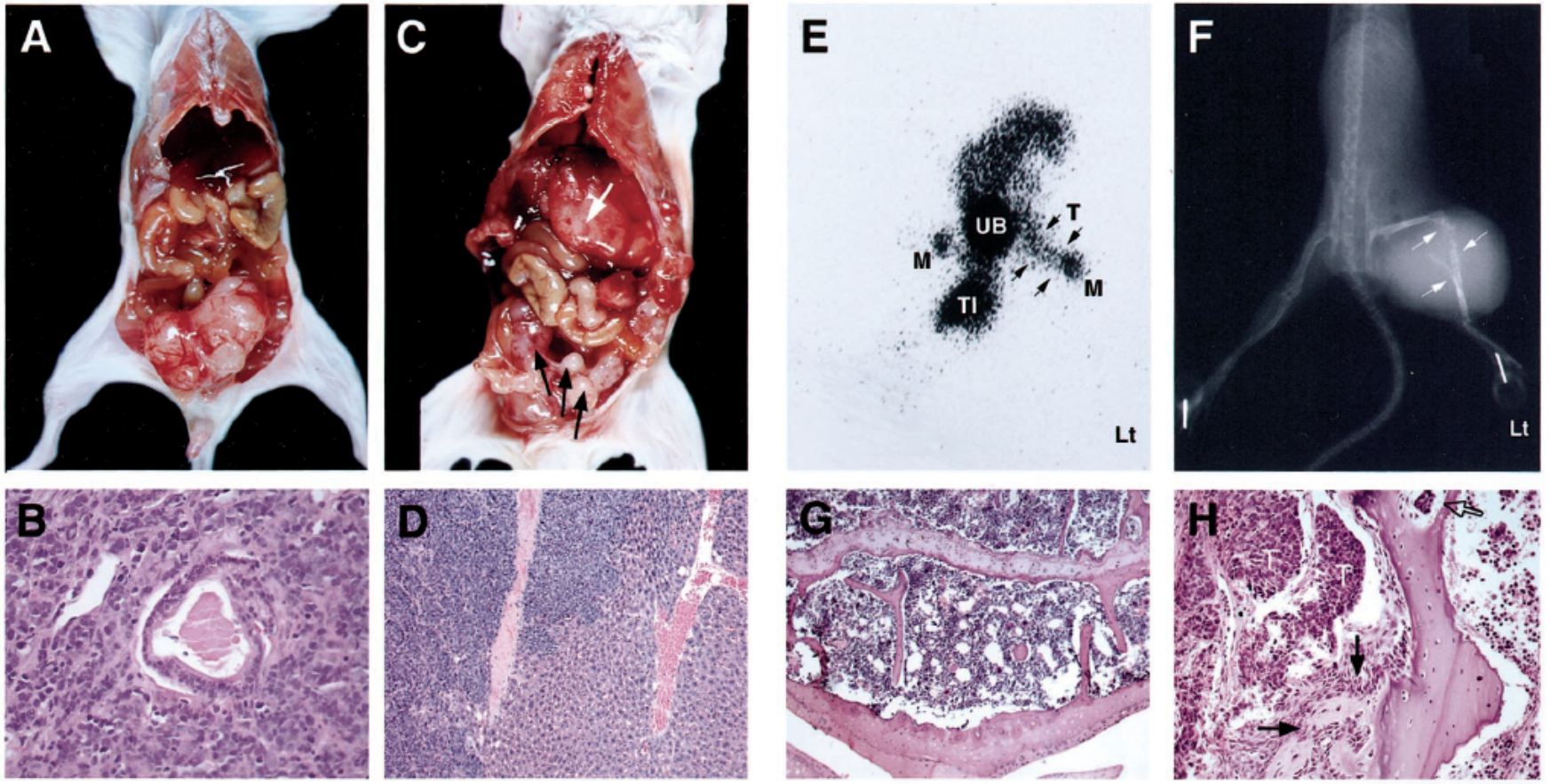
- Androgens enhance the growth of the AR-negative xenograft, probably via an indirect effect on the surrounding stroma

Table 1 *Phenotypic features of WISH-PC2*

Feature	Expression
DNA ploidy	Aneuploid
Proliferative Index (Ki-67)[a]	High
Bcl-2[a]	Positive
Mutated p53[a]	Positive
MDR1 gene product[a]	Negative
PSA[a,b]	Negative
PSCA[a,b]	Negative
PSMA[b]	Negative
PAP[a]	Negative
AR[a,b]	Negative
STEAP[b]	Positive
PCTA-1/galactin-8[b,c]	Positive
Cytokeratin 8[b]	Positive
Cytokeratin 18[b]	Positive
Chromogranin A[a,d]	Positive
NSE[a]	Positive
Synaptophysin[a]	Positive
Her-2/neu[e]	Positive
Her-3/neu[e]	Positive
Her-4/neu[e]	Positive
MHC class-I[e]	Positive

[a] Determined by immunohistochemistry.
[b] Determined by RT-PCR.
[c] Determined by Western blot analysis
[d] Determined by ELISA of murine host plasma.
[e] Determined by FACS analysis.

Pinthus et al. Cancer Res 2000



Pinthus et al. Cancer Res 2000

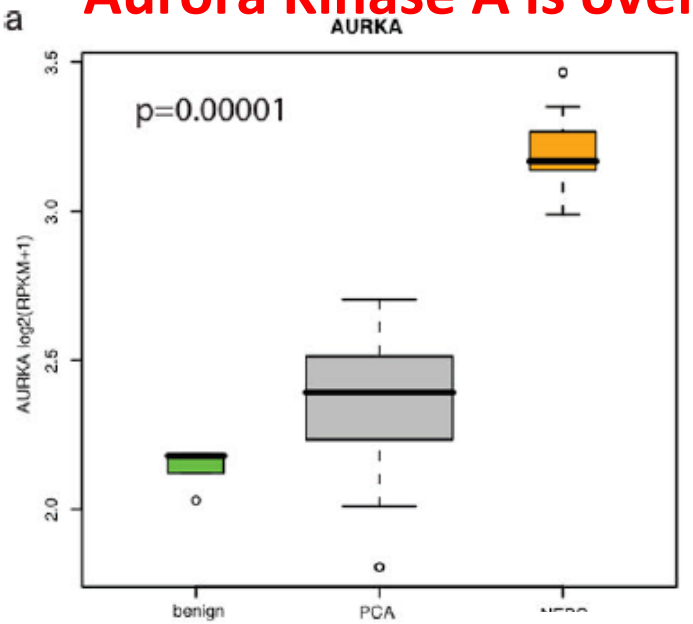
NE cells can function as endocrine-paracrine cells of the prostate

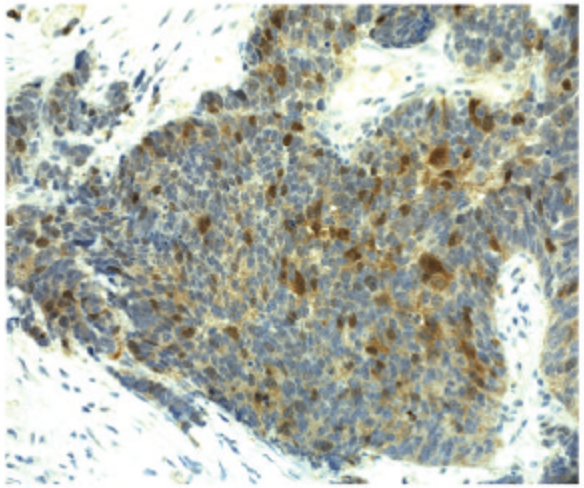
- A suggested role as intraepithelial regulatory cells displaying hybrid epithelial, neural, and endocrine characteristics.

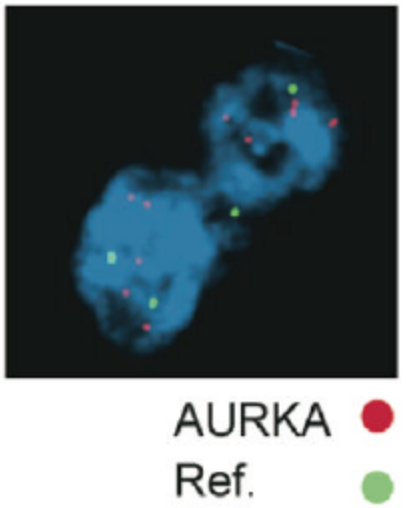
- secreting alternative growth factors such as bombesin, serotonin, somatostatin, calcitonin, and parathyroid hormone-related protein.

- SCCP is composed of an enriched population of androgen-independent cells whose growth is sustained through alternate paracrine and autocrine pathways

Aurora Kinase A is over-expressed (gene amplification) in NEPC

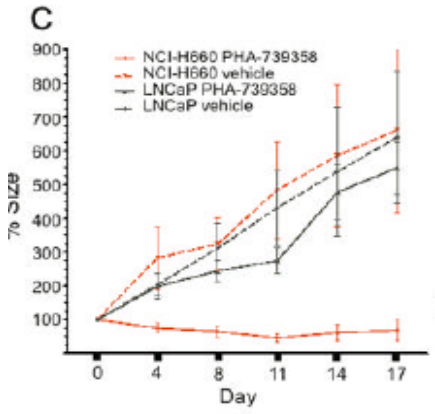


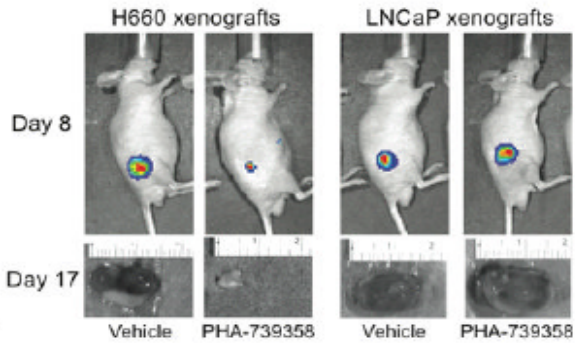


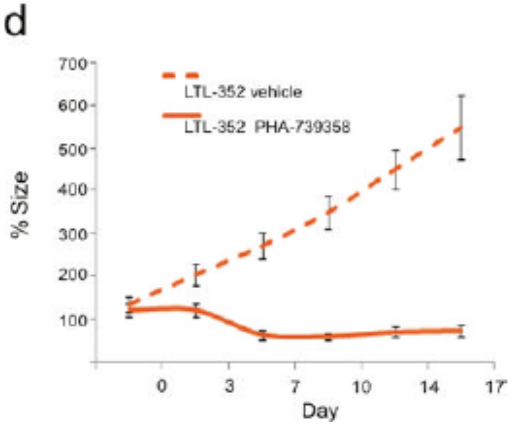


	Total	Aurora A IHC	AURKA Amplification
Benign	22	0	0
PCA	117	12%	6%
NEPC	29	76%	38%

Inhibition of AURKA (PHA-730358) suppress the growth of NEPC







Beltran et al Cancer Discov 2011

Aurora Kinase A Inhibitor MLN8237

Treatment

- Orally administered Aurora kinase A inhibitor.
- 50 mg twice daily for 7 days repeated every 21 days.
- Multi-institutional single-arm, open-label Phase 2 trial in patients with metastatic castrate resistant and NEPC (SCPC, adenocarcinoma plus > 50% immunohistochemical staining for NE markers. Response and progression (primary end point) are evaluated by CT/MRI scan and bone scan after every 3 cycles

Prevention?

•AURKA amplification in primary adenocarcinoma of the prostate predicts for late stage development of NEPC in CRPC patients

(Terry and Beltran 2014)

Take home massages (cognitive doggy bag)

- NEPC develops from adeno-carcinoma of the prostate
- This process is a result selective pressure (ADT and cytotoxic agents) and involves specific molecular pathways (PCDH-PC, AURAKA, REST)
- An increase incidence of NEPC in the current era of novel ADT agents is suspected.
- NEPC cells secret alternative growth factors such and thus can promote AR independent growth.
- Potential for targeted therapy (Aurora kinase A inhibitors)