Catechine del tè verde ed Oncoprevenzione

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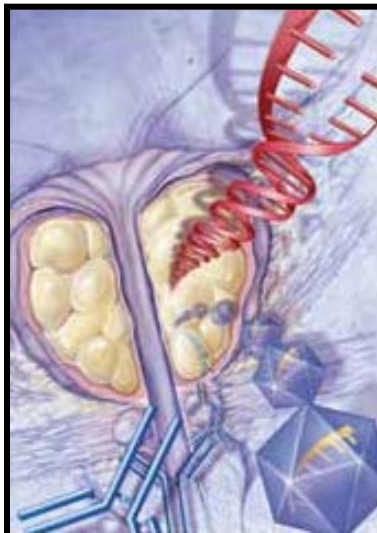
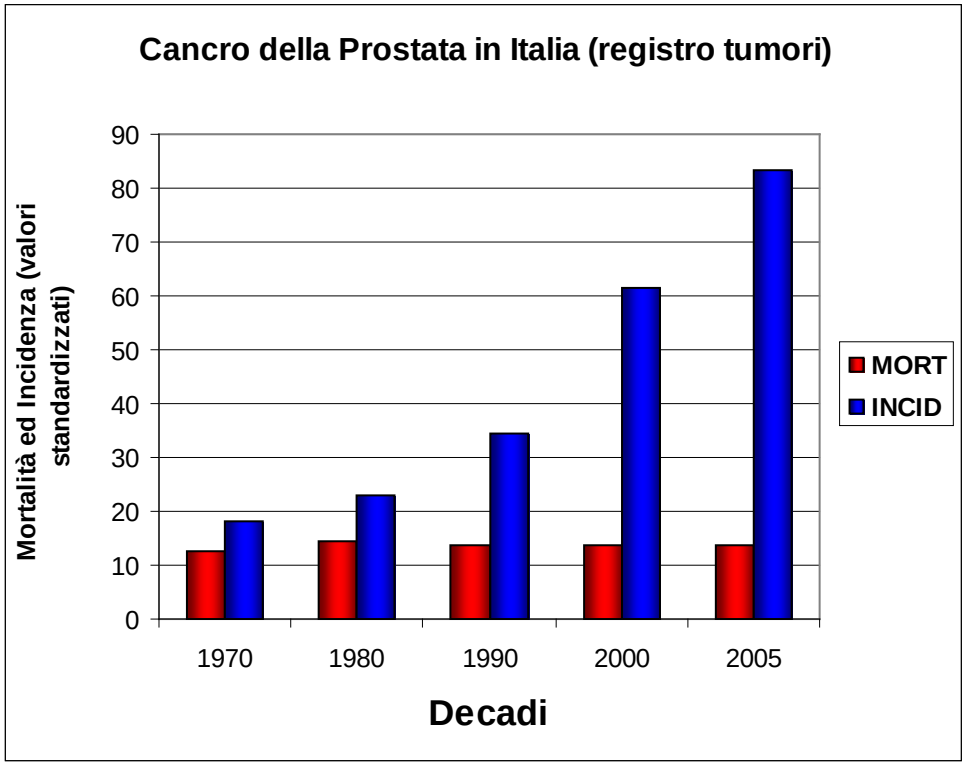


Prostate Cancer in Italy: National Cancer Registry

Sede	Sesso	Periodo	Regioni	Età	mort_eur	inc_eur	prev_eur
Prostata	U	1970	Italia	0-84	12.41	18.19	47.06
Prostata	U	1980	Italia	0-84	14.37	23.05	54.28
Prostata	U	1990	Italia	0-84	13.67	34.57	101.36
Prostata	U	2000	Italia	0-84	13.54	61.30	220.87
Prostata	U	2005	Italia	0-84	13.55	83.22	326.85



INCIDENCE: ↑

MORTALITY: ↔

GOOD, OR BAD NEWS?

- ✓ ***PCa is biologically heterogeneous: polyclonal from the very beginning***

- ✓ ***PCa is an elusive disease: molecular determinants of onset and progression are still unclear***

- ✓ ***Prediction of outcome is a problem***

- ✓ ***Efficacy of therapy in advanced disease (metastatic) is unsatisfactory***

Rate of PCa diagnosis and life-time risk in western Countries

- Life-time risk of **PCa diagnosis** is ~**1:6**
- Life-time risk of **PCa-specific death** is ~**1:30** (unfortunately, very stable with time…)

- **Much better not to have PCa, than to cure it!**

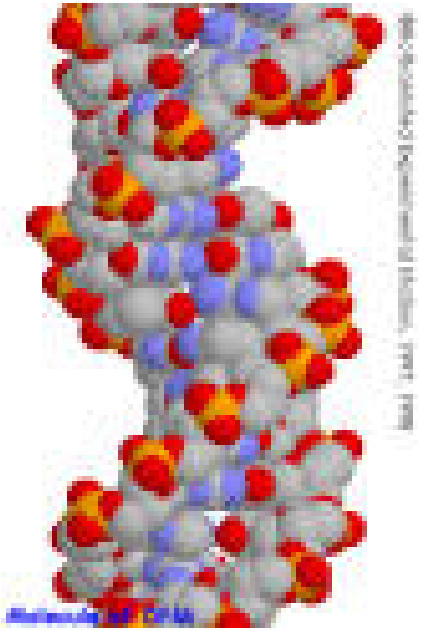
- the relative incidence in USA is about **10 times higher** than in Italy and Japan
- Italian or Japanese men migrated to USA for generations: PCa incidence identical to USA men

- Genetic background is not the issue:

  → **LIFE STYLE?**

Prostate cancer: risk factors and prevention

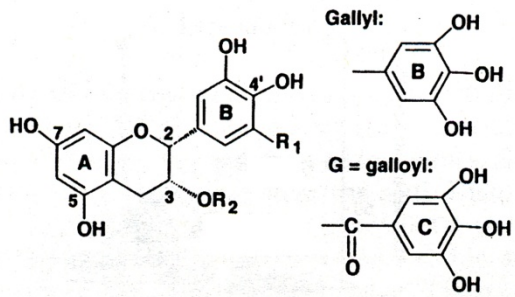
Genetics (x2)



Age, life style and food (x>10)

→ PCa is an excellent candidate for application of chemoprevention strategies, because of slow progression and late diagnosis in elderly men.

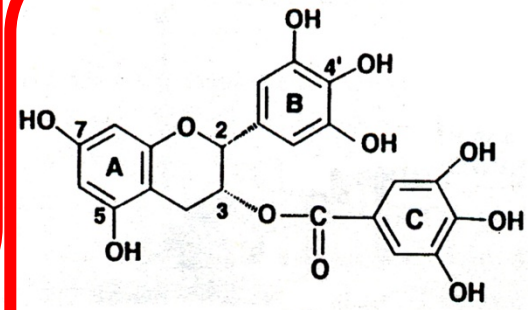
Green Tea Catechins extract (GTE): purity is assessed by HPLC (EGC 5.5%; EC 12.2%; EGCG 51.9%; ECG 6.1%; total GTE 75.7%; caffeine < 1.0%)



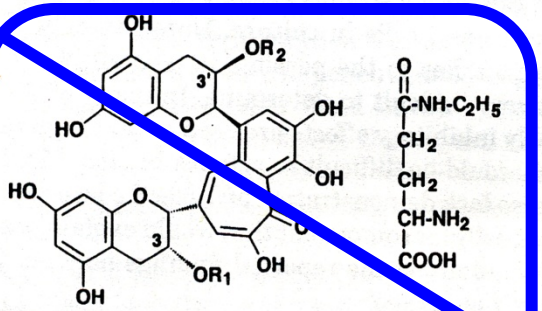
	R_1	R_2
(-)Epicatechin (EC)	H	H
(-)Epigallocatechin (EGC)	OH	H
(-)Epicatechin-3-gallate (ECG)	H	G
(-)Epigallocatechin-3-gallate (EGCG)	OH	G
(-)Gallocatechin-3-gallate (GCG)*	OH	G

* Ring-B is attached to C-2 in the trans position in respect to the attachment of the gallate substitution at C-3.

S. LIAO, Y-H. KAO, AND R. A. HIIPAKKA



EGCG



Theaflavins
(R_1, and R_2, can be H or a galloyl group)

Theanine
(γ-glutamylethylamine)

FIG. 2. Structures of catechins and theanine in green tea and theaflavins in fermented tea.

Why Green Tea, and not other teas or vegetables?

PRE-CLINICAL STUDIES:
Caporali A et al. Carcinogenesis 25, 2217-24, 2004

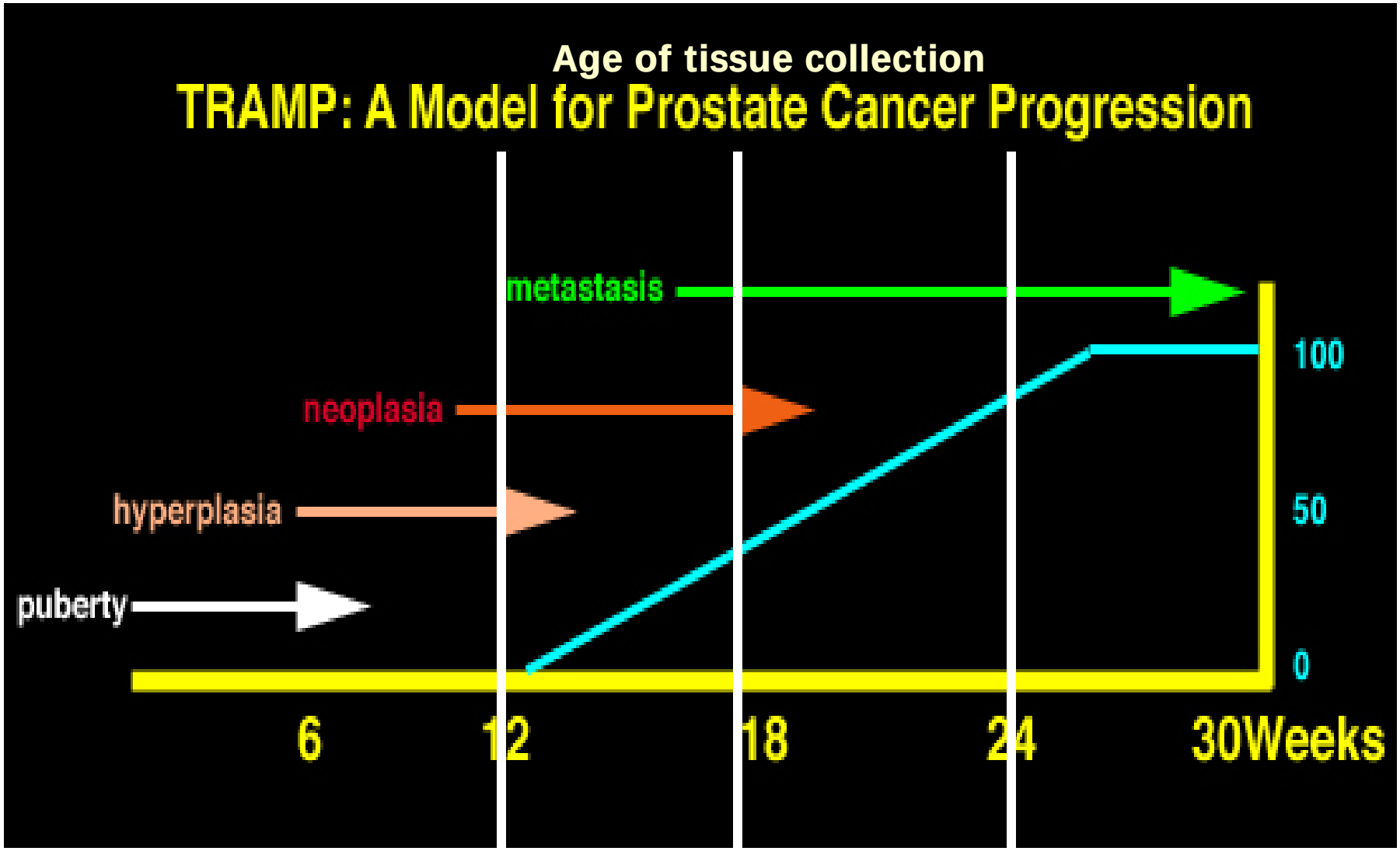
IC_{50} values for EGCG (μg/ml) as assessed in benign human prostate epithelial cells (controls), SV40-immortalized (PNT2 and PNT1A) and tumorigenic (DU145 and PC-3) prostate cell lines

differentiation

	Controls	PNT2	PNT1A	DU145	PC-3
Mean	N.D.	31,8	83,6	99,4	202,3
SD	N.D.	1,3	1,3	0,58	3,2

→Selective action of EGCG on early stages of transformation

TRAMP (PB-SV40 T antigen transgene): PCa progression model

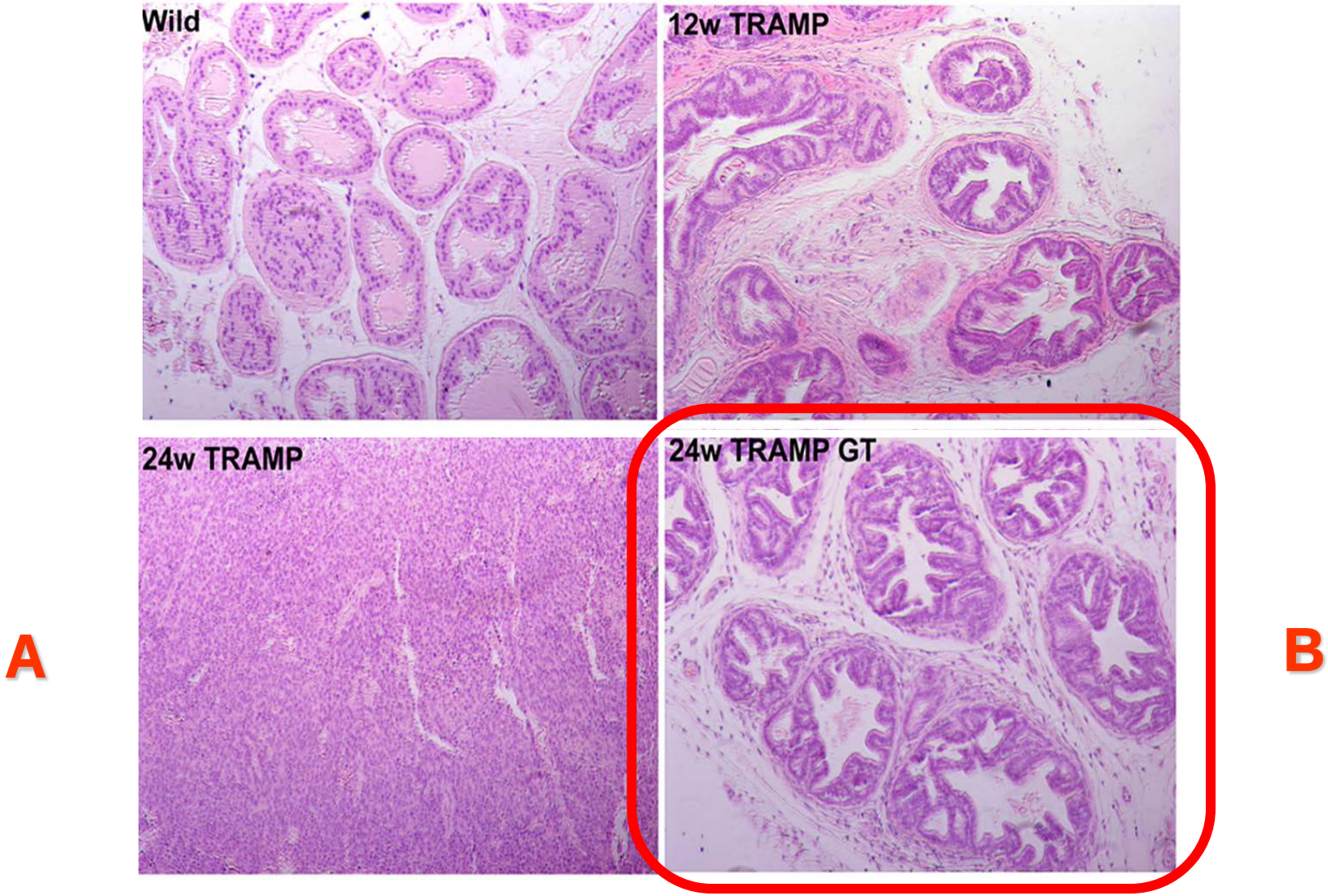


Chemoprevention of PCa in the TRAMP model: results (now: n = 200)

(Caporali et al, Carcinogenesis 2004)

Age (weeks)	PCa/n°	PCa/n°
	control	GTE
12 w	0/20	0/20
17 w	1/20	0/20
24 w	20/20 (=100%)	4/20 (=20%)

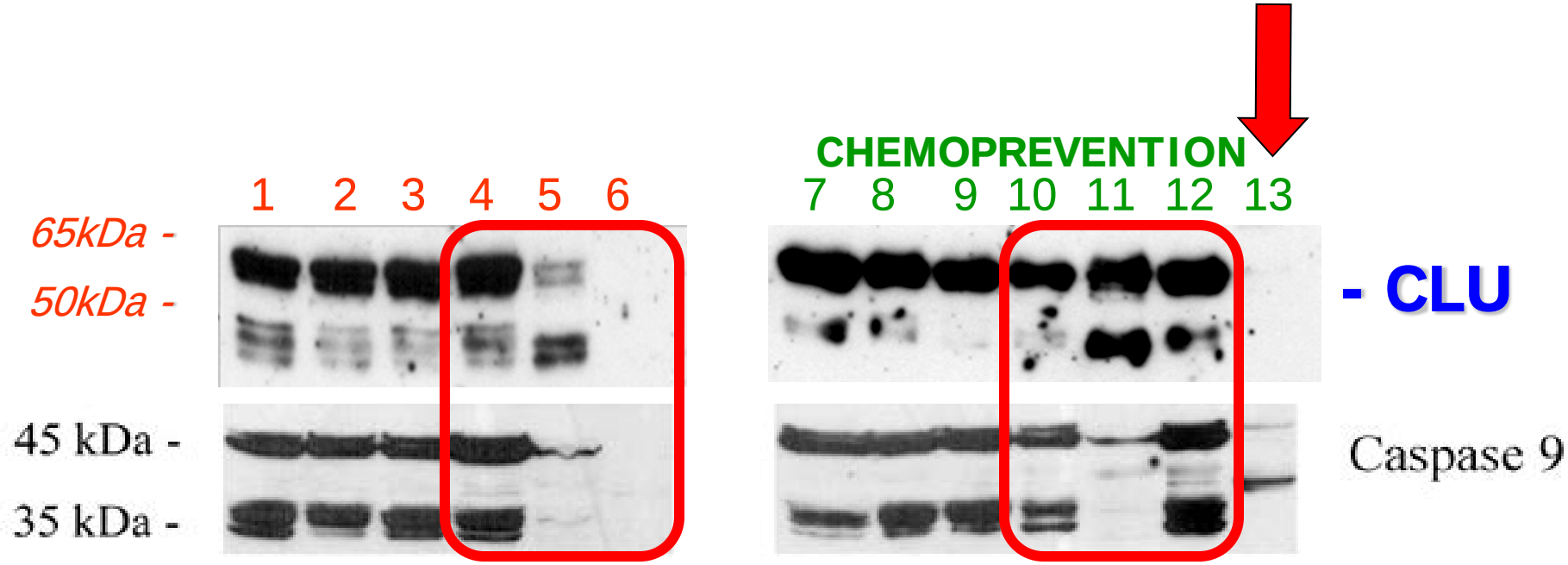
Only confined PCa !



A) H/E staining of a representative prostate section of a 24w-old TRAMP mice developing PCa;

B) Efficacy of GTE administration (chemoprevention) at inhibiting PCa progression in age-matched TRAMP mice.

Expression of CLUSTERIN and CASP-9 in the TRAMP model



1. 12w wild
2. 17w wild
3. 24w wild
4. 12w TRAMP
5. 17w TRAMP
6. 24w TRAMP (CaP)

7. 12w wild
8. 17w wild
9. 24w wild
10. 12w TRAMP
11. 17w TRAMP
12. 24w TRAMP
13. 24w TRAMP (PCa)

Caporali et al.
Carcinogenesis
25, 2217-24, 2004

Clinical trial: STUDY DESIGN

Bettuzzi et al., Cancer Research 66: 1234-1240, 2006

- **Following the pre-clinical results, we performed a proof of principle clinical trial to assess the possible efficacy of GTE at preventing PCa progression.**

- **This information had to be reached as quick as possible and at low cost: the answer was a selected population of human volunteers with HGPIN.**

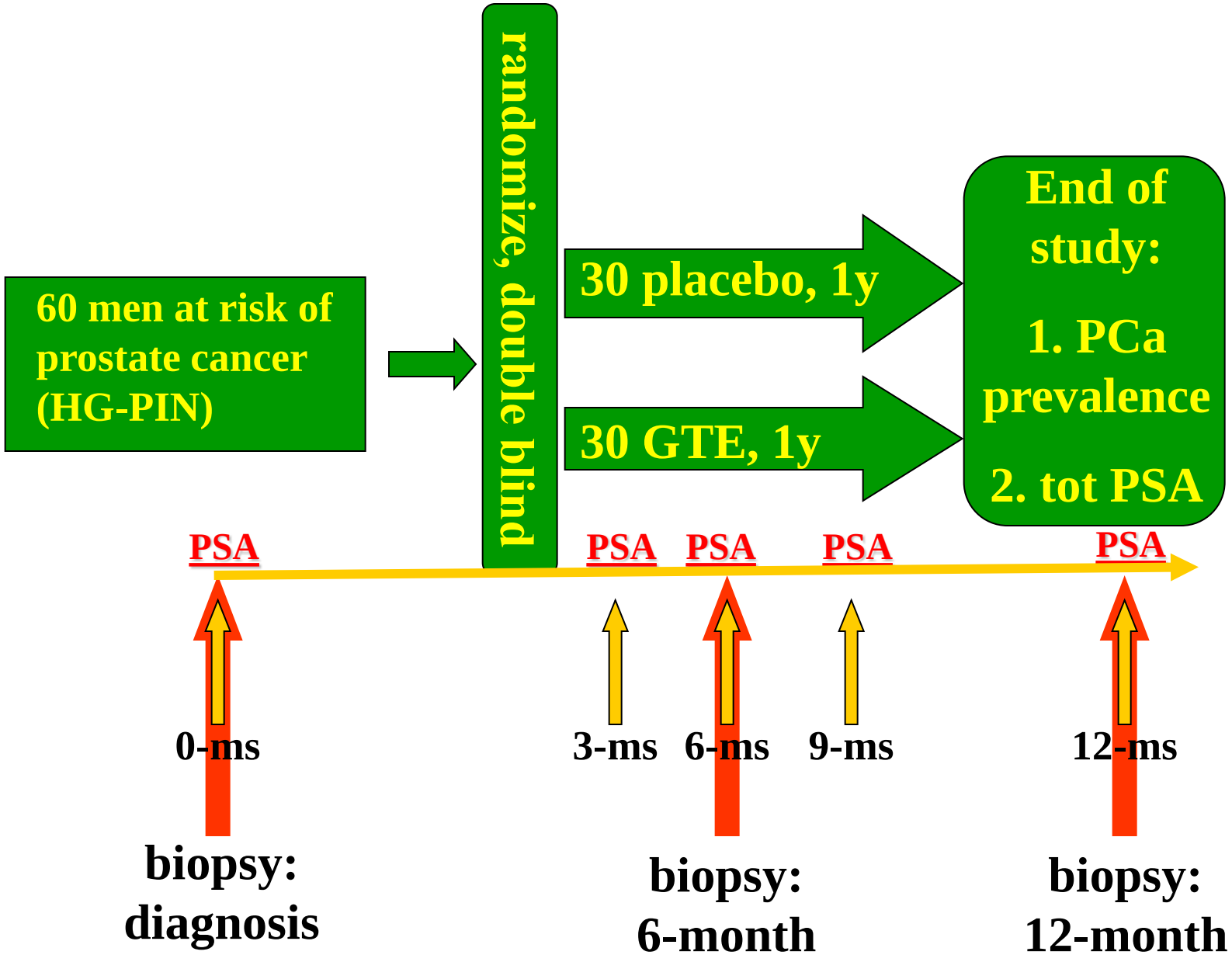
- **HGPIN is considered the most likely pre-invasive stage of PCa.**
At present, no treatment is given to these patients until PCa is diagnosed.

- **Our study is also an attempt to fill this therapeutic void.**

- **The primary end point of the study was the possible difference in the prevalence of PCa in the GTE-treated arm in comparison to placebo.**

- **Possible changes in total serum PSA values were also studied.**

STUDY DESIGN, endpoints: PCa incidence + tot PSA



All subjects were volunteer, aware of the study and agreed to sign an informed consent form.

- **Eligibility criteria: men of 45-75 years of age with HG-PIN found after collection of 8-18 needle biopsies according to prostate volume.**

- **Exclusion criteria: men aged over 75 years, consuming green tea or derived products, vegetarians, taking anti-oxidants and following anti-androgenic therapy.**

- **DOSE: 3 capsules per day containing 200 mg of GTE each, for a total of 600 mg of GTE/day. GTE were given as a high quality preparation whose content was determined by HPLC: EGC 5.5%; EC 12.24%; EGCG 51.88%; ECG 6.12%; total GTE 75.7%; GTE extract was virtually caffeine-free (< 1%).**

In case of cancer diagnosis, subjects were excluded from the chemoprevention trial (→ failure of chemoprevention) and recommended to clinical management.

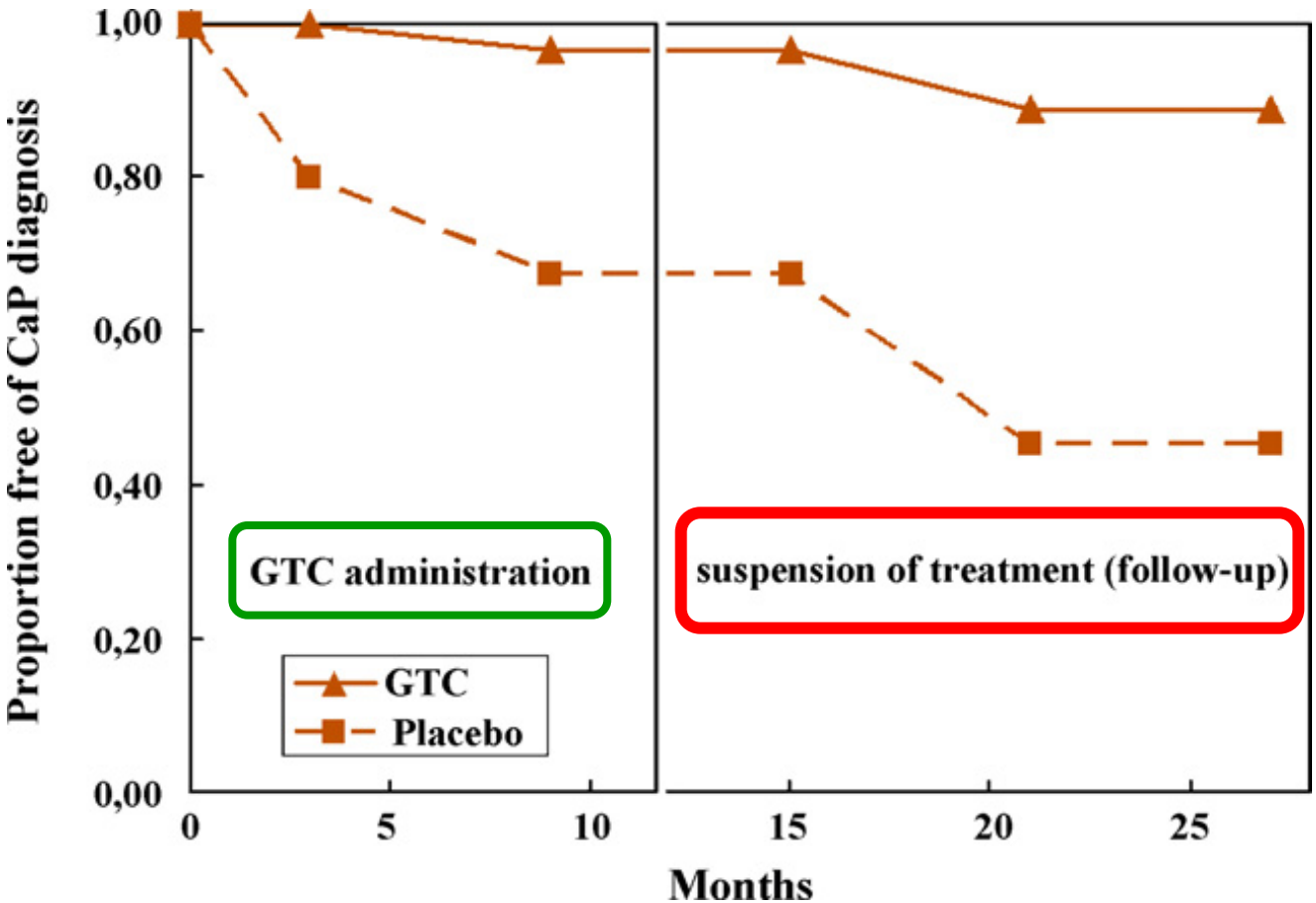
RESULTS (Bettuzzi et al., Cancer Research 66: 1234-1240, 2006)

PREVALENCE OF PROSTATE CANCER
placebo-arm versus GTE-arm
at the 6- and the 12-months biopsy checkpoint
(i.e. at the end of the 1-year study).

ARM	6-months biopsy check	12-months biopsy check	Total number of PCas diagnosed	Total number of recruited subjects	Prevalence of prostate cancer
PLACEBO	6	3	9	30	30.0%
GTE	0	1	1	30	3.3% $p < 0.01$

Chemoprevention of Human Prostate Cancer by Green Tea Catechins: Two Years Later. A Follow-up Update

Brausi M., Rizzi F. and Bettuzzi S. Eur Urol, 2008



GTE: 2

Placebo: 11

Kaplan-Meier analysis of final result showing the prevalence of prostate cancer at 6- and 12-mo check (previous study) and 2 years later after suspension of GTE administration (follow-up study).

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Green tea catechins suppress the DNA synthesis marker MCM7 in the TRAMP model of prostate cancer

Susan McCarthy[1,4], **Andrea Caporali**[2,4], **Steve Enkemann**[1], **Maurizio Scaltriti**[2], **Steve Eschrich**[1], **Pierpaola Davalli**[3], **Arnaldo Corti**[3], **Alice Lee**[1], **Jimmy Sung**[1], **Timothy J. Yeatman**[1*], and **Saverio Bettuzzi**[2*]

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Abstract

Green tea catechins (GTCs) exert chemopreventive effects in many cancer models. Several studies implicate the DNA synthesis marker minichromosome maintenance protein 7 (MCM7) in prostate cancer progression, growth and invasion; representing a novel therapeutic target. In this study, we investigated the effect of GTCs on MCM7 expression in the transgenic adenocarcinoma mouse prostate model (TRAMP). DNA microarray, immunohistochemistry and western blot analysis showed that GTCs significantly suppressed MCM7 in the TRAMP mice treated with GTCs. Our study indicates that the cellular DNA replication factor MCM7 is involved in prostate cancer (CaP) and MCM7 gene expression was reduced by GTCs. Together, these results suggest a possible role

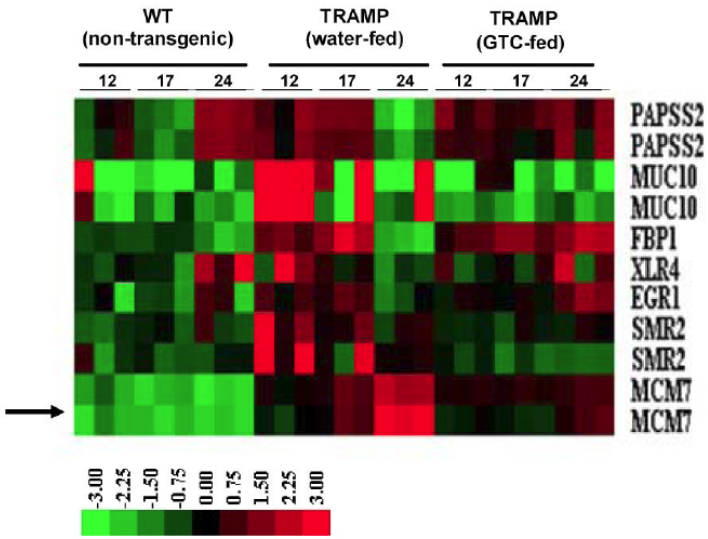
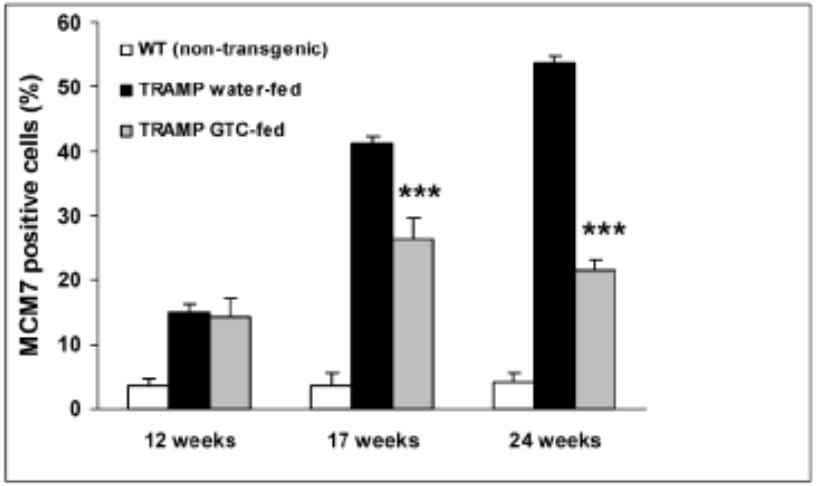
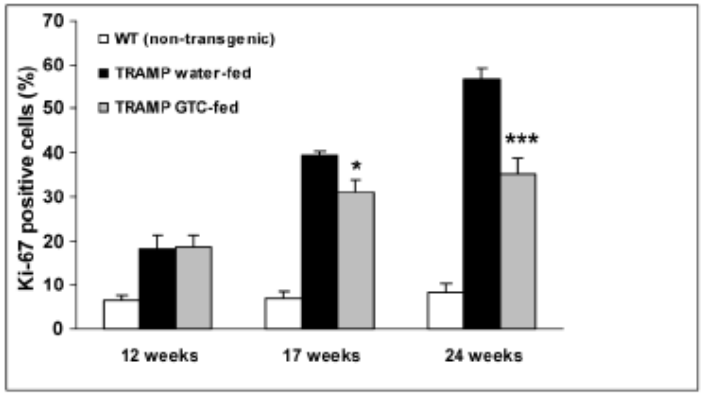


Figure 1.
Gene expression changes in the prostates of WT (non-transgenic), TRAMP water-fed and TRAMP mice treated with GTCs. Gene expression values were normalized, $\log_2$-transformed, and mean-centered to produce relative values. Each column represents pooled samples from 3 different prostate glands (dorsolateral) taken from WT, TRAMP water-fed and TRAMP treated with GTCs at the 12, 17, and 24 week time point. Some genes have multiple Affymetrix probe sets. Genes that are upregulated appear in red, and those that are downregulated appear in green. A color bar (bottom) relates to the intensity of the differences in gene expression.

A.



B.



INTERNATIONAL JOURNAL OF ONCOLOGY 39: 1521-1527, 2011

Chronic administration of green tea extract to TRAMP mice induces the collapse of Golgi apparatus in prostate secretory cells and results in alterations of protein post-translational processing

PIERPAOLA DAVALLI[1*], FEDERICA RIZZI[2*], GAETANO FELICE CALDARA[3], SERENA DAVOLI[2], ARNALDO CORTI[1], ALESSANDRO SILVA[2], SERENELLA ASTANCOLLE[1], MARCO VITALE[3], SAVERIO BETTUZZI[2], MARIALUISA ARCARI[3] and GIACOMO AZZALI[3]

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Carcinogenesis vol.25 no.11 pp.2217–2224, 2004
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The chemopreventive action of catechins in the TRAMP mouse model of prostate carcinogenesis is accompanied by clusterin over-expression

Andrea Caporali[1], Pierpaola Davalli[2], Serenella Astancolle[2], Domenico D'Arca[2], Maurizio Brausi[3], Saverio Bettuzzi[1,4,*] and Arnaldo Corti[2,*]

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Introduction

Prostate cancer (CaP) is the second leading cause of cancer-related death among men in Western countries after lung cancer. Truly organ confined CaP can be cured by radical prostatectomy or radiation therapy, but in the case of advanced cancer, androgen ablation, proposed by Charles Huggins >60 years ago (1), remains the most generally used CaP chemotherapy. Indeed, by causing apoptotic death of androgen-dependent cells, this treatment substantially reduces tumor growth in 70–80% of patients. However, in all too many

Oncogene 28, 4344-4352, 2009

Oncogene (2009), 1–9
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www.nature.com/onc

ORIGINAL ARTICLE

Genetic inactivation of ApoJ/clusterin: effects on prostate tumourigenesis and metastatic spread

S Bettuzzi[1], P Davalli[2], S Davoli[1], O Chayka[3], F Rizzi[1], L Belloni[1], D Pellacani[2], G Fregni[2], S Astancolle[2], M Fassan[4], A Corti[2], R Baffa[4,5] and A Sala[3]

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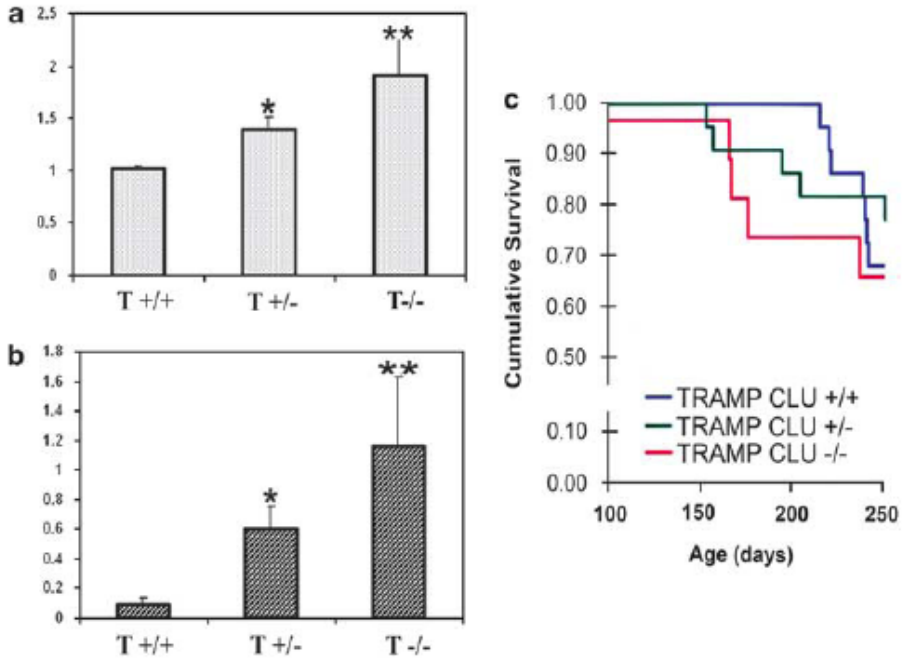
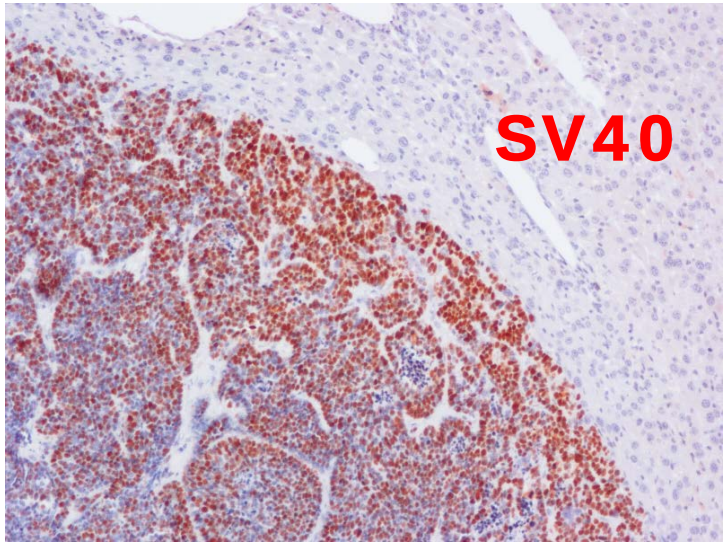


Figure 4 Enhanced tumourigenesis and metastatic spread in TRAMP mice with reduced dosages of ApoJ/Clusterin (CLU). Quantification of primary tumours formation in TRAMP mice according to the CLU genotype. The number of mice for each genotype is the following: wild-type TRAMP (T+/+) $n=43$, TRAMP/heterozygous CLU knockout (CluKo) (T+/−) $n=20$; TRAMP/homozygous CluKo (T−/−) $n=12$. (**a**) The average number of primary tumours per animal is indicated on the y axis. Bars indicate standard deviation. $*P=0.05$; $**P=0.01$, one-way analysis of variance (ANOVA). (**b**) The average number of metastases per animal is indicated on the y axis. Bars indicate standard deviation. $*P=0.05$; $**P=0.01$, one-way ANOVA. (**c**) Kaplan–Meier analysis of TRAMP/CluKo mice survival. Log-rank test indicates that there is a significant difference in survival up to 200 days from birth (+/+ vs −/−, $P=0.006$; +/+ vs +/−, $P=0.038$). Afterwards the difference becomes non-significant and the curves start to merge.

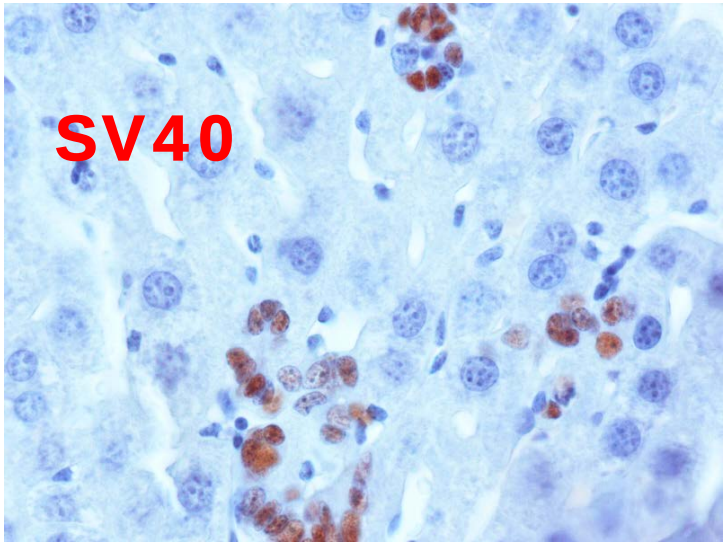
Metastatic behaviour

TRAMP/CluKO +/- (heterozygous)



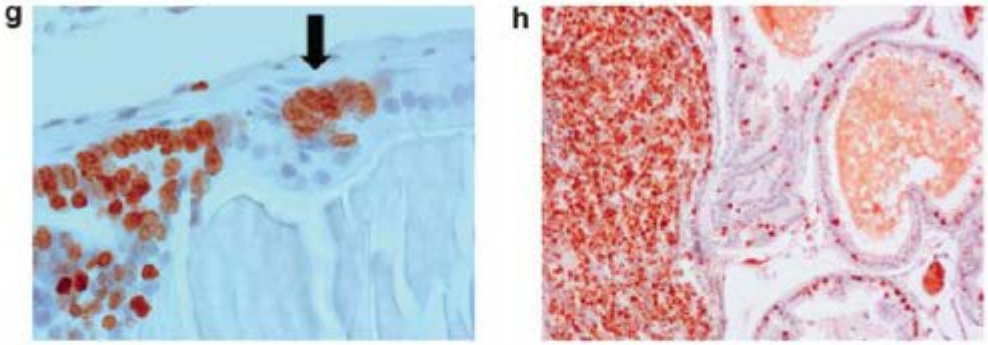
Tendency toward differentiation

TRAMP/CluKO -/- (homozygous)



Highly invasive: micrometastases

Ki67



Ki67

Figure 5 Increased Ki67 staining in the untransformed portion of prostatic tissue of TRAMP/CluKo mice. (**a**) PIN (prostate intraepithelial neoplasia) lesion in a wild-type TRAMP prostate, ×10; (**b**) and (**d**) cribriform prostate cancer in a TRAMP mouse (**b**, ×10; **d**, ×40); (**c**) poorly differentiated prostate cancer in a TRAMP mouse, ×20; (**e**) normal prostate tissue and adjacent adenocarcinoma in a TRAMP/CluKo+/− mouse, ×20; (**f**) normal prostate tissue and adjacent adenocarcinoma in a TRAMP/CluKo−/− mouse, ×10; (**g**) detail showing a nest of actively proliferating cells in the prostate of a TRAMP/CluKo+/− mouse, ×40; (**h**) undifferentiated prostate carcinoma (left) highly reactive to the Ki67 antigen detected in the same prostate shown in **f**, ×10.

The TS Needle innovative technology

The **Twin Sample (TS) biopsy needle** http://twinsample.com/ from EPITOME (www.epitomepharm.com/research07.htm)

The TS needle retrieves **two adjacent samples in one pass**: one for the pathologist, while the second is used for molecular assays.

Epitome Pharm. Ltd is partnering with Gallini SpA (Mirandola) and Kaneco (Japan) to produce TS needles.

The Twin Sample needle is manufactured under **GMP** and posses a **FDA approval** and **CE Mark**.

File Modifica Visualizza Cronologia Segnalibri Strumenti Aiuto
Twin Sample™ Biopsy Needle
twinsample.com
Twin Sample (TS) biopsy needle
Più visitati Come iniziare

Twin Sample™
Biopsy Needle

Addresses the need for fresh tissue for molecular assays.

Two samples from one pass, one for pathology and one for your lab.

- Operates like a conventional biopsy needle – no training needed.
- Available in typical lengths and gauges
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- ASCUT disposable automatic actuator
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Paired samples with minimal intrusion

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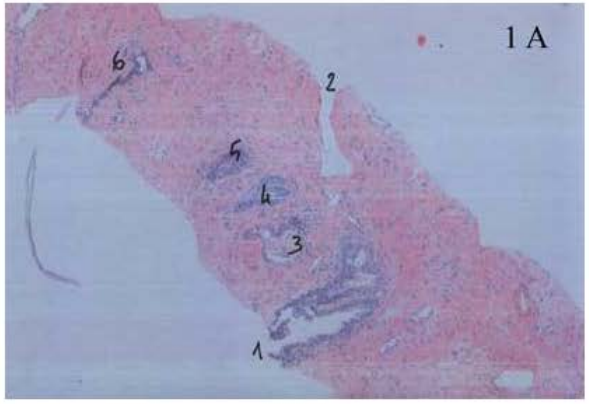

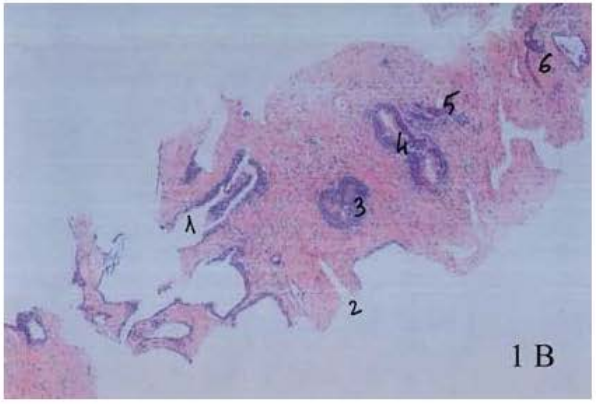

Slides from twin samples of fresh ex-vivo prostate.

One sample satisfies pathology and ethical requirements for patient treatment, leaving the second available for molecular assays. Since the second mirrors the first, the pathologist's findings illuminate areas of interest, as illustrated above.

Sample Custody

Your own sample is sent from the biopsy procedure directly to your lab. No more tracking a single biopsy or finding the remaining block inadequate. Make getting tissue from every biopsy, on every patient, routine.

High quality tissue

Fresh tissue stays intact in modern transport media. A median size of 5 mg per sample provides enough mRNA for a complete gene expression assay.

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Gene signatures

Sono un gruppo di "**geni informativi**" che sono coordinatamente co-espressi e correlati ad una particolare situazione biologica.

Shaffer A.L. et al. "Signatures of the Immune Response" Immunity 15, 375-385 (2001)

I predittori non sono geni singoli, ma le componenti della "gene signature".

Importanti per scopi **diagnostici**, possono fornire informazioni indirette riguardo a particolari meccanismi biologici.

Usate per costruire modelli di **evoluzione clinica: prognosi**, tempo di **sopravvivenza**, **risposta a chemioterapia**.

Conta il livello di **espressione genica** (dato quantitativo).

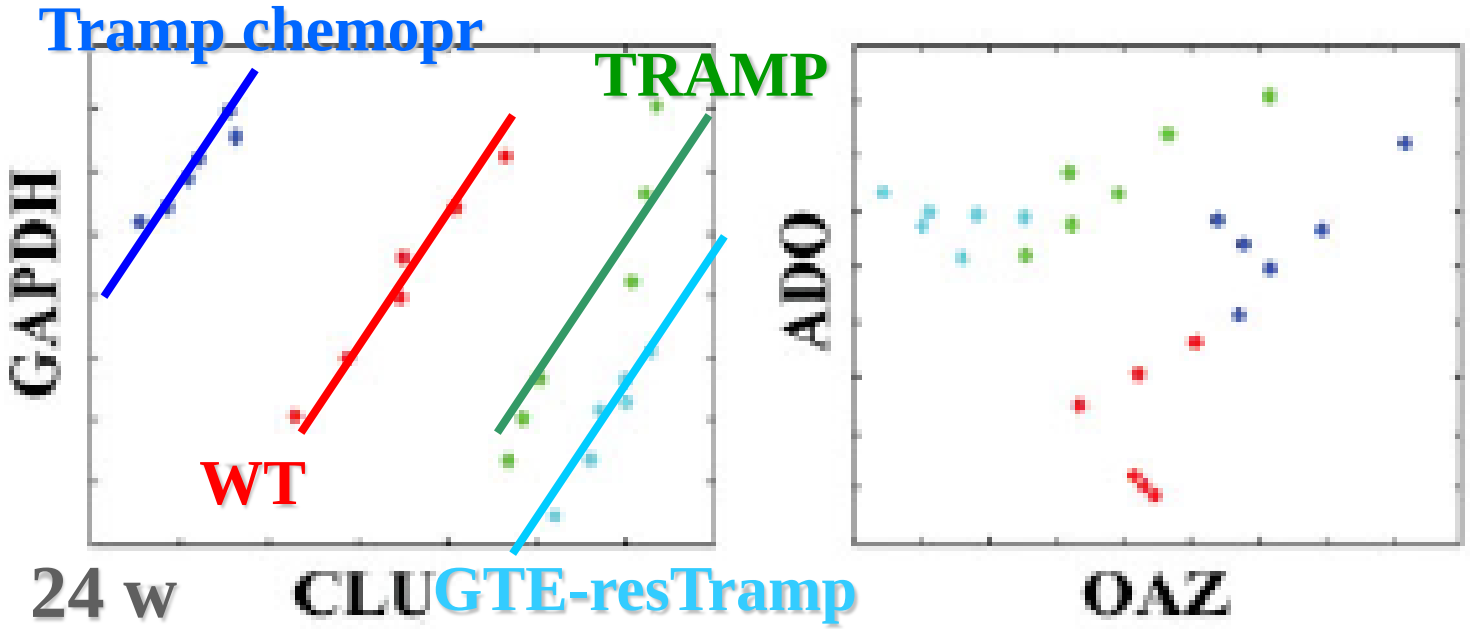
Fondamentale è la **correlazione statistica**, mentre non è determinante il nesso causale/meccanicistico/biologico.

Importantissima la qualità del campione biologico.

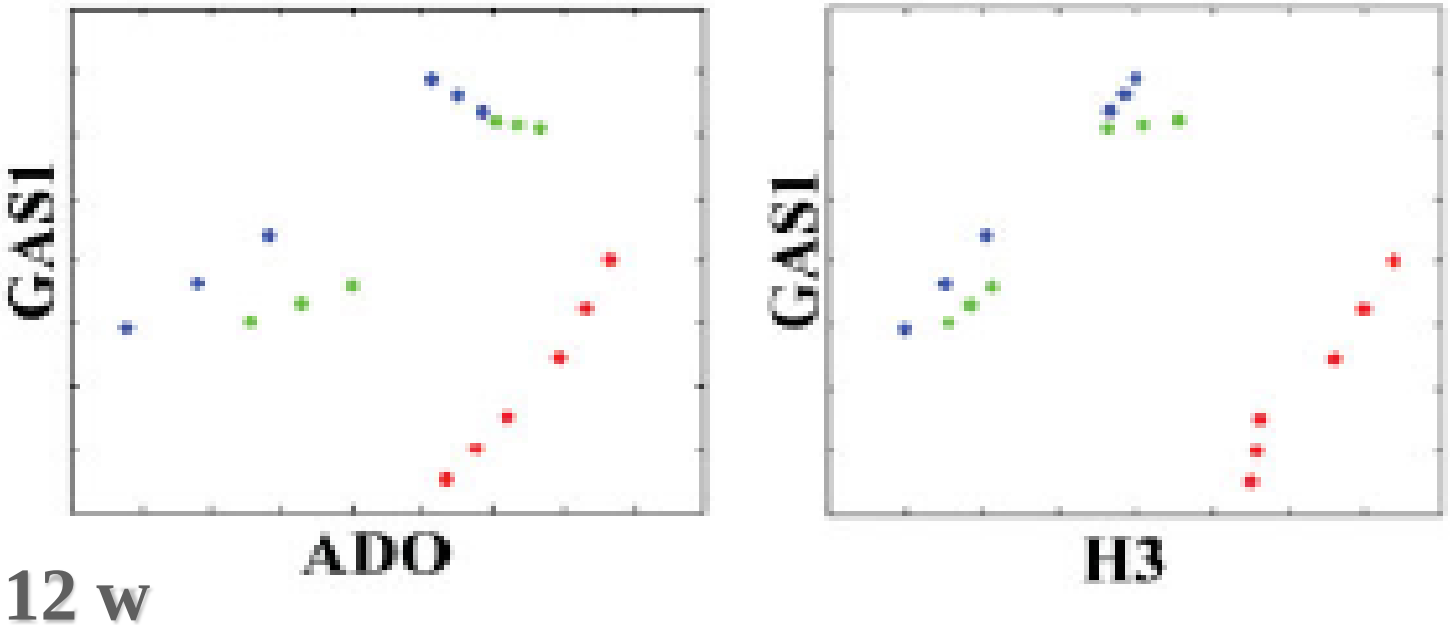
Medicina di precisione, medicina personalizzata.

Real-Time quantitative PCR

Gene signature is made of 8 different genes including CLU



LDA with different combinations of 2 genes



Gene signature for PCa

by real time quantitative PCR (RT-qPCR)

- **Diagnostic power:** validated in more than 250 patients; fresh tissue specimens and FFPE (Rizzi et al PLoS One 2008)

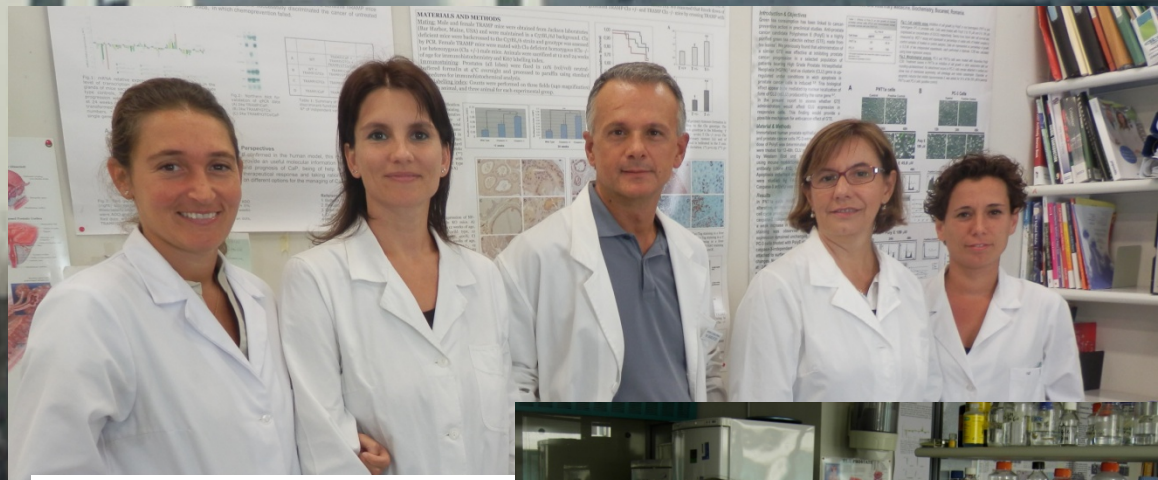
- **Prognostic power:** published, proof of principle trial: prediction of recurrence in 95.7% of patients; n = 23; only 1 missed; follow-up 5 years.
  (Bettuzzi et al Cancer Res 2003)

- **Prognostic power:** validation ongoing in 300 patients, 10 years follow-up, from pathology archives

BIOCHEMISTRY, MOLECULAR AND TRANSLATIONAL ONCOLOGY

Dept. of Medicine and Surgery (DiMeC)

University of Parma, Italy



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