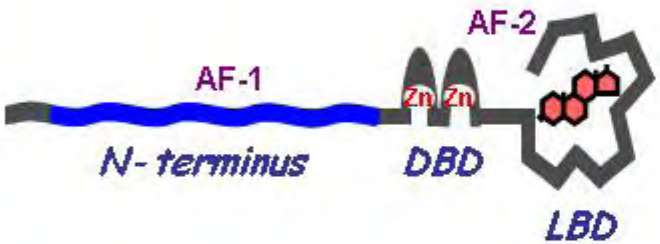
Androgen Receptor Variation and Prostate Cancer in Humanized AR Mice

- Q tract polymorphisms associated with cancer risk
  ("Length Matters")

- AR variations associated with tumor progression

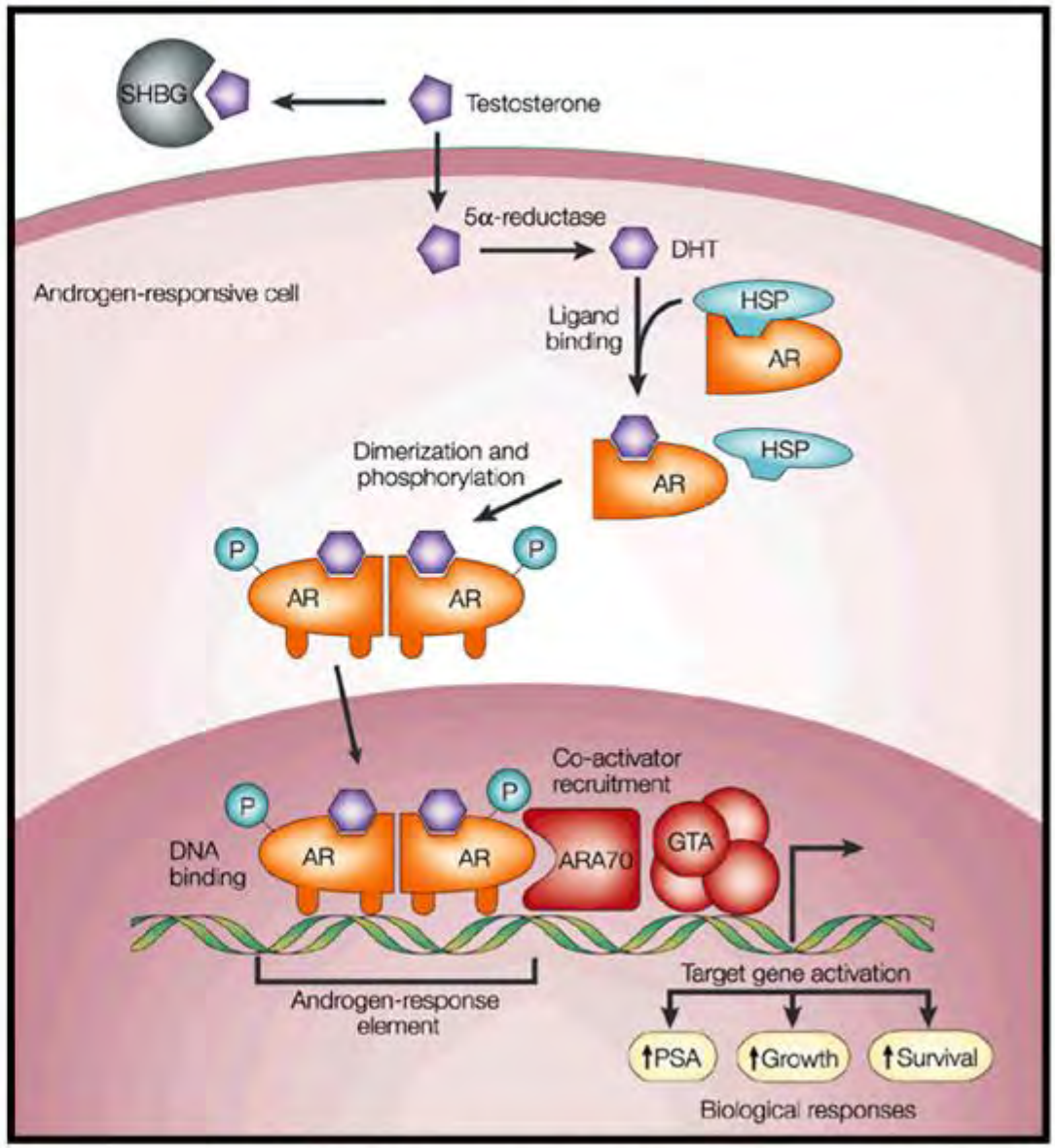
Prostate Cancer is an Androgen Dependent Disease

- AR is a ligand-activated transcription factor central to prostate health & disease



- In prostate cancer androgen acts via AR to activate oncogenes
  (*TMPRSS2:ETS fusions?*)

- This initial androgen dependence allows PCa to respond to anti-A therapy
  (which ultimately fails…)



(Feldman and Feldman, Nat Rev Cancer 2001)

Variation in AR and Risk of Prostate Cancer

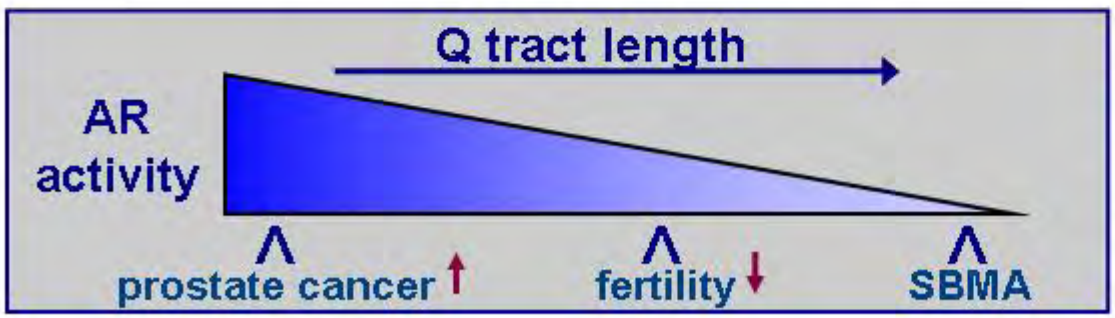
AR Q tract length is polymorphic

- CAG trinucleotide repeat - glutamine (Q)
- 9 - 37 Qs in "normal" population
- Expansion causes spinobulbar muscular atrophy (SBMA, Kennedy)
- Shorter Q tract AR is more active *in vitro* :

  increased mRNA & protein stability

  increased N/C interaction

  greater association with RAN/ARA24



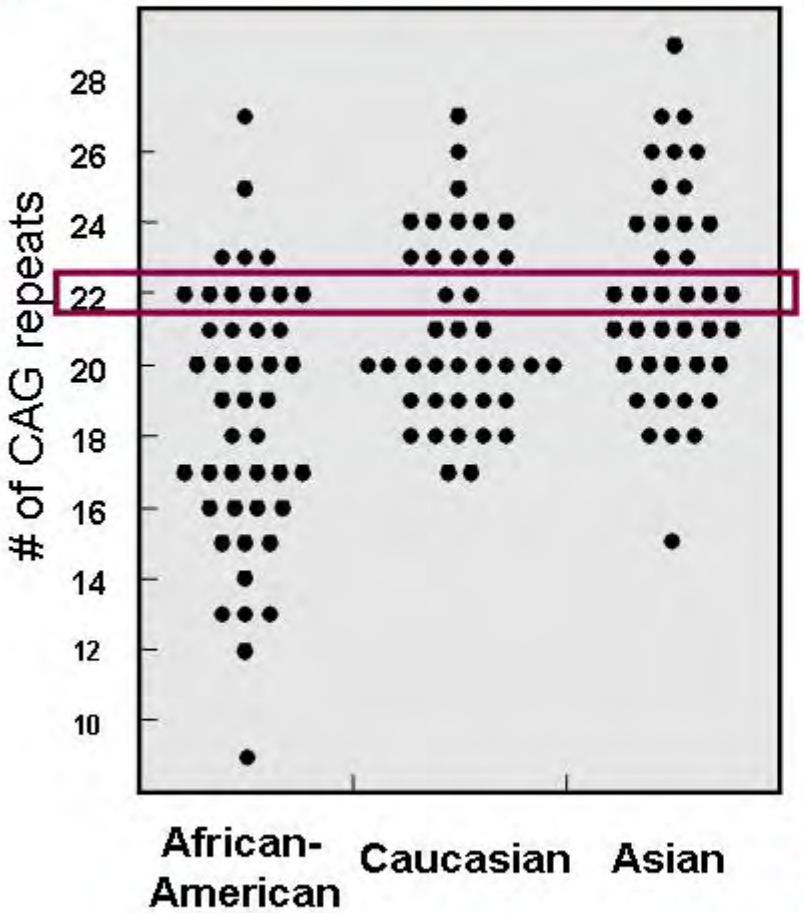
(AR is X-linked - single copy in males so effect of variation is physiologically evident)

? risk by population correlates inversely with Q tract length

AR's CAG Repeat and Prostate Cancer Risk

(Irvine et al., 1995; Ingles et al., 1997)

Population distribution of AR-CAG repeat



< 22 rpts **75%** **62%** **49%**

P = 0.046

AR-CAG and PCa Risk

Subjects	Odds ratio (95%CI) AR-CAG 'short'
Controls (n=169)	1.00
PCa cases (n=57)	2.00 (1.07-3.75)
Localized (n=31)	1.74 (0.78-3.87)
Advanced (n=26)	2.36 (1.02-5.49)

Greater risk associated with stronger AR?

Then the deluge... controversy re cancer link, association with any trait

What confounding factors underlie controversy ?

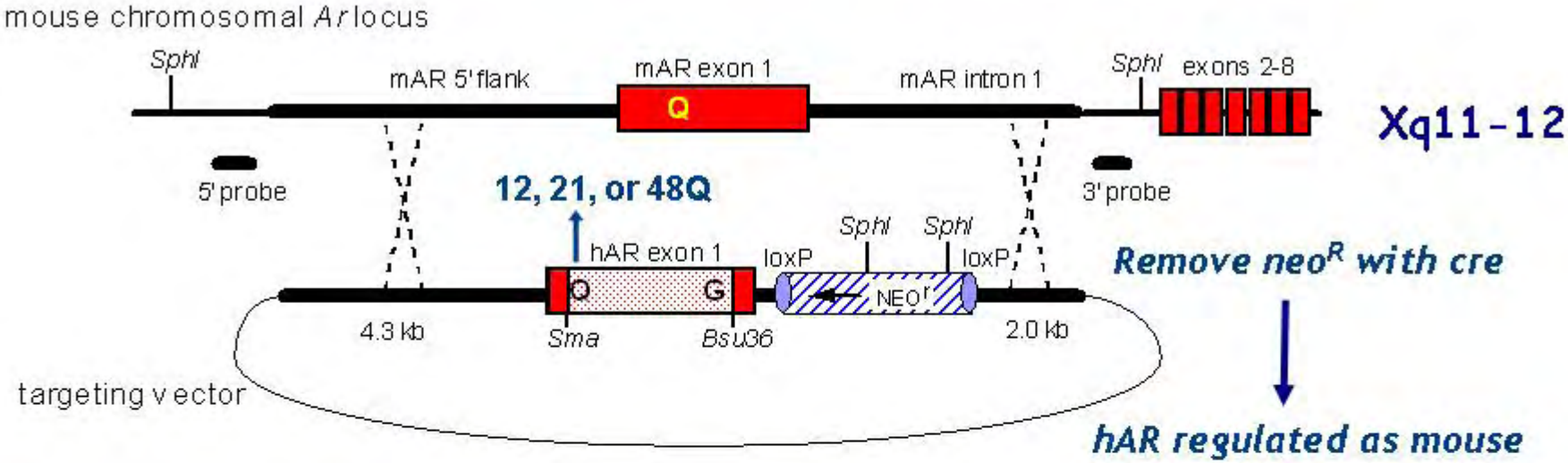
Human studies: multiple interacting genes (e.g., androgen axis)
gene-environment interactions (e.g., diet)
ascertainment bias (pre- or post-PSA testing)
small sample sizes
population diversity ("race" ?)

Complex biology: multiple hormones, multiple receptors (A/E, α/β)
changes in development and in aging

An animal model would be helpful -

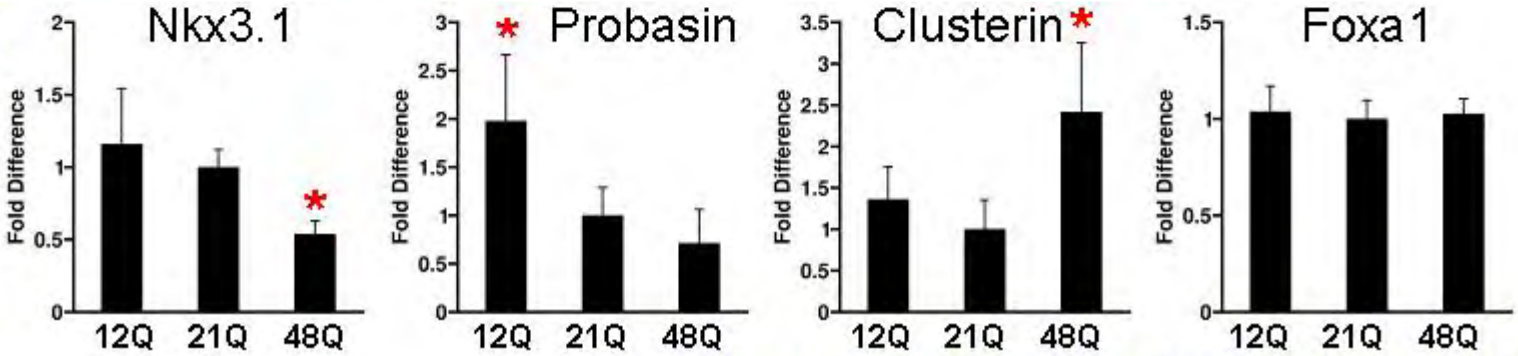
- Mouse/human AR are identical in DBD & LBD, differ ~15% in N-termini
  (hAR N-terminus ~ 2X transcriptional activity of mAR)
- Mice have distal Q tract (+His), no G tract, rarely get PCa

"Humanizing" the Mouse AR Gene



- Created h/mAR allelic series varying in Q tract length - 12Q, 21Q, 48Q
- Each strain normal in fertility, # pups, reproductive tract, T levels
- Differential h/mAR strengths detectable at molecular level:

RT-Q-PCR of AR Prostate Target Genes



Microarray analysis - few differences > 1.5-fold

TRAMP: Transgenic Adenocarcinoma of the Mouse Prostate

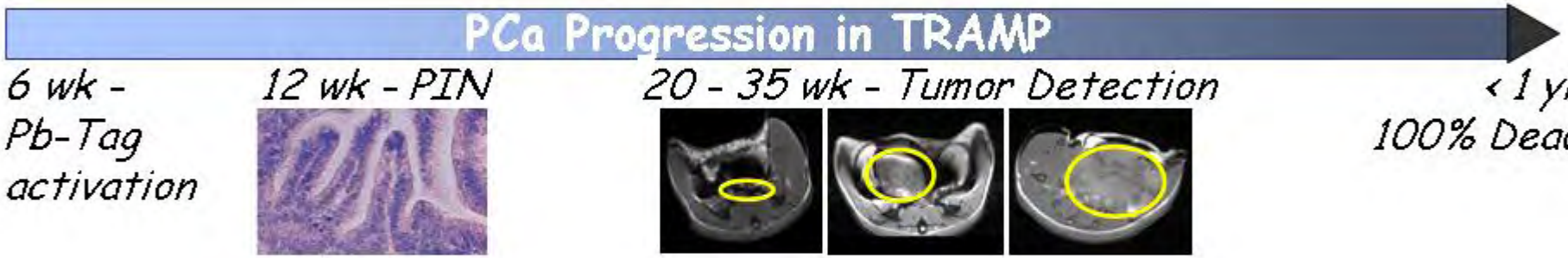
Created by Norm Greenberg, FHCC

Probasin promoter - prostate epithelial specific, A-responsive

SV40 T-antigen oncogene:

T - blocks p53 & Rb function

t - modulates PP2A function



Like human PCa: PIN early (prostatic intraepithelial neoplasia)
distant site metastases (lung, liver)
initially A-dependent, progresses to A-independent
phenotype varies with genotype

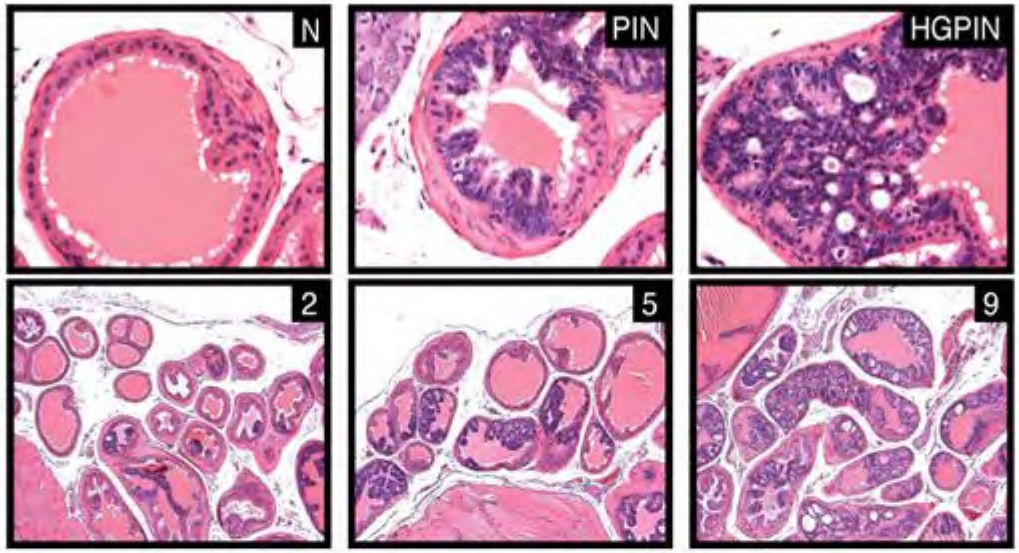
Unlike human PCa: mouse prostate morphology - lobular
aggressivity (mostly neuroendocrine in some strains)

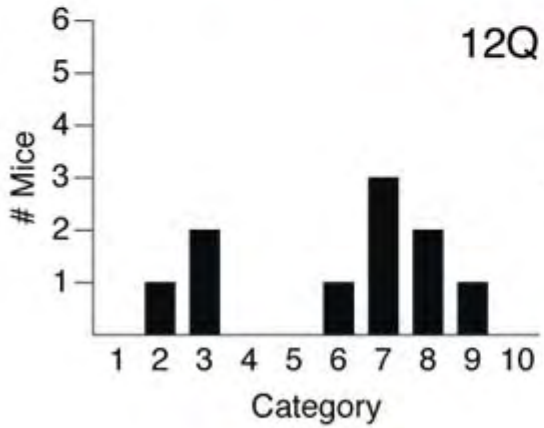
Q Tract Length Subtly Impacts Cancer Initiation

(12 wks)

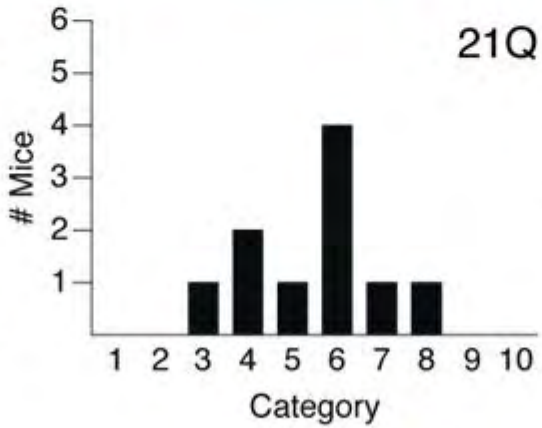
PIN Grade

PIN Extent

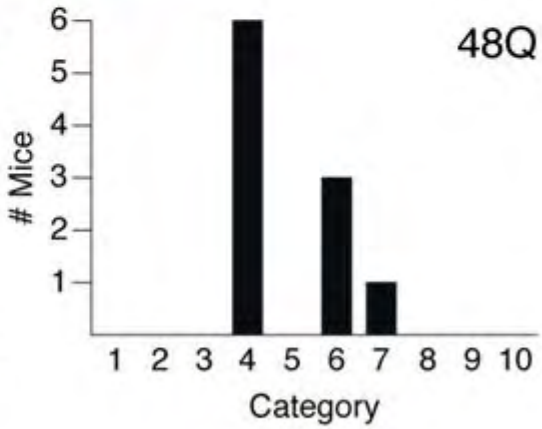




Mean - 6
Median - 7
PIN 2,3

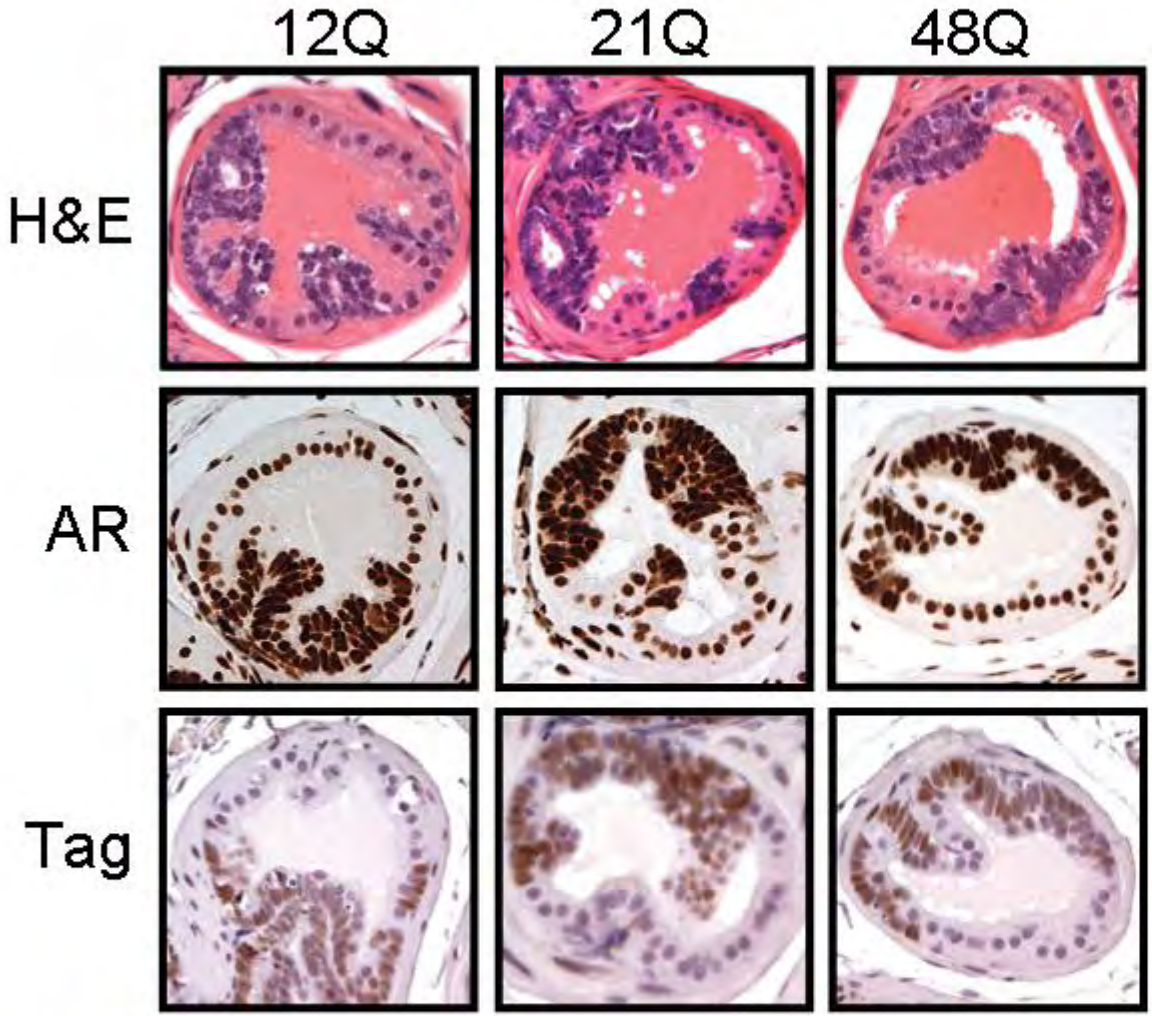


Mean - 5.5
Median - 6
PIN 2, 3



Mean - 4.9
Median - 4
PIN 2 only

Tag Expression is Associated with PIN



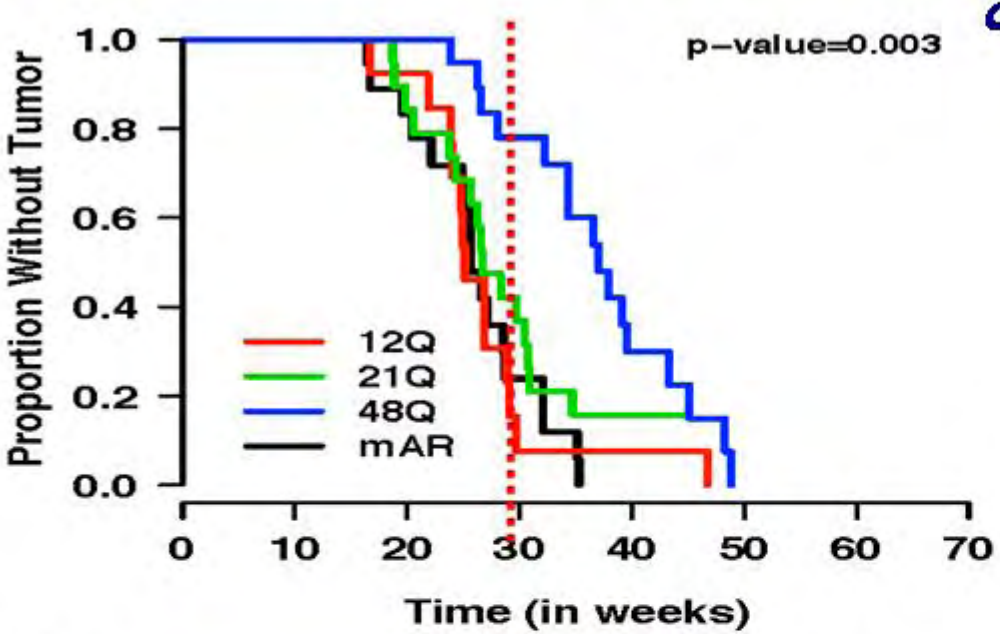
AR expression is similar in all epithelial cells regardless of genotype.

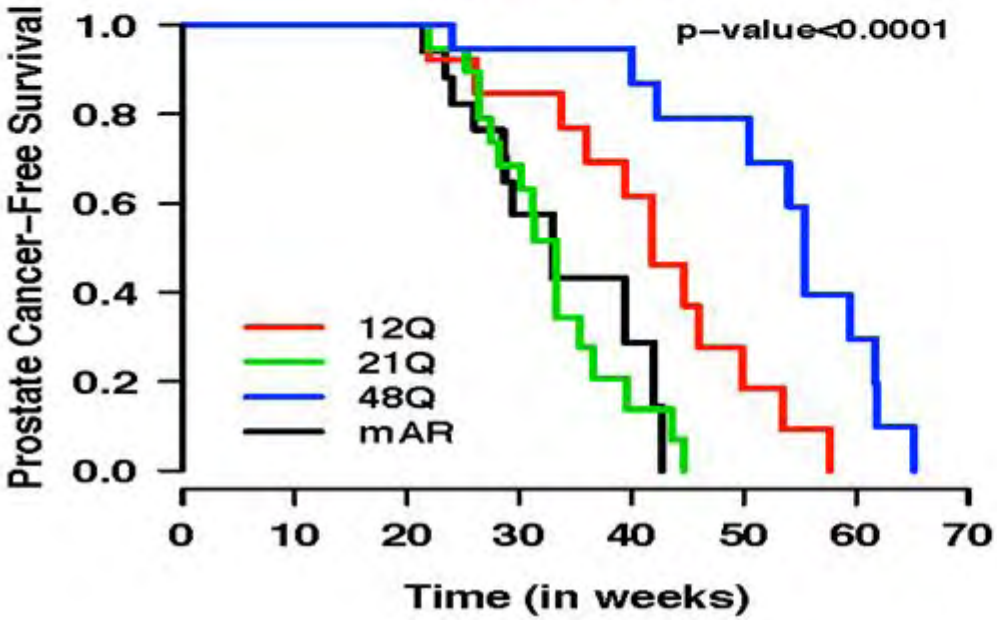
Tag oncogene expression is much higher in PIN.

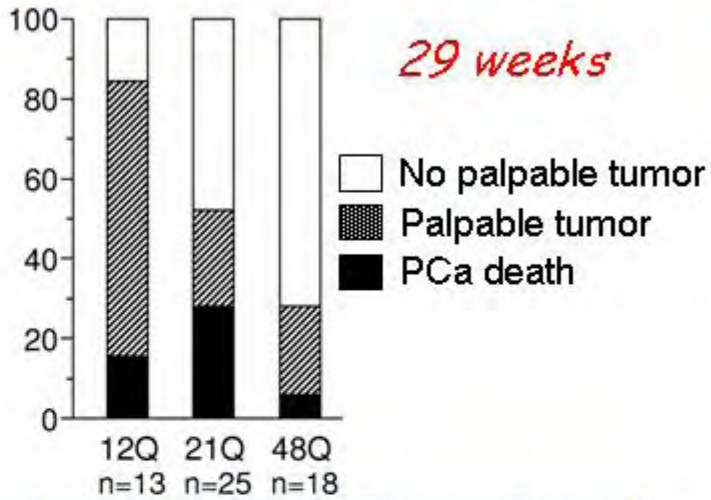
By 12 wks, Tag level affected by more than AR (cf. normal vs. PIN) - other promoter factors, post-tx effects, stromal AR effects?

Differences between normal and PIN greater than those due to genotype.

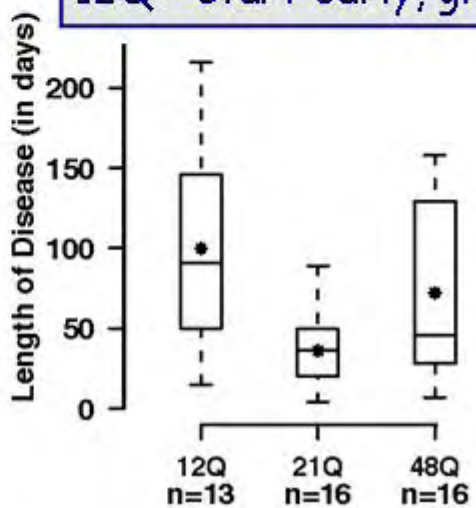
Q-Tract Length Affects Tumor Detection (by palpation) & Survival in Intact Mice



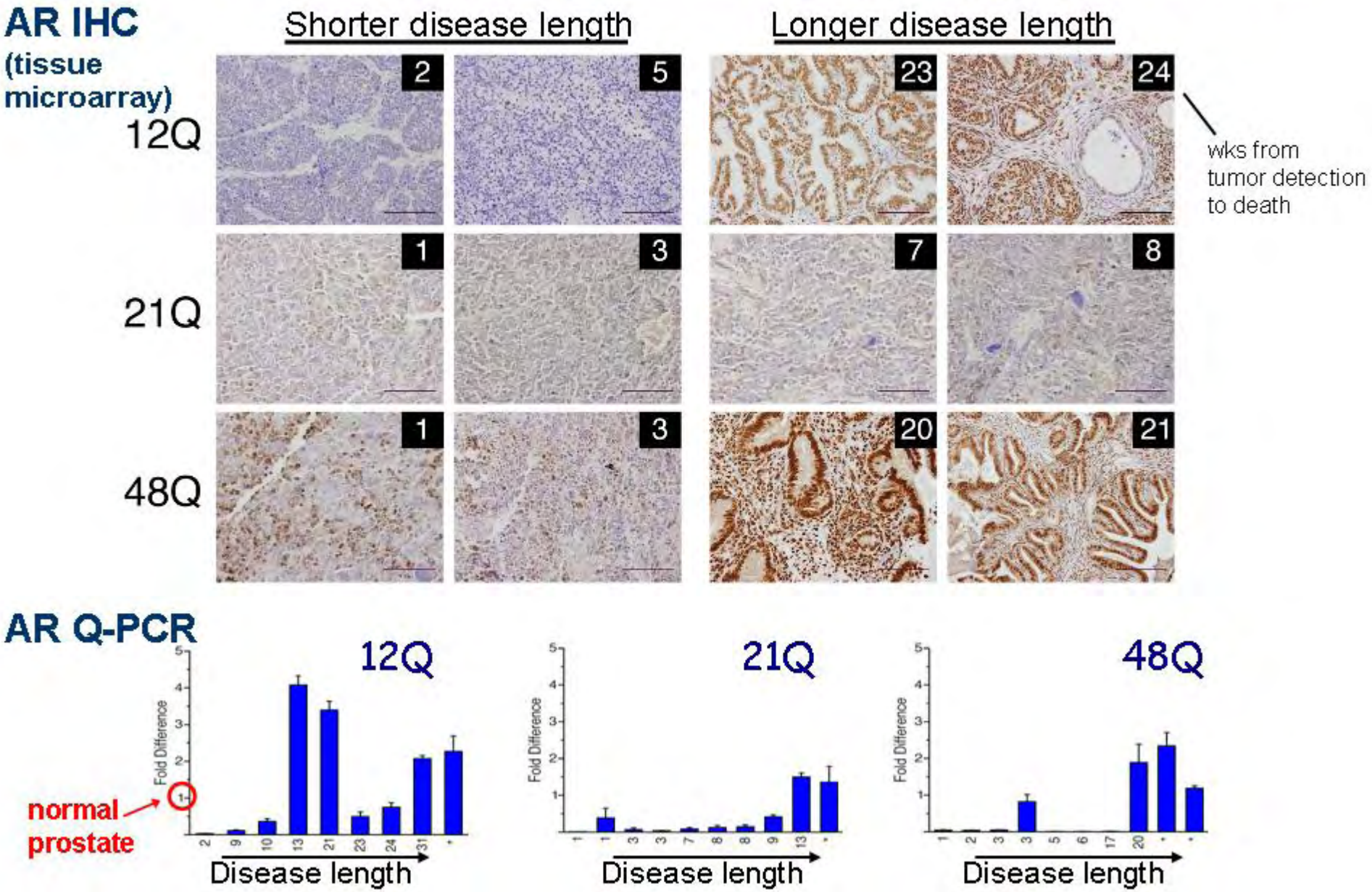




Heterogeneity for each but:
48Q - start late, survive long
12Q - start early, grow slow

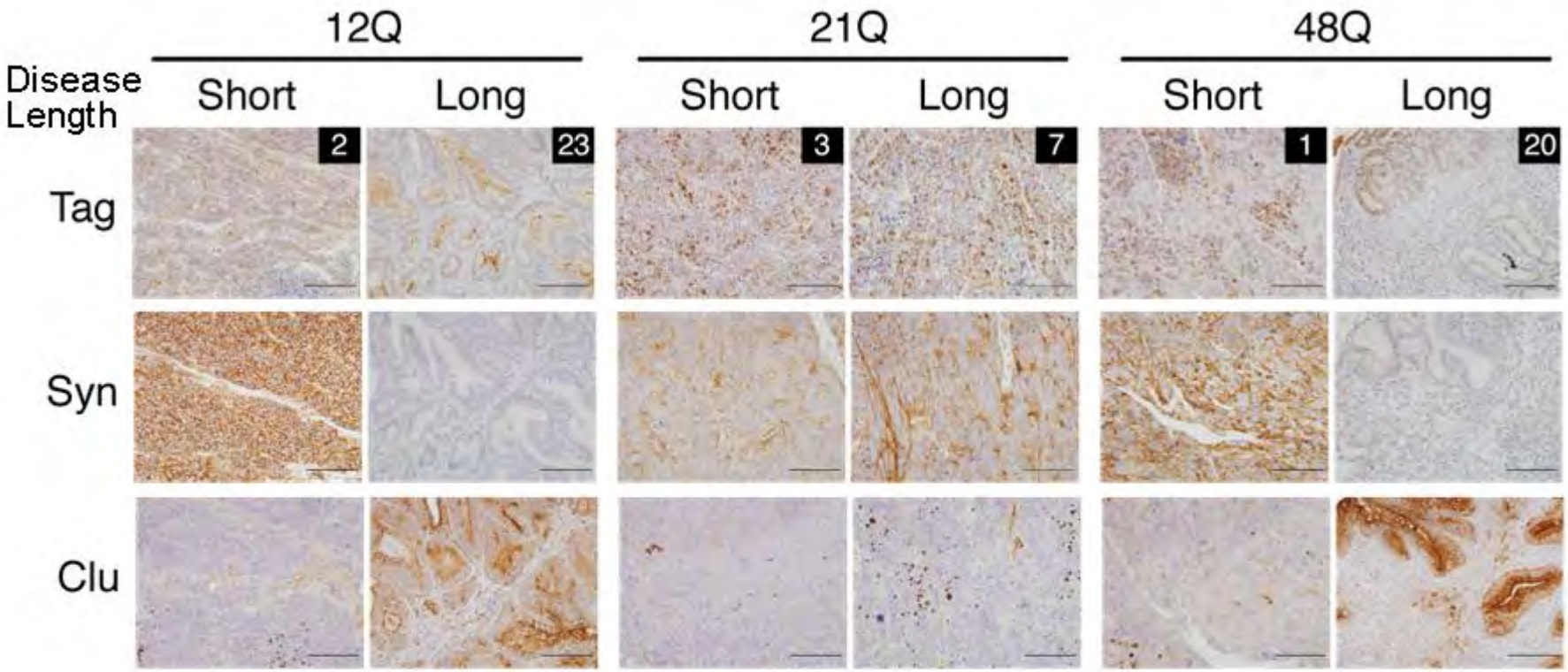


Longer Disease Length is Associated with More AR Expression and More Differentiated Tumors



Differentiation Markers in Prostate Tumors of Intact Mice

(endstage tissue microarray)

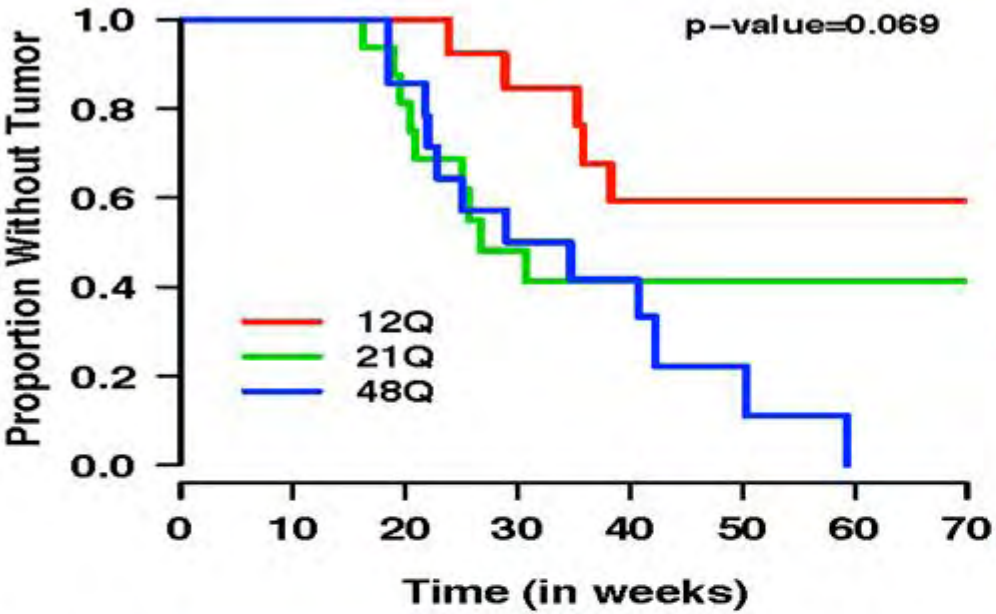


Synaptophysin expression is associated with aggressive tumor progression, while clusterin expression is associated with more differentiated tumors

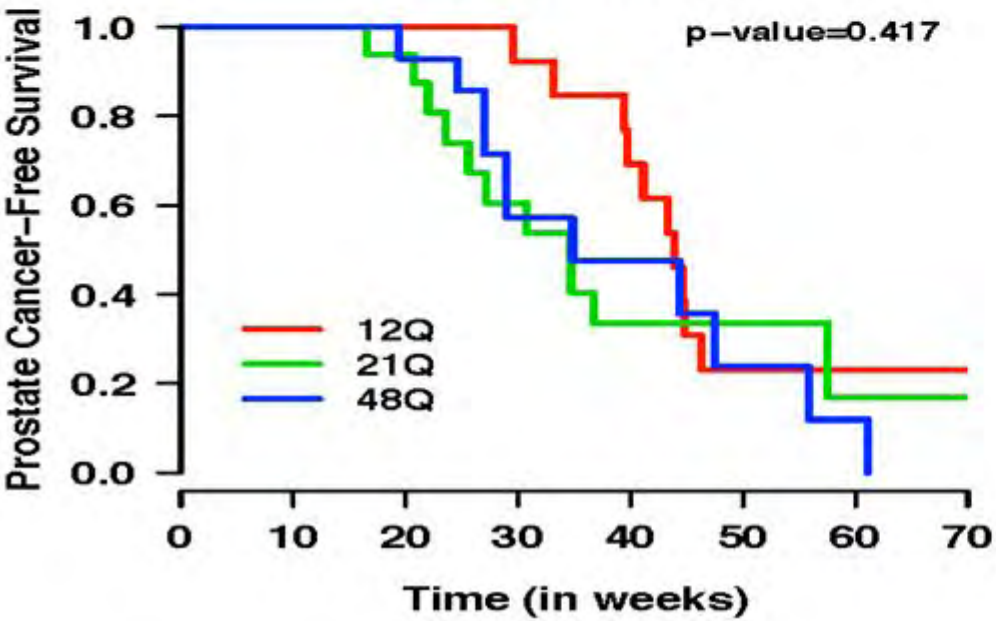
	AR^+Syn^-	AR^+Syn^+	AR^-Syn^+	AR^-Syn^-
12Q	5	0	5	0
21Q	1	6	3	0
48Q	5	2	2	1

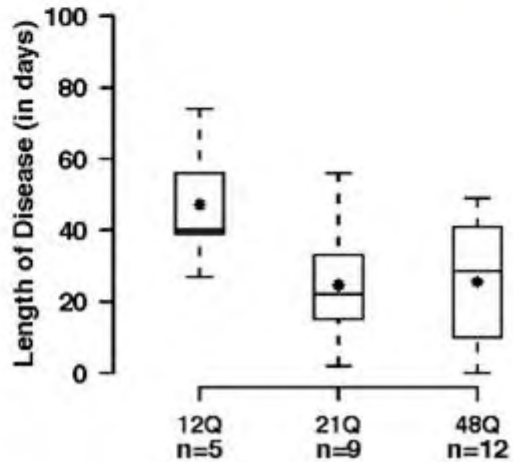
12Q tumors tend to be positive for either AR or synaptophysin, whereas 21Q tumors tend to express both AR (lower levels) and synaptophysin.

Q-Tract Length Affects Tumor Detection and Survival Differently in Castrates

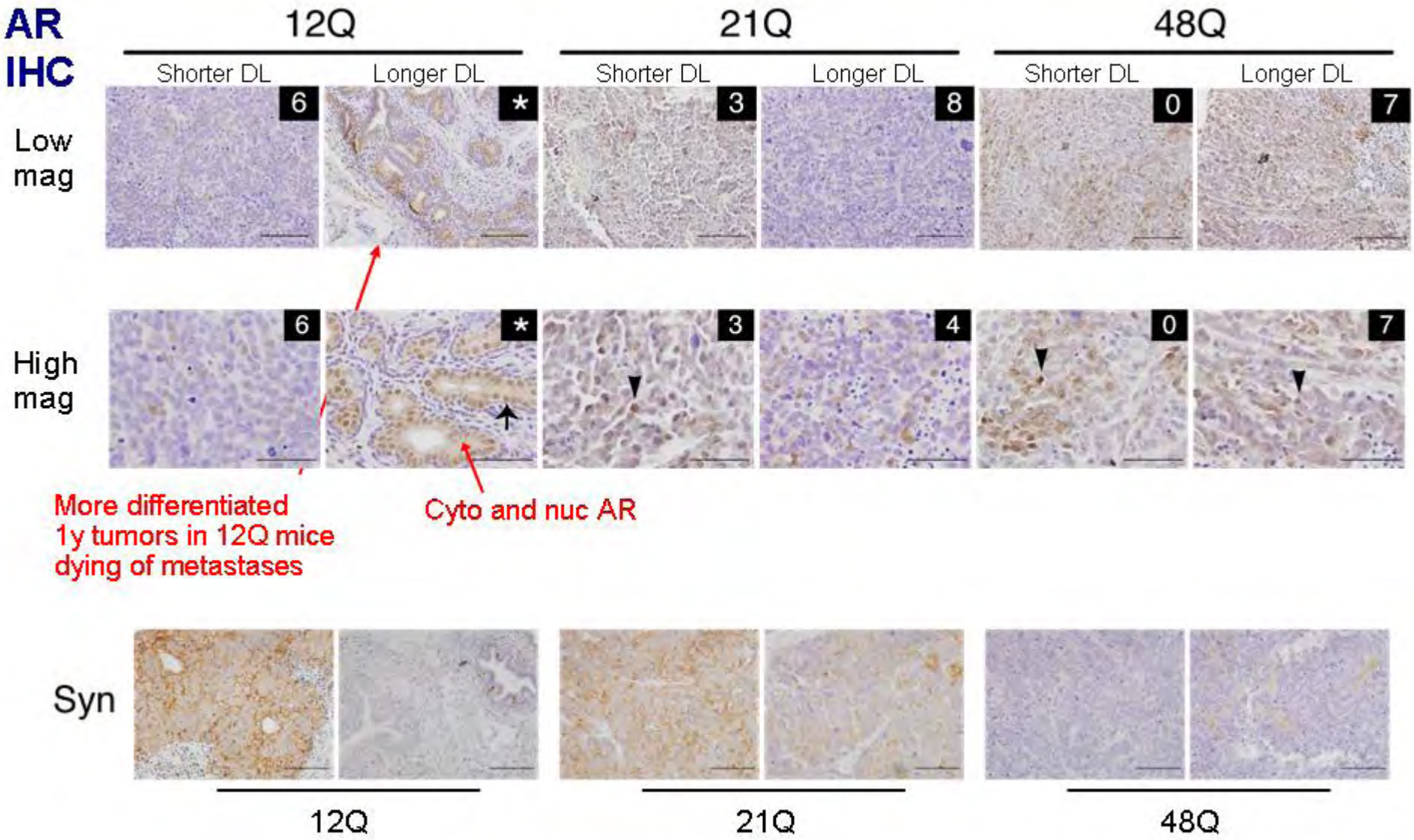


- Castrate at 12 wks - early stage - allelic differences imply role of AR (& Q) even without ligand.
- 12Q castrates develop tumors later; ~1/2 die of metastasis prior to detection of primary tumor.
- Disease length (palpation to death) brief in castrates; longest in 12Q.

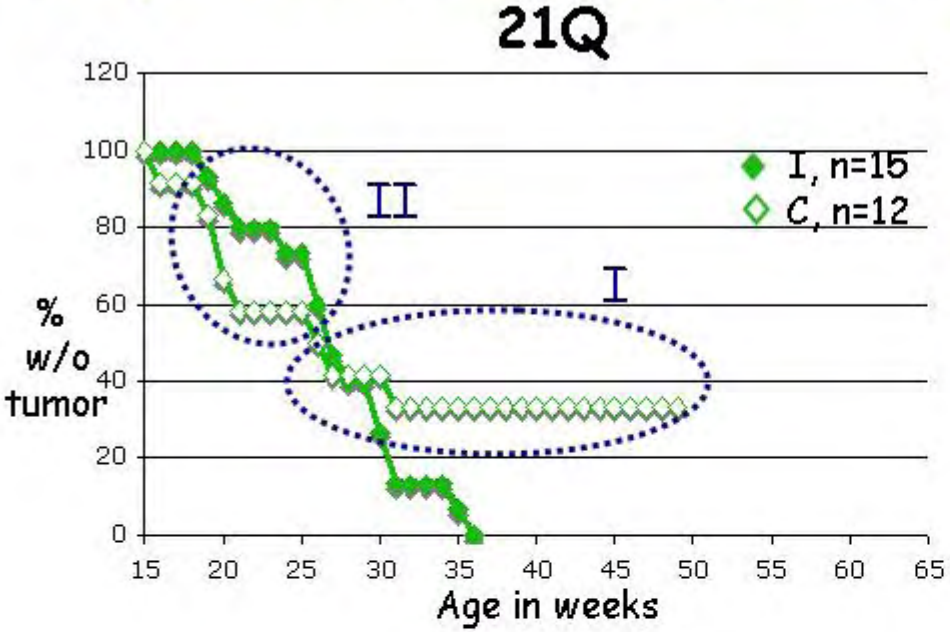




Tumor Differentiation & AR Expression in Castrated Mice



Response to Castration varies with Q-tract Length



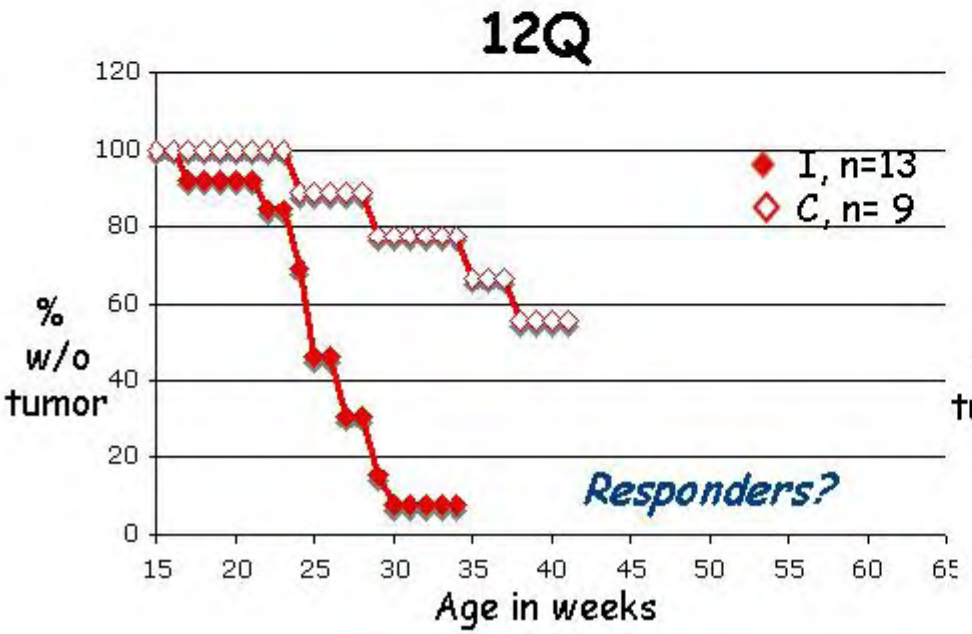
Two classes of response to castration:

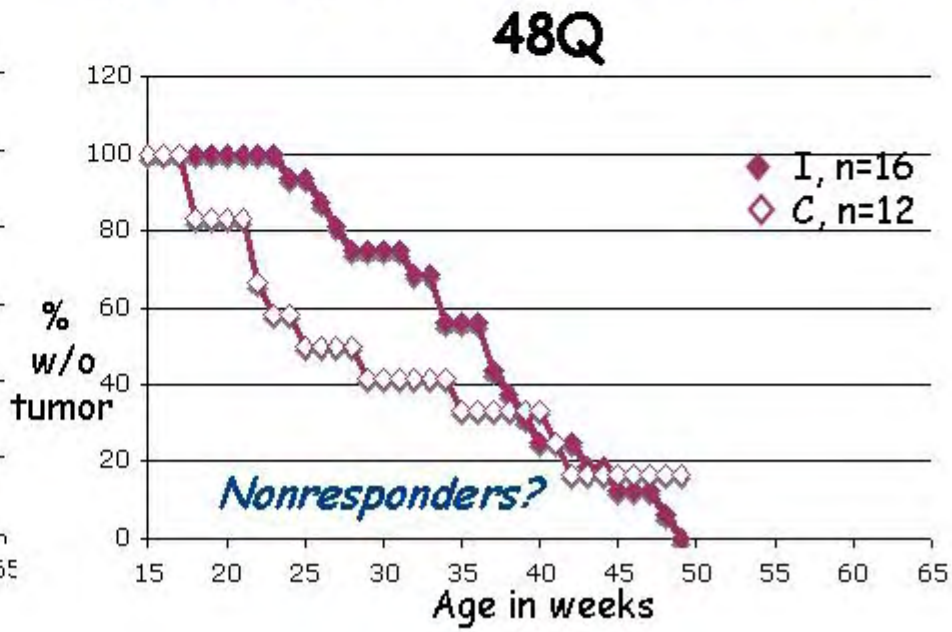
I) A-Dependent - slowed by ablation, benefit from therapy

II) A-Independent - pre-existing, synchronized for growth by ablation, failed response

(similar to finasteride trial?)

Early events may differ from later - little survival benefit for 12Q *C*, significantly worse survival for 48Q *C*





Q-Tract Conclusions

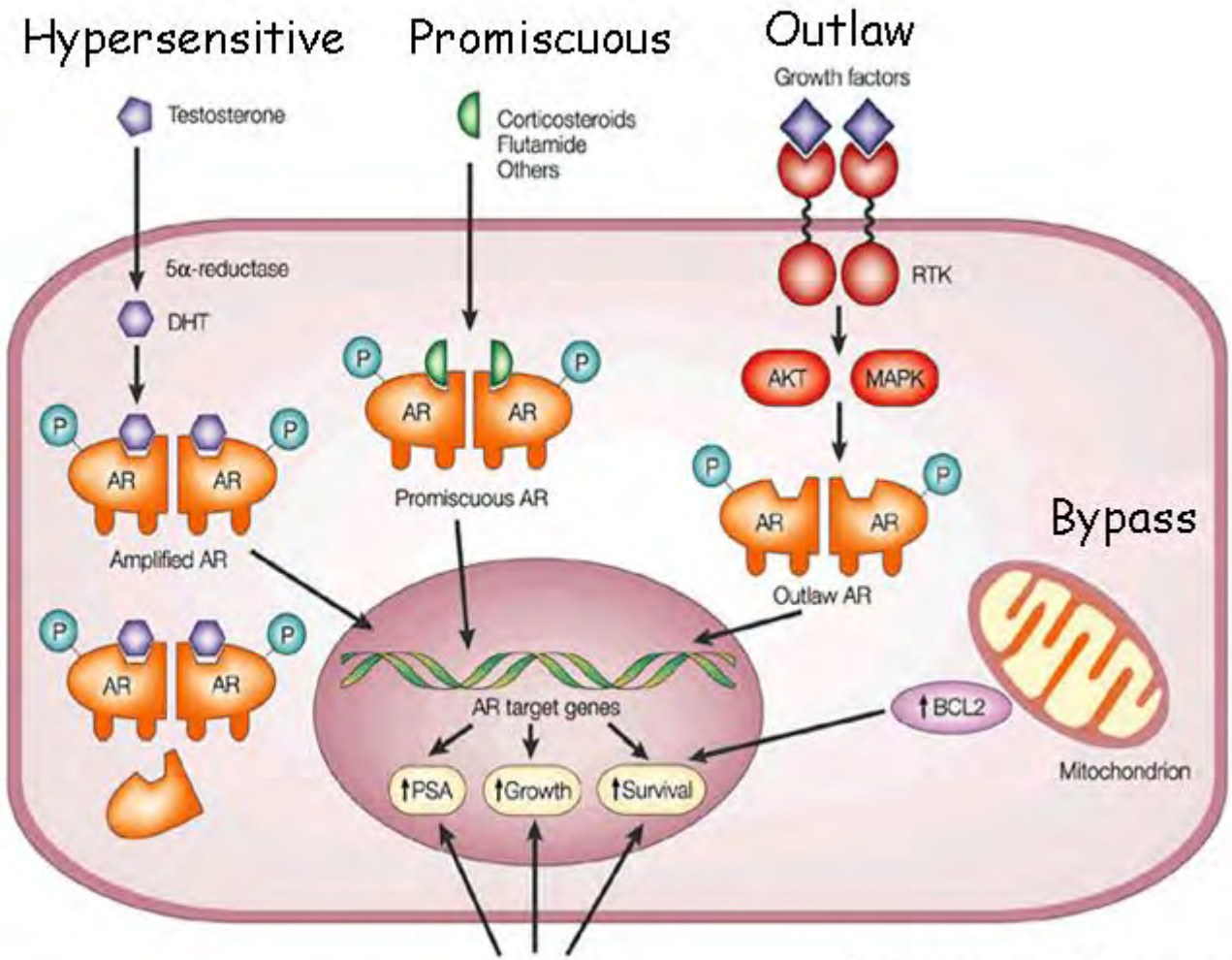
- h/mAR Q-tract variants are physiologically normal but small differences in AR target gene expression may have cumulative effects over time.
- Genetic homogeneity of mice and the stress of oncogenesis may experimentally amplify the effects of varying Q-tract length.
- In man, Q-tract effects may be most significant at extreme lengths - similar to differences in T level - one of several variables in the A axis.
- Recent studies in more homogeneous populations of genes in the A axis (*Ar*, *cyp17*, *SRD5a2*) reveal variant haplotypes with differential risk.
- Q-tract length impacts androgen-independent as well as -dependent PCa, emphasizing the continuing role of AR despite A depletion.
- The Q allelic series in mice models differing strength of the A axis, and may distinguish roles of AR that vary with disease stage. **(A little A good?)**

Context-dependent opposing functions of AR:

stage (development vs. oncogenesis)
cell-type (stroma vs. epithelia)
targets (proliferation vs. differentiation)

PCa Progresses to Androgen Independence during Treatment

- Despite "androgen-independence", AR and down-stream signaling persist
- Resistance is independent of androgen, but dependent on AR
- Mechanisms:
  a) changes to AR (mutate, amplify)
  b) changes in AR cofactors
  c) GF activation of AR w/o ligand
  d) pathways that bypass AR



(Feldman and Feldman, Nat Rev Cancer 2001)

AR is central to every phase of PCa – initiation, progression and treatment response

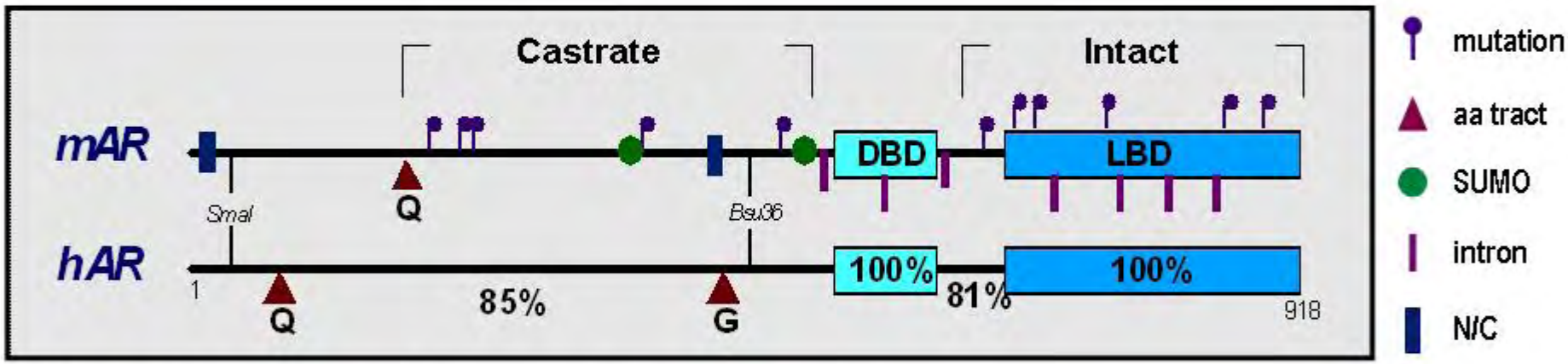
AR Mutations in Prostate Cancer

Rare in 1^{y} tumors, but found in ~ 50% late stage tumors & metastases - does treatment provide selection for mutations?

Some are gain of function -

relaxed ligand specificity, altered cofactor interaxns, *Ar* amplification

Problems with human studies - small sample sizes, diverse treatments....



Norm Greenberg (Han et al., PNAS 102:1151) -

~10% of TRAMP AR cDNAs mutated - site varies with hormonal status:

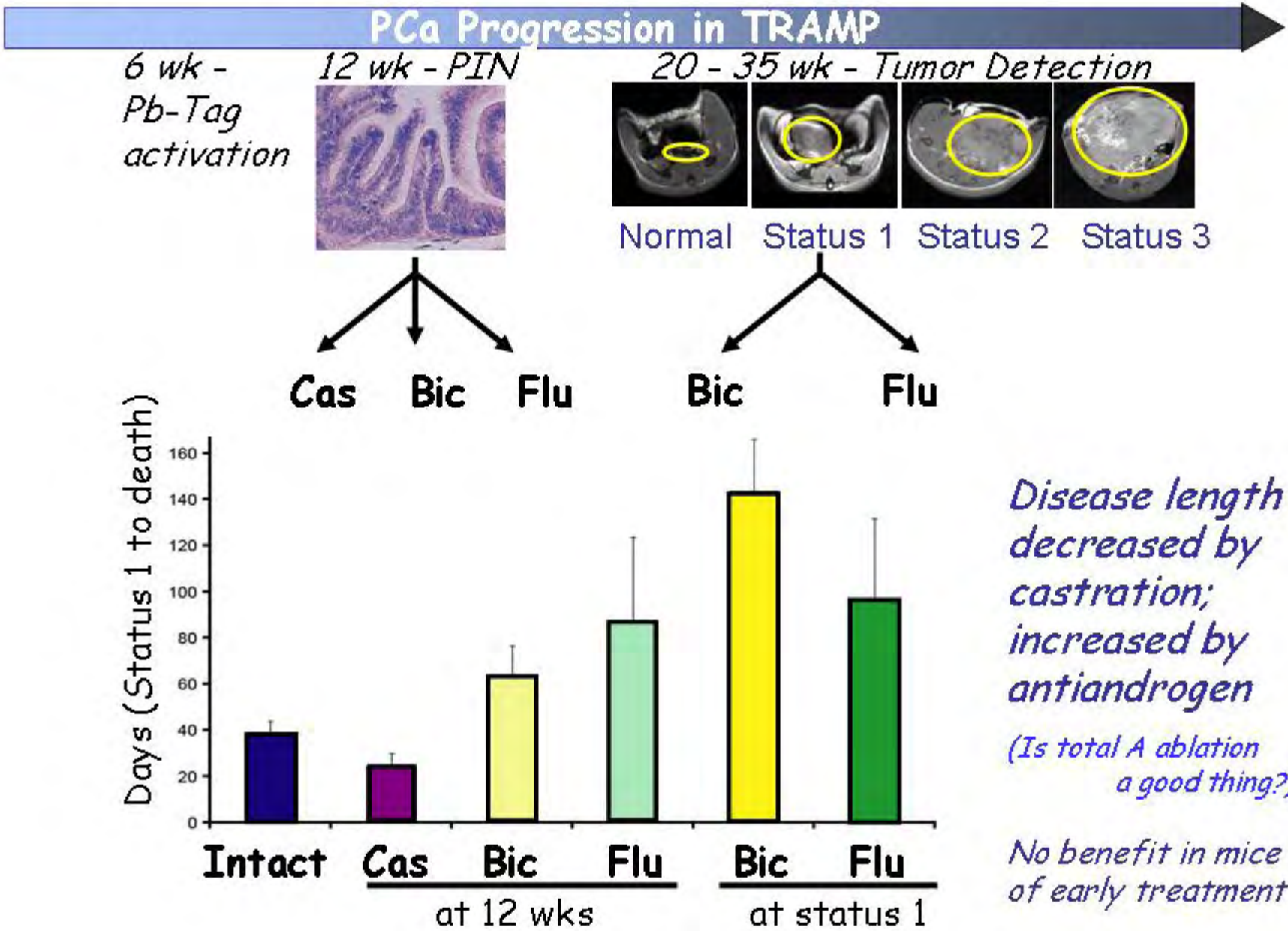
Intact - mutations in LBD - reduce response and ligand specificity

Castrate - mutations in NTD - greater ligand-independent action

Interactions at these sites may reveal new therapeutic targets

- confirm more directly in humanized AR mouse

Do AR Mutations Vary with PCa Treatment?



Disease length - decreased by castration; increased by antiandrogen

(Is total A ablation a good thing?)

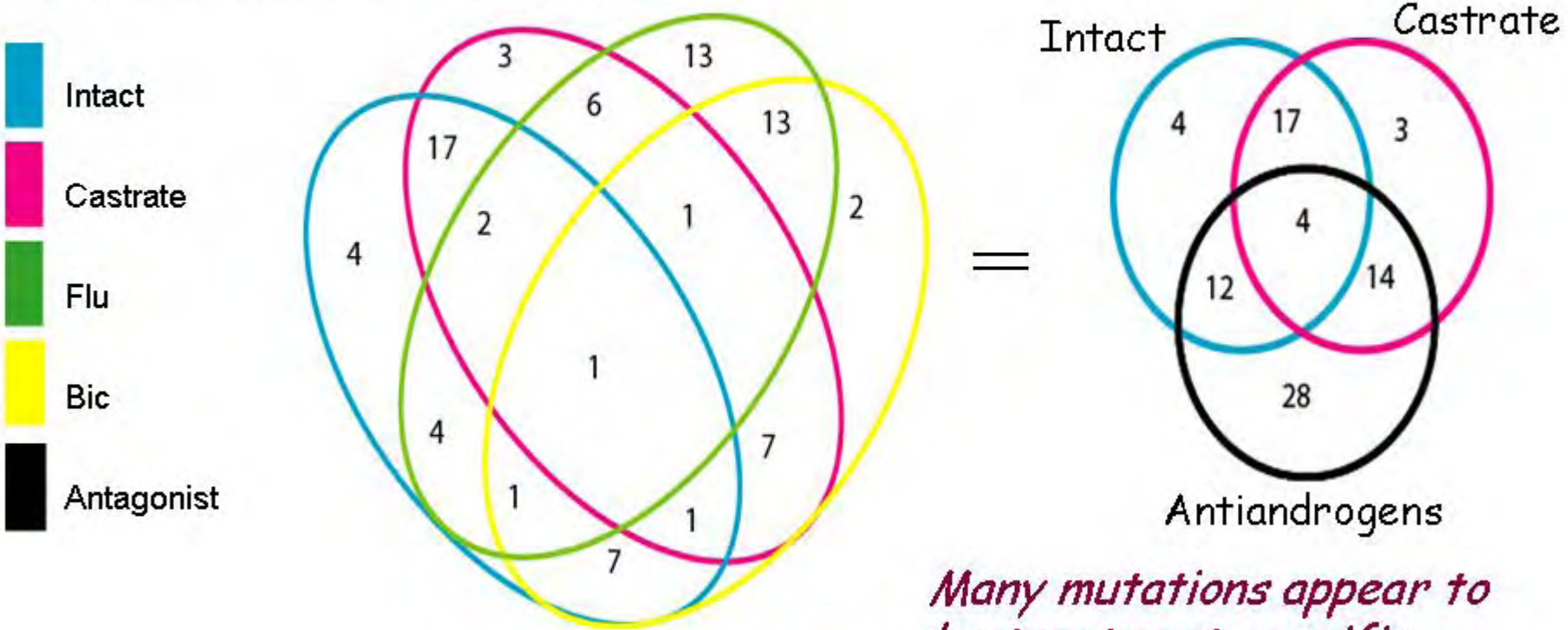
No benefit in mice of early treatment

Exact Recurring Mutations

(to same base, not just same amino acid)

Includes 2 RT, >10% clones

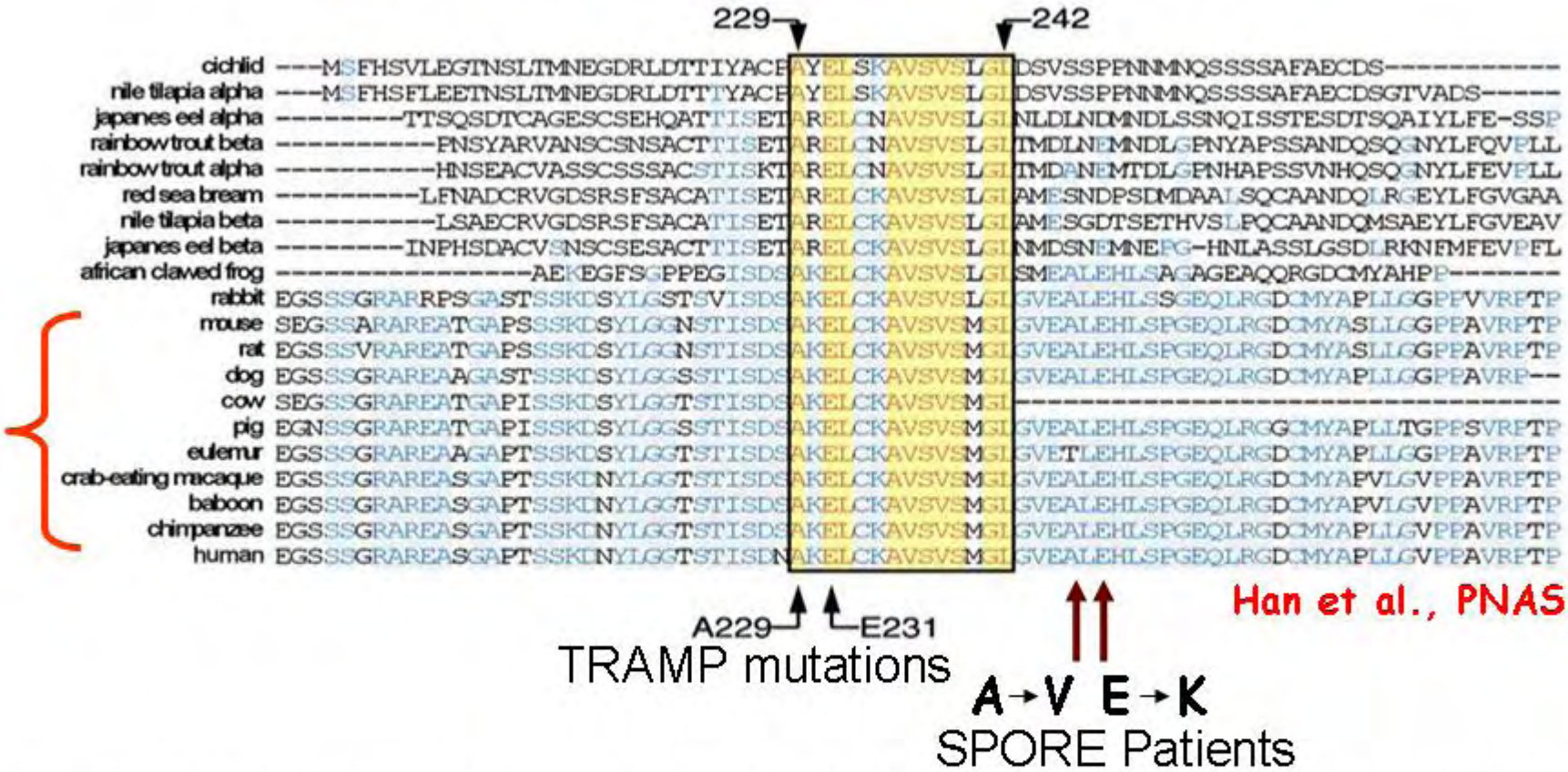
Excludes indels in Q and G tract, silents



Many mutations appear to be treatment-specific, suggesting they are selected.

Very few mutations are more than 10% of tumor or in more than a few mice....

Mutations in the CHIP Interaction Domain of AR in Tumors from Mice and Men



Han et al., PNAS 2005

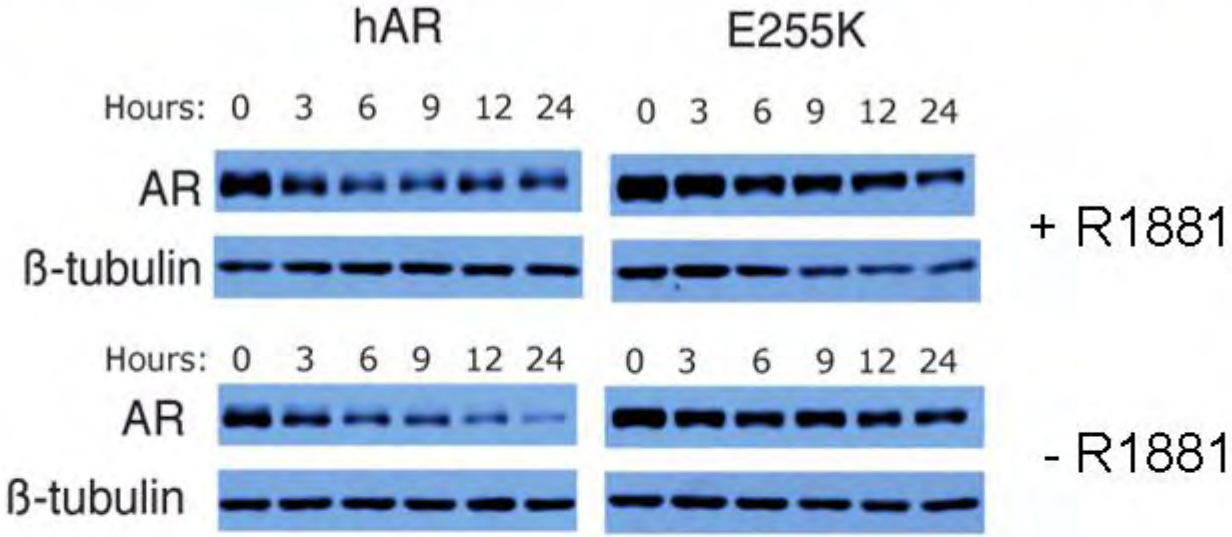
CHIP (COOH terminus of the Hsp70-interacting protein) functions as a negative regulator of AR by promoting AR degradation.

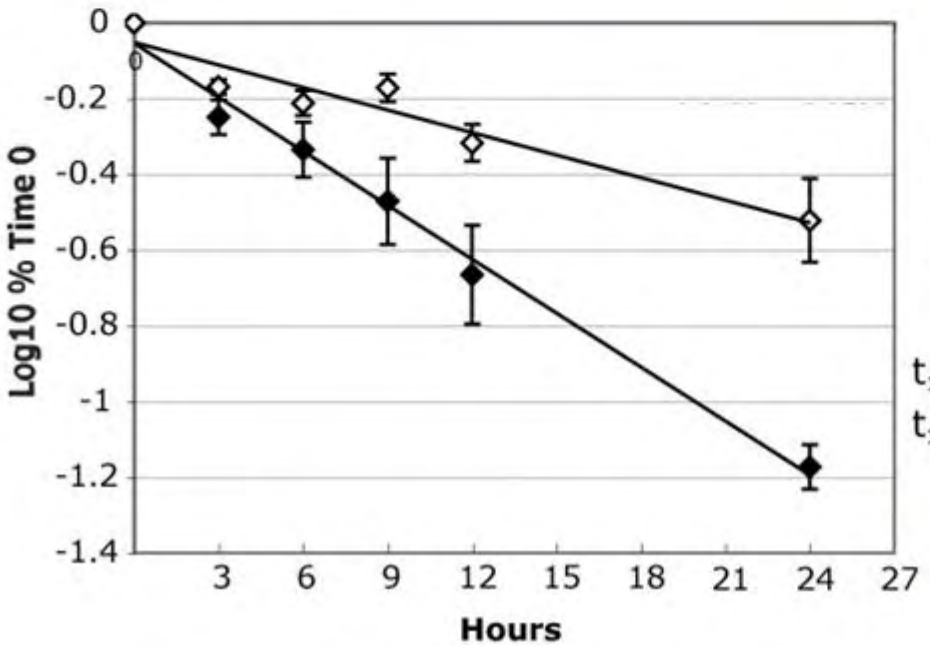
Mutations could slow degradation and increase AR protein levels.

Transgenic prostate-targeted AR-E231G causes oncogenesis in mice.

E255K is More Stable than WT in the Absence of Ligand

Cycloheximide degradation assay:



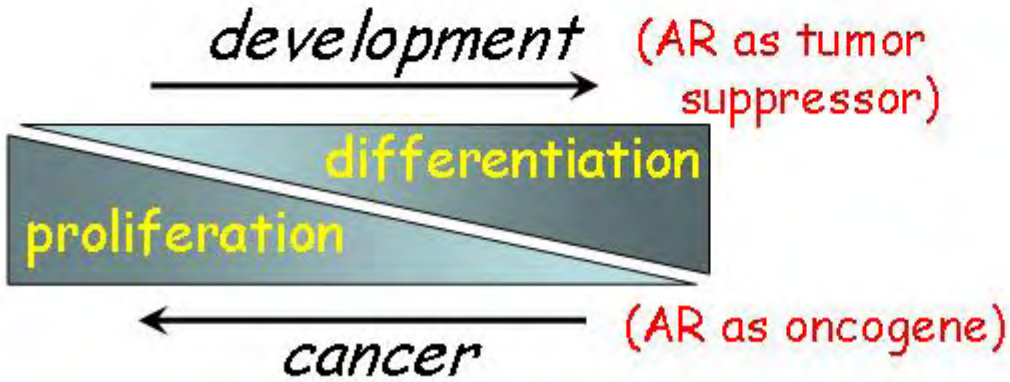


$t_{1/2}$ hAR = 5.2 hrs.
$t_{1/2}$ E255K = 12.5hrs

Proof of Principle – TRAMP is a great model!

Conclusions

- Mutations occur in AR in a variety of prostate cancer models, but at low frequency (most < 10% of tumor mRNA).
- Many mutations cluster in functionally important AR domains.
- They suggest gain rather than loss of function, and possible significance for disease progression.
- Factors interacting at these sites are potential therapeutic targets (for wild type as well as mutant receptors).
- The h/mAR mice bridge relevance of mouse to man and allow preclinical testing of novel therapies directed against the human AR.



Context-dependent opposing functions of AR

Prostate group

Megan Albertelli
Michele Brogley
Arno Scheller
Orla O'Mahony
Mara Steinkamp

Jeff Tosoian
Jennifer Gerber
Salina Olmsted

Acknowledgments

DOD-PCRP
UMCCC Prostate Cancer SPORE
UM Transgenic Animal Core

U.M. collaborators

Kirk Wojno - Pathology
Stephanie Daigneault - Statistics
Andy Lieberman - Kennedy disease