Tumour Oxygen, DNA Repair and Prostate Cancer: The *LEGEND* Program

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University Health Network

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Spatio-Temporal Targeting Amplifying Radiation Response

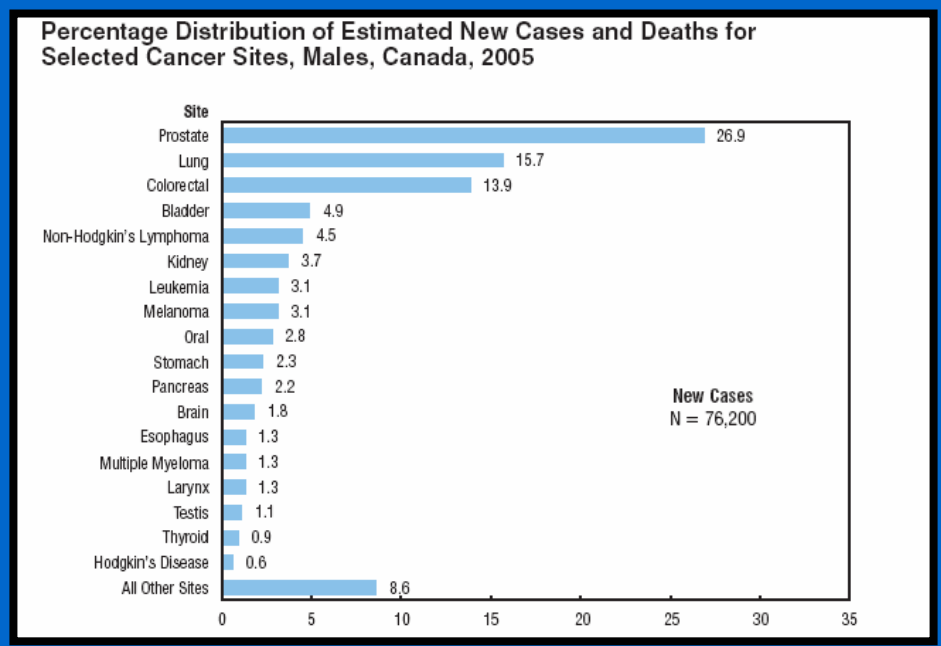
Canadian Cancer Society / Société canadienne du cancer

HP5 PMH-Terry Fox Hypoxia Program

Canadian Cancer Society / Société canadienne du cancer

Prostate statistics:

- most common cancer in men with 20,500 men diagnosed and 4,300 dying in Canada in 2005
- 1 in 7 men over the age of 70 will develop prostate cancer during their lifetime; mostly after the age of 70. 1 in 26 will die from it.



The Changing Statistics of Prostate Cancer

- Men under the age of 60 are fastest growing group of patients
- Men are being diagnosed with earlier stage tumours and at lower PSA values (< 10ng/ml)
- Death rate for prostate cancer is decreasing over the last decade
  - Earlier detection
  - Better treatments

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Risk Groupings and Prognostic Factors

PROGNOSTIC FACTORS

- Traditional: T-stage, PSA, Gleason Score
- Newer: Percent Positive Biopsies, Ki-67, PSA DT < 10 months
- Promising: **p53,** BAX-BCL2, EGFR,MDM2, SURVIVIN, **$p16^{INK4a}$, Hypoxia, Repair**

RISK GROUPS

- LOW: T1/T2; PSA <10; GS 4-6 (*Brachy, EBRT; Surgery, WW*)
- INTERMEDIATE: T1/T2; GS 7; PSA 10-20 (*Brachy/EBRT +/- Hormones; Surgery, WW*)
- HIGH: PSA > 20; GS 8-10; T3-T4 (*EBRT + Hormones+/- Chemo; rarely surgery)*

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Key Concepts & New Approaches in The 21st Century: Individualization

- Build on Radiotherapy **Technical Precision** with **Biological Precision & Escalation**
- Use **Genetic Fingerprinting** for individualized treatment choices and **Preventing Side-effects**
- Develop **Molecular-Targeted Drugs** to add to precision radiotherapy +/- surgery/chemo
  - Added value to current radiotherapy-chemotherapy programs in head and neck cancer, cervix cancer, lung cancer, brain cancer and bladder cancer

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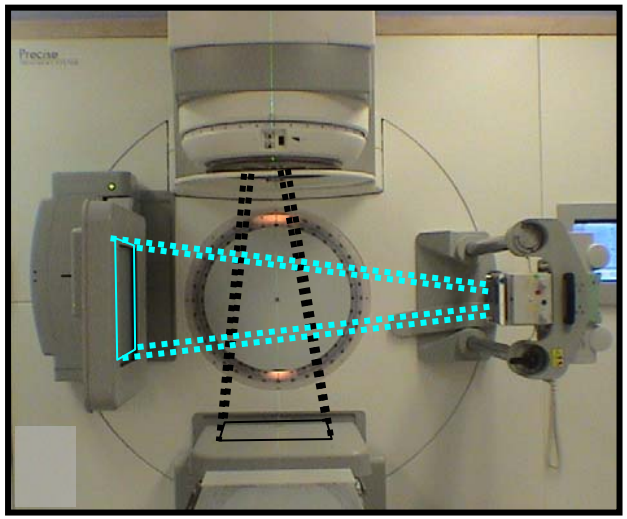
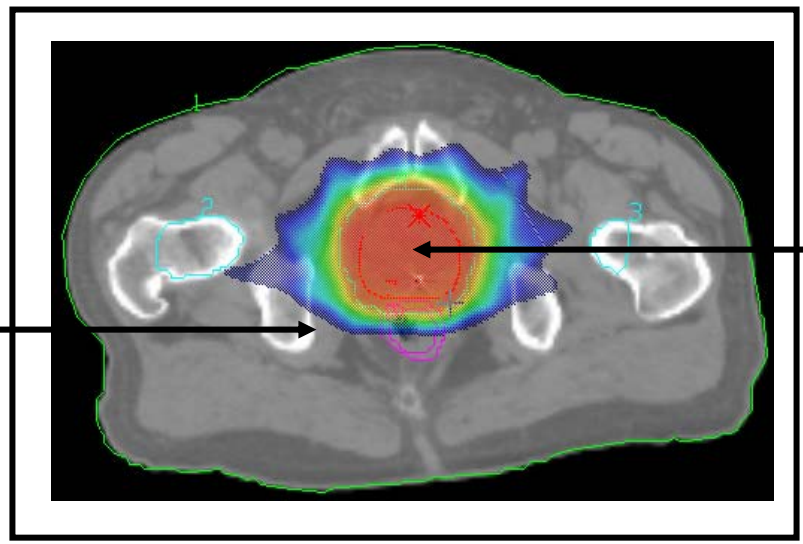
Precision-Guided Radiotherapy to Kill Cancer Cells and Protect Normal Cells





Low Dose To Normal Cells

High Dose To Cancer

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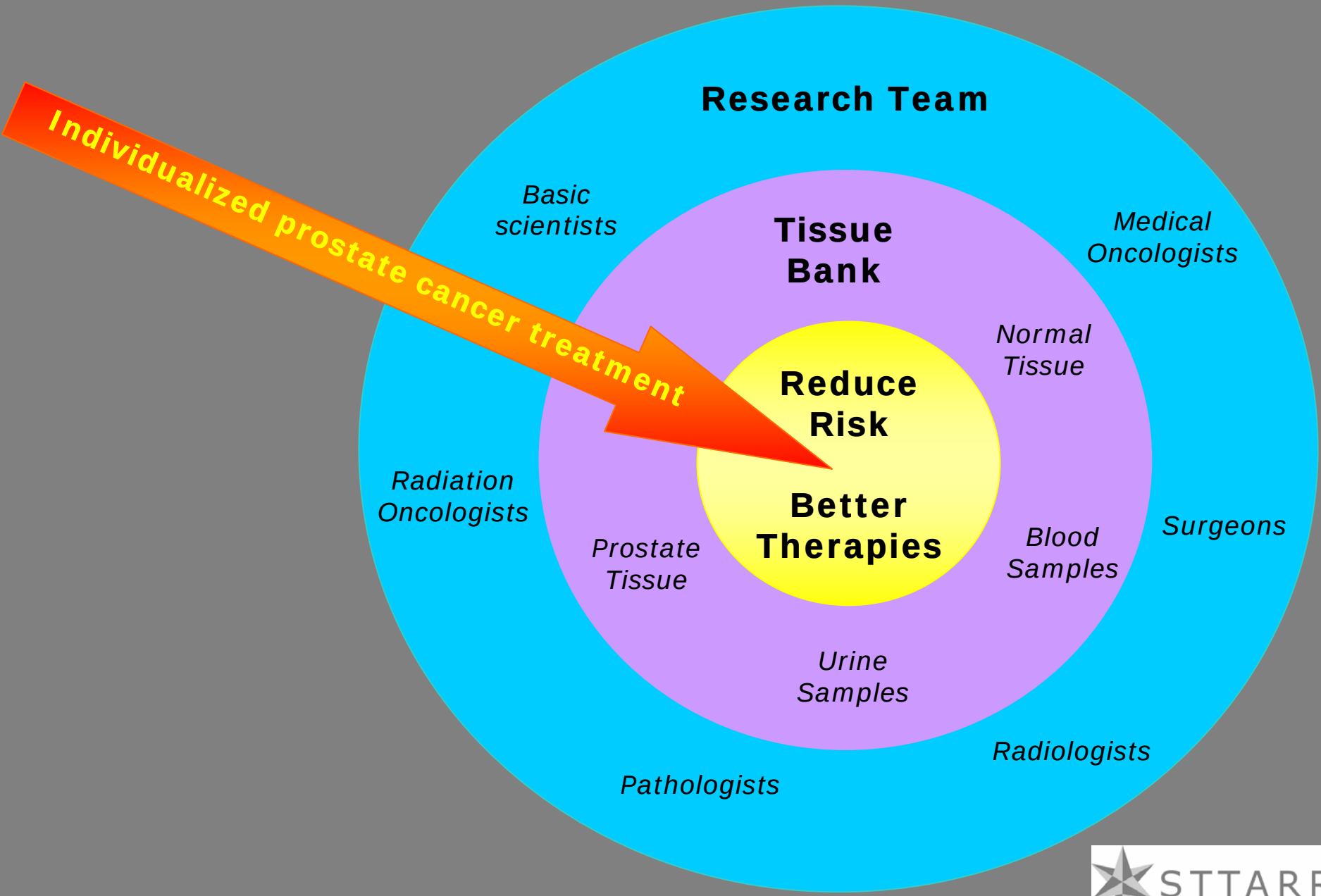
The PMH Prostate Program



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The New Era of Prostate Cancer Research

- The 20th century approach to cancer: Seek and destroy
- The 21st century approach: target and control
- Personalized genetic medicine
- To treat patients with fewer side effects.
- To prevent deaths in patients who are currently incurable.

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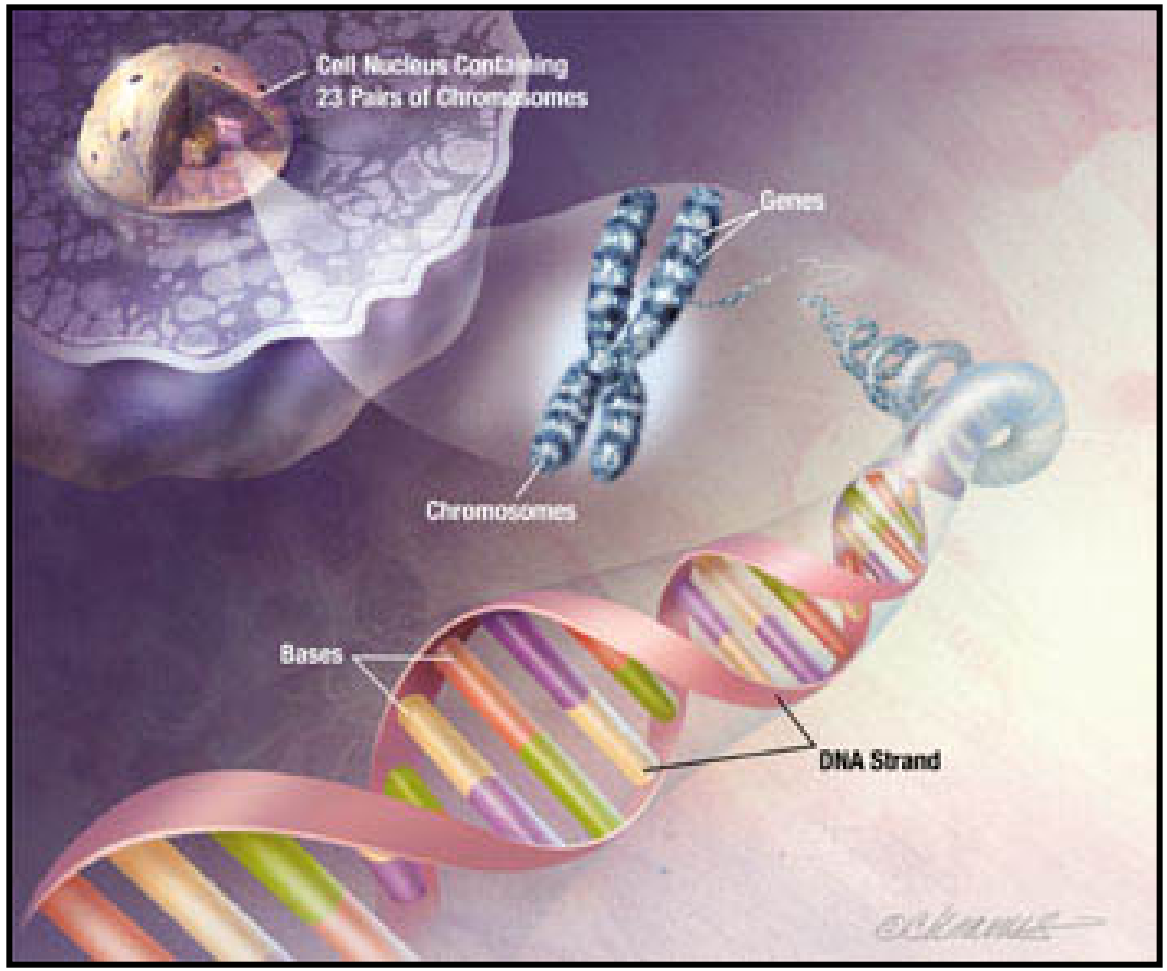
Genetic Studies: Chromosomes and DNA



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GENETICS OF PROSTATE CANCER

- Men with a brother or father affected are twice as likely to develop prostate cancer
- Men with 3 affected first-degree relatives are 11 times more likely to develop prostate cancer than other men.
- Earlier age of onset ?
- Genes (multiple-not one and environment) ?

The study of DNA and genetics can give information regarding the **risk** of prostate cancer, the **aggressiveness** of prostate cancer and the **response to therapy** (RT, HT, CT)

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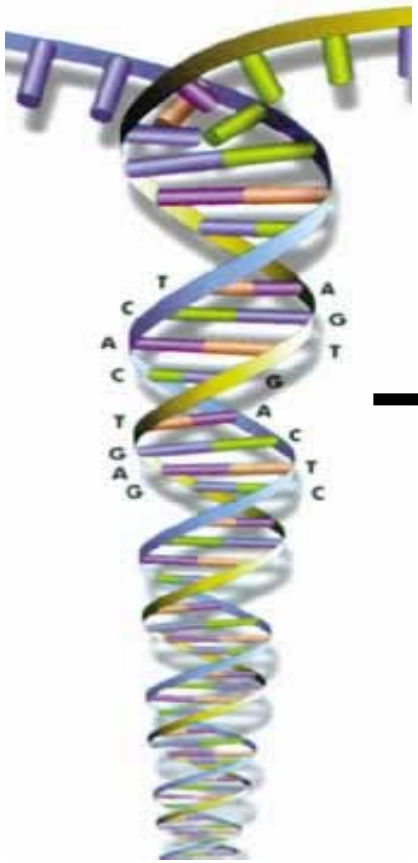
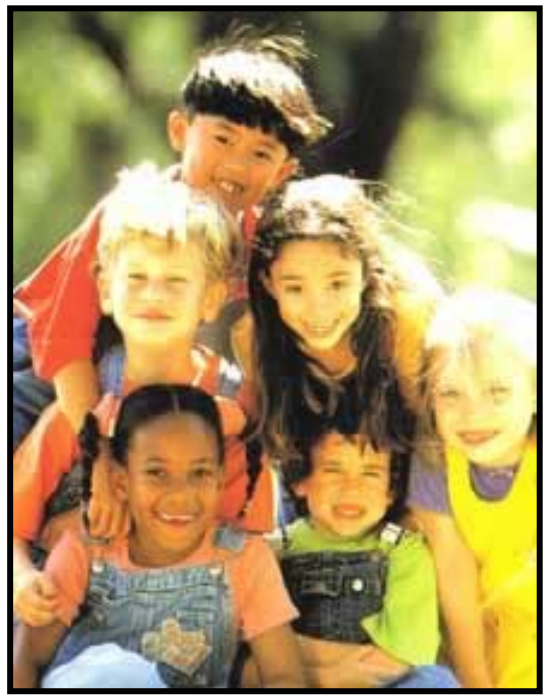
GENETIC MUTAIONS & PROSTATE CANCER





However, the letters (bases) of the code can be scrambled or mutated in cancer cells and alter the cell's behaviour

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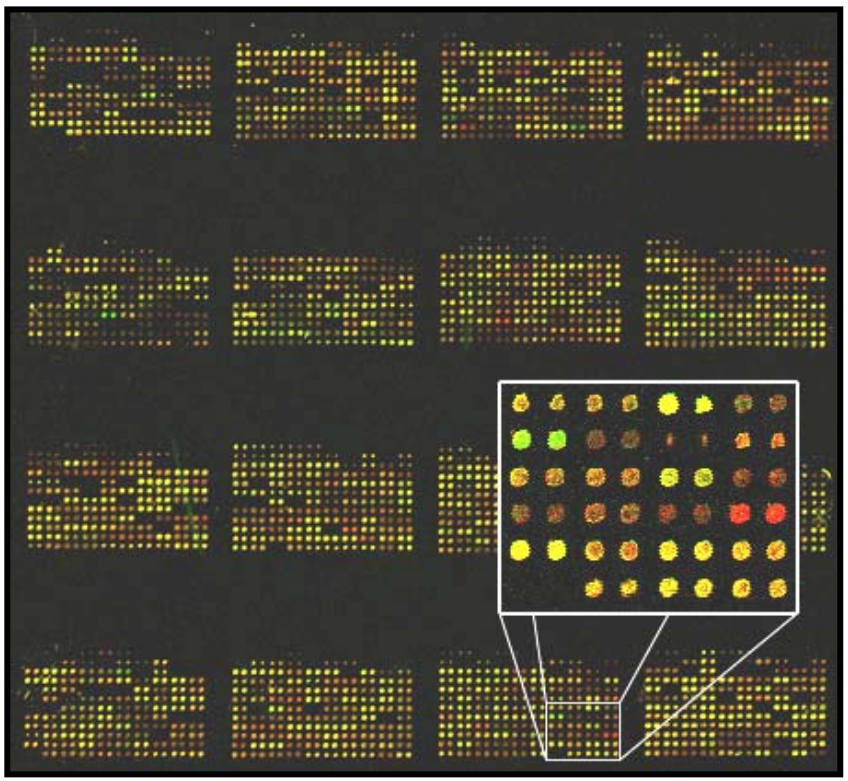
Gene Profiling: Like Fishing-BUT in a Fish Farm (rather than *Expedition*)



Genetics: gives extra information beyond Gleason score and PSA-an individual signature!

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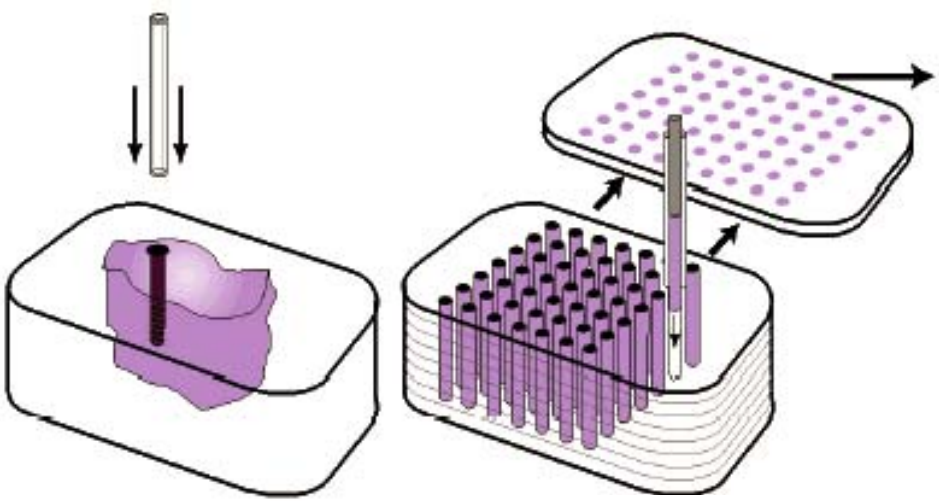
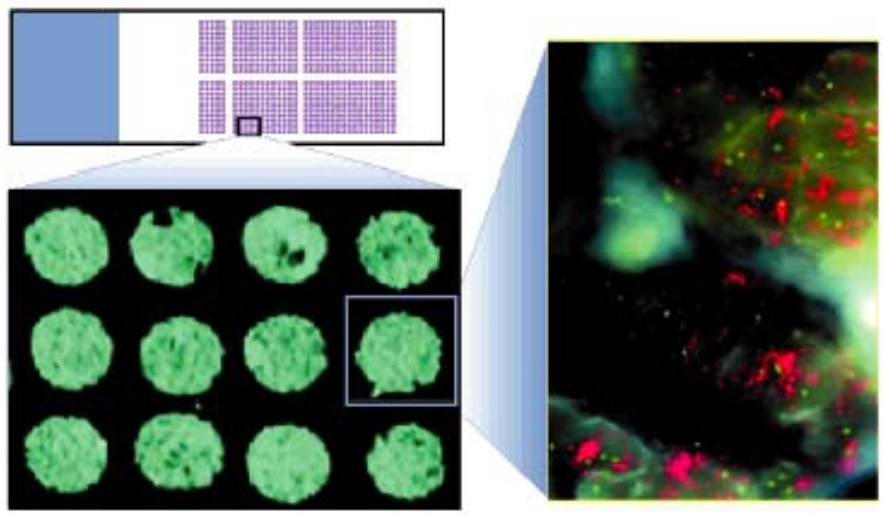
Tissue Arrays to Compare Similarly-Treated Patients:

"Why do some patients respond and others do not ?"



One Patient's Tumour → Hundreds of Patients' Tumours →



Test Significance of New Markers

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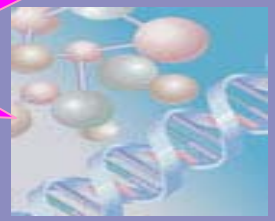
Individualization of Prostate Cancer Treatment

Prostate Biomarkers

Tissue analysis

Novel Therapies



Genetics & Family History

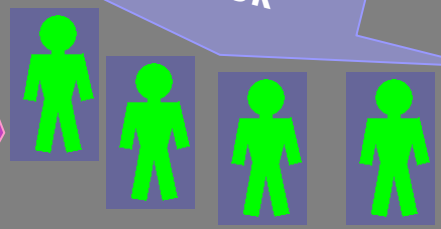
Medical History

Novel Therapies

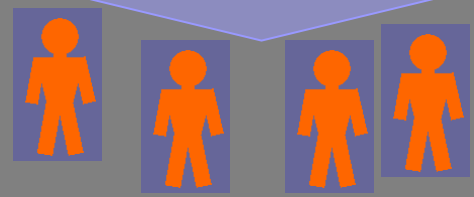
Predict Increased risk

Predict Resistant prostate cancer

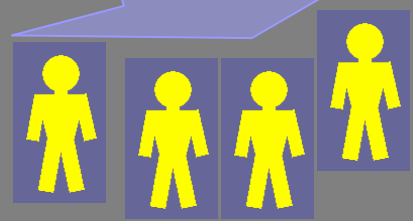
Predict Increased Side Effects



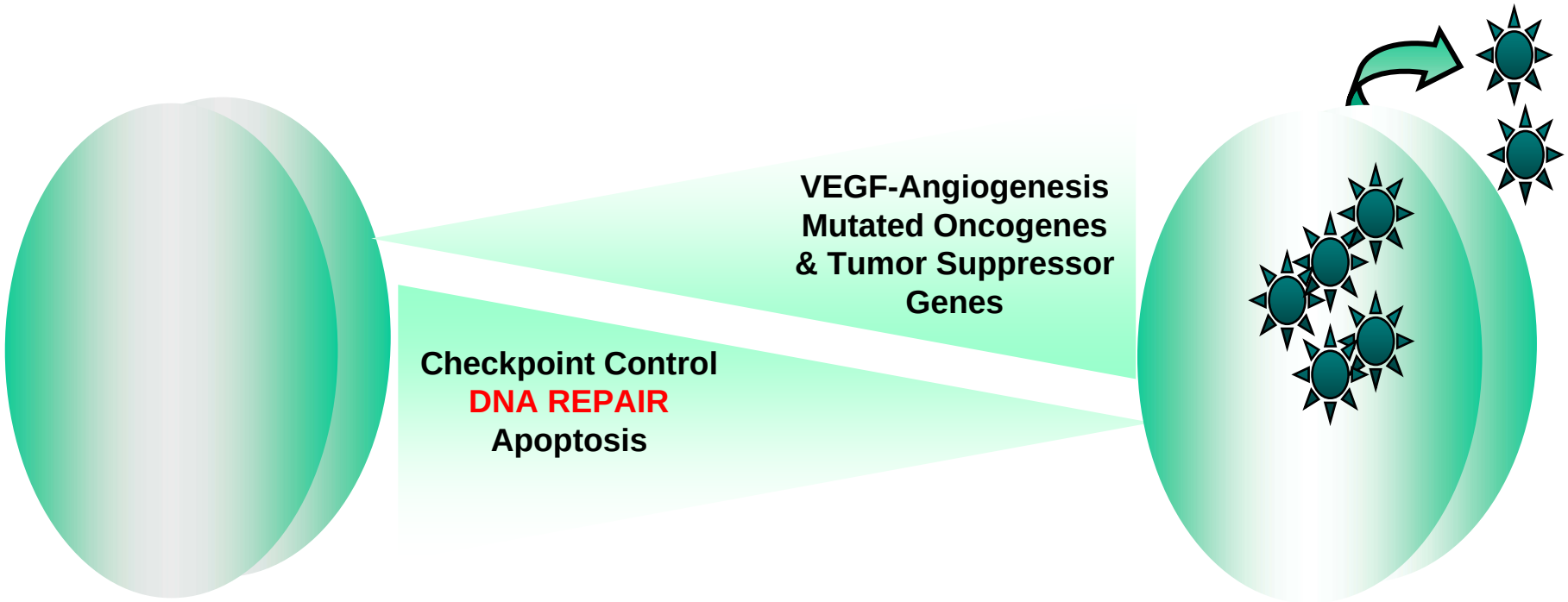
Prevent Cancer with Chemoprevention



Improve Cancer Cure with New Treatments



Improve Quality of Life with Targeted Therapies



Pre-Cancer
(Increasing Mutations)

Increasing Hypoxia

- **Anti-VEGF/HIF1α therapies**
- **Precision Radiotherapy to hypoxic areas**
- **Hormone Therapy**
- **Target Repair-deficient cells**
- **Hypoxic cell toxins (e.g. tirapazamine)**

Aggressive Cancer
(Fast growing; Hormone-Independent; Radioresistant and Metastatic)

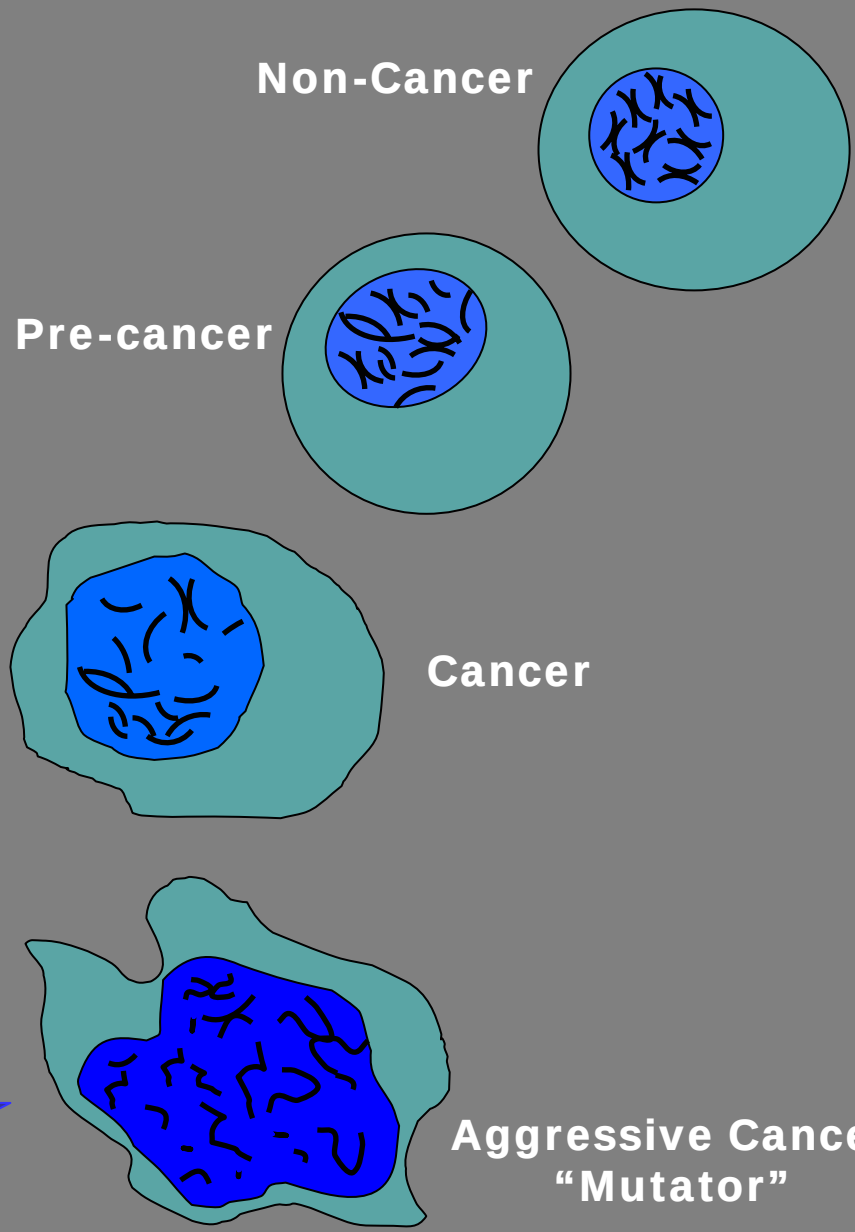
Chan, Milosevic and Bristow, Future Oncology, 2007

LEGEND Cell & DNA Repair Program

Faulty Repair of DNA

Primary and Secondary Mutations in Growth Regulating Genes

Low Oxygen Levels and other Factors

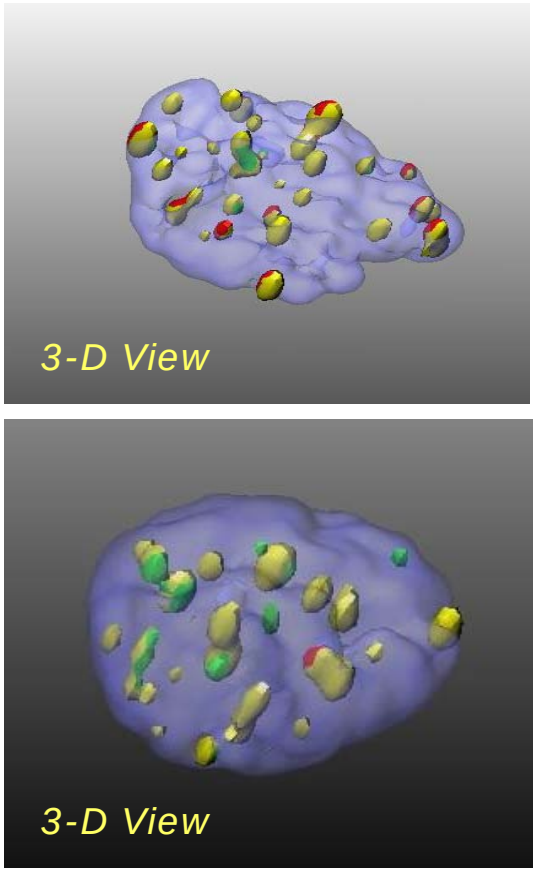


New Therapies

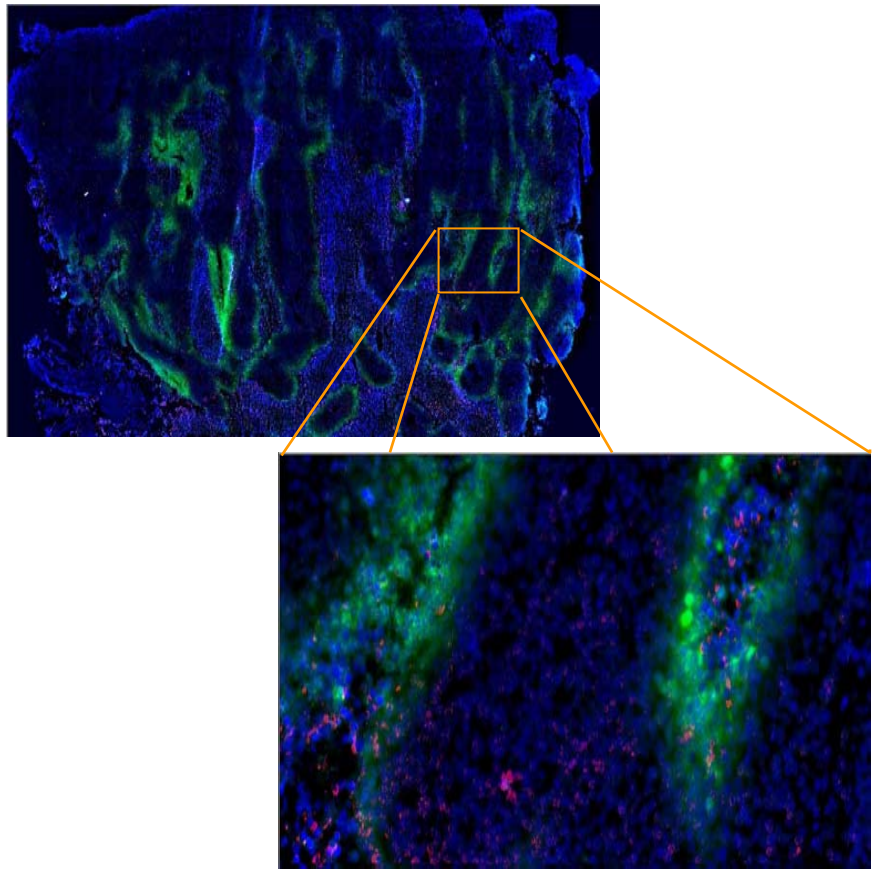
Improve Cell Repair or Kill Poorly Repairing (Mutator) Cells = Reverse Cancer Back to Non-Cancer (Chemoprevention)

Kill Mutated Cells and Hypoxic Cells = Prevent Local resistance and Distant Spread (Novel Drugs)

Discovery of New Repair Biomarkers



Cellular Biomarkers of Repair



Tissue Biomarkers of Repair and Hypoxia

The *LEGEND Repair Program* is developing new ways to determine the repair capacity of normal and cancer cells. On the ***left*** are two cells in 3-D where the individually coloured ovals are DNA breaks being actively repaired in the cell nucleus. On the ***right*** are the cells at a tissue level where the repair (in red) can be tracked in oxic or hypoxic cells (green). Analyses of these repair factors in patients may help determine cancer risk and response to therapy.

DNA Repair and Prostate Cancer

- Faulty DNA repair may give rise to prostate cancer pre-malignant cells which are genetically unstable = **MEASURE OF RISK ?**
- An increasing cascade of mutations in the prostate cell's DNA leads to the cell transforming into cancer = **MEASURE OF AGGRESSION ?**
- Similar mutations may also determine the response of the prostate cancer to radiotherapy, hormone therapy or chemotherapy = **MEASURE OF RESPONSE ?**

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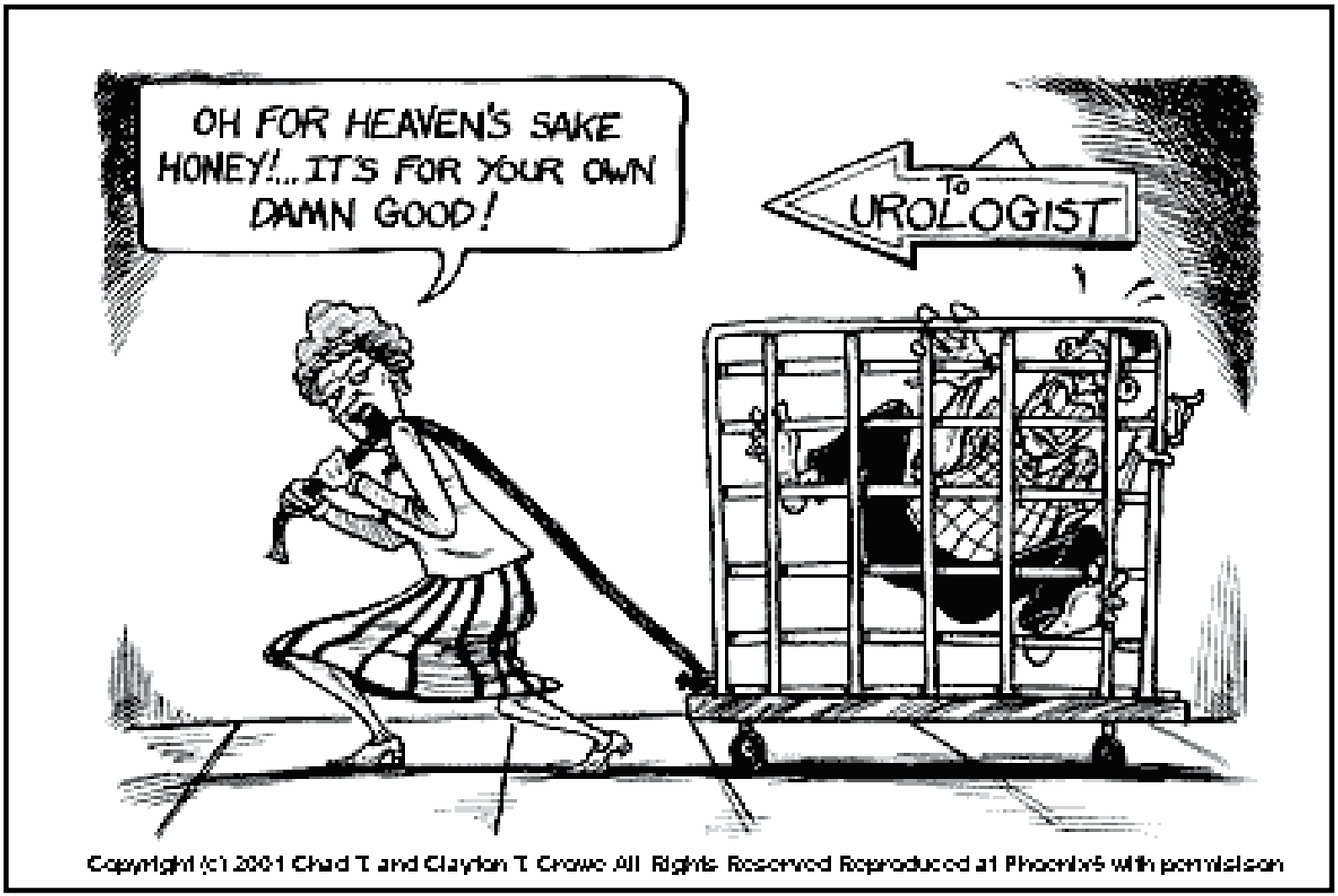

DNA Repair and Cancer Therapy: Key Questions

- Is the level of DNA-dsb repair gene expression different in normal and cancer cells ?
- If so, are there new treatments that could be deigned to targeting faulty DNA repair as a new strategy ?
- How could we track DNA repair in cancer cells with new drugs plus radiation ?

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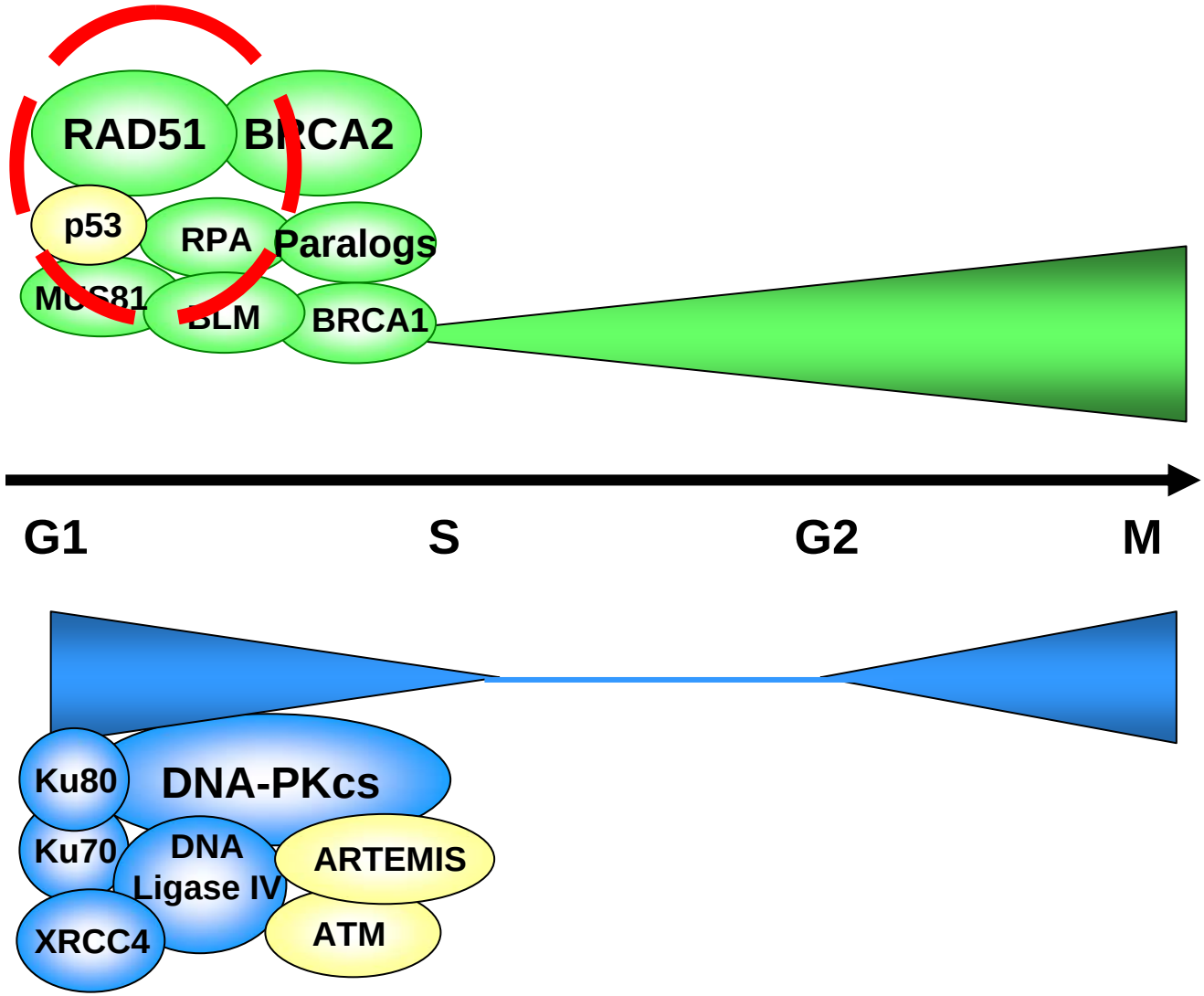
DNA-dsb Repair Pathways



HR:

- Homologous template
- Error free
- S & G2 predominates

NHEJ:

- No homology
- Error prone
- G1 predominates

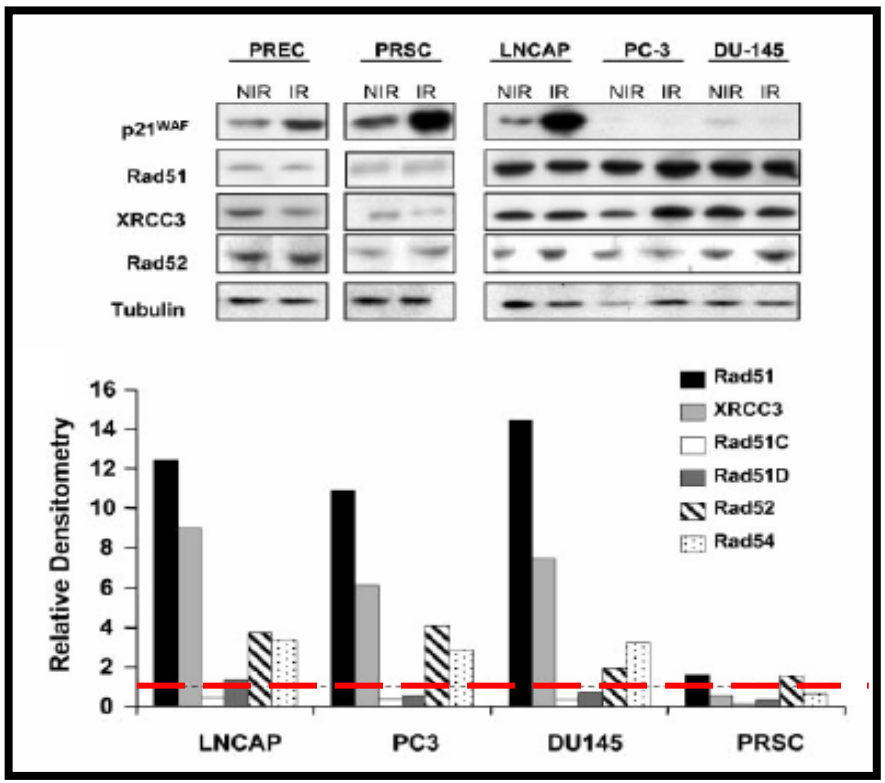
Important for Carcinogenesis/Aggression/Response !

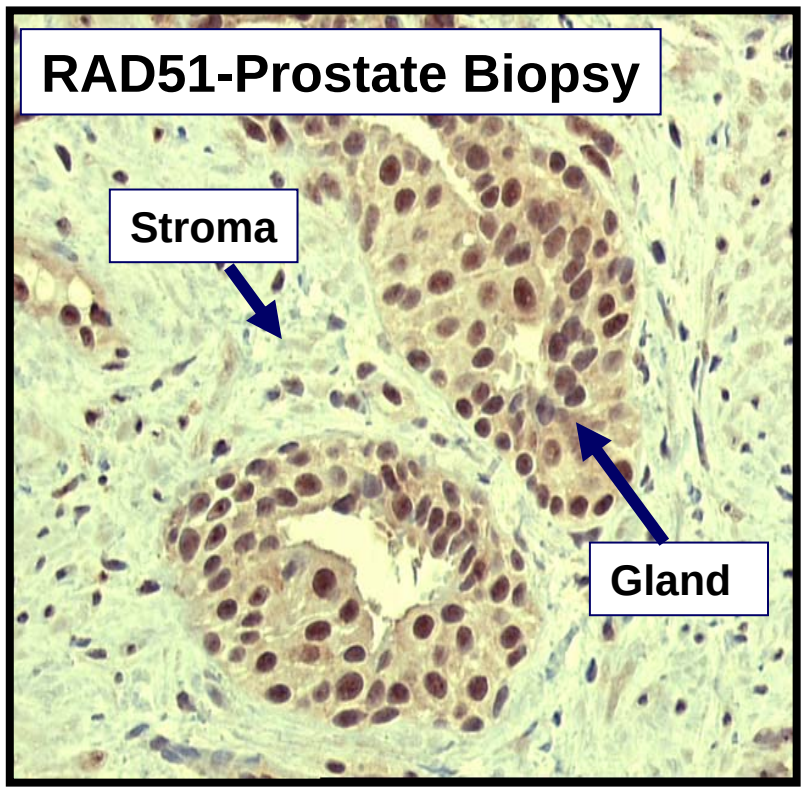
[CANCER RESEARCH 64, 8526–8533, December 1, 2004]

Defective DNA Strand Break Repair after DNA Damage in Prostate Cancer Cells: Implications for Genetic Instability and Prostate Cancer Progression

Rong Fan,[1] Tirukalikundram S. Kumaravel,[1] Farid Jalali,[1] Paula Marrano,[1] Jeremy A. Squire,[1,3,4] and Robert G. Bristow[1,2,4]

[1]Ontario Cancer Institute/Princess Margaret Hospital, University Health Network; and Departments of [2]Radiation Oncology, [3]Pathology, and [4]Medical Biophysics, University of Toronto, Toronto, Ontario, Canada





Fan, Bristow et al. Cancer Res; 2004
Cole, Sweet, Phan, van der Kwast, Bristow-2006

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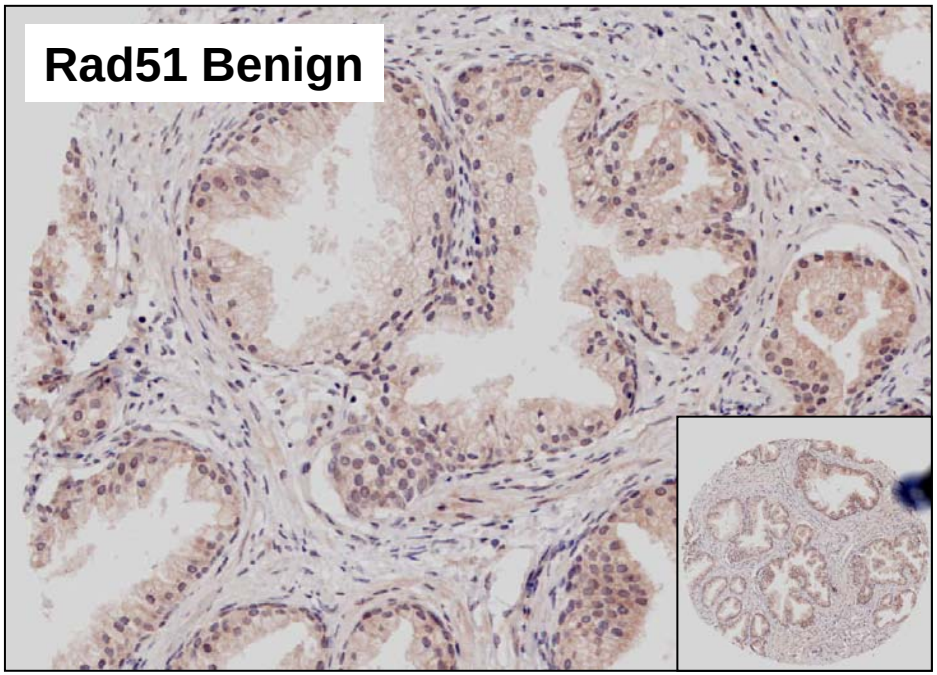

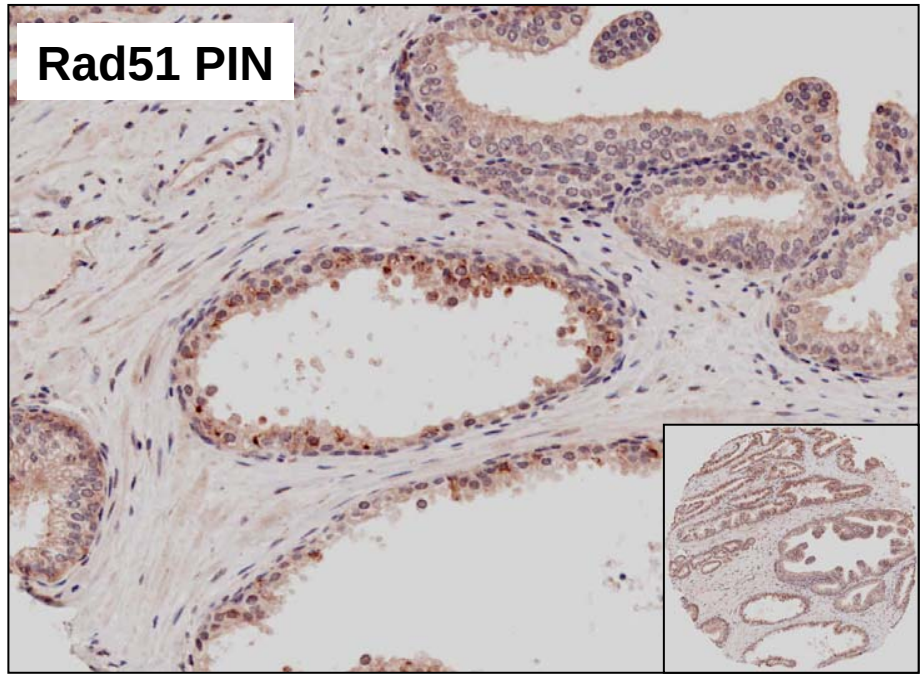

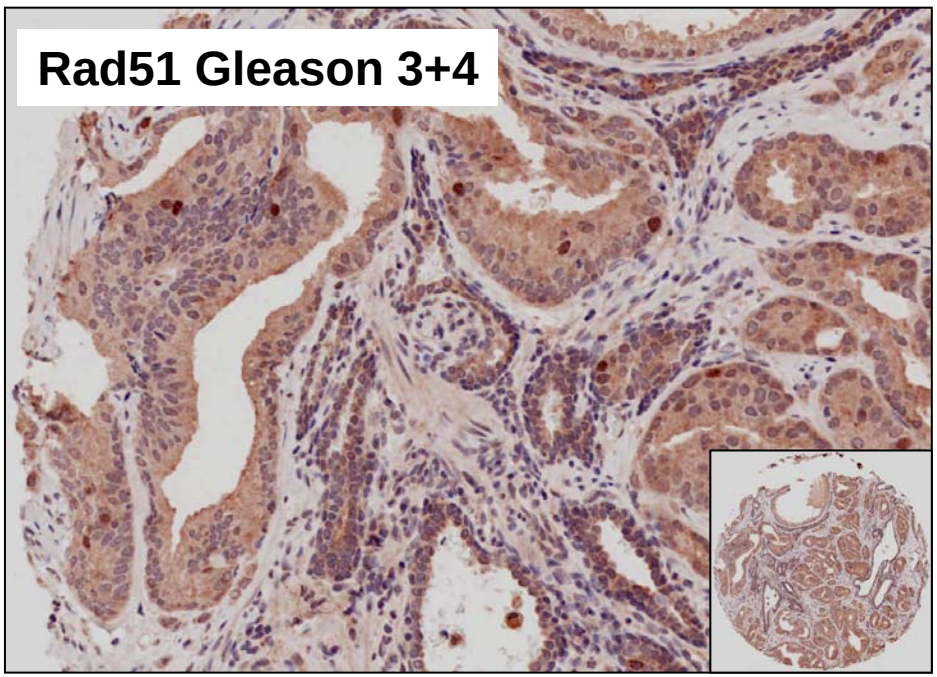

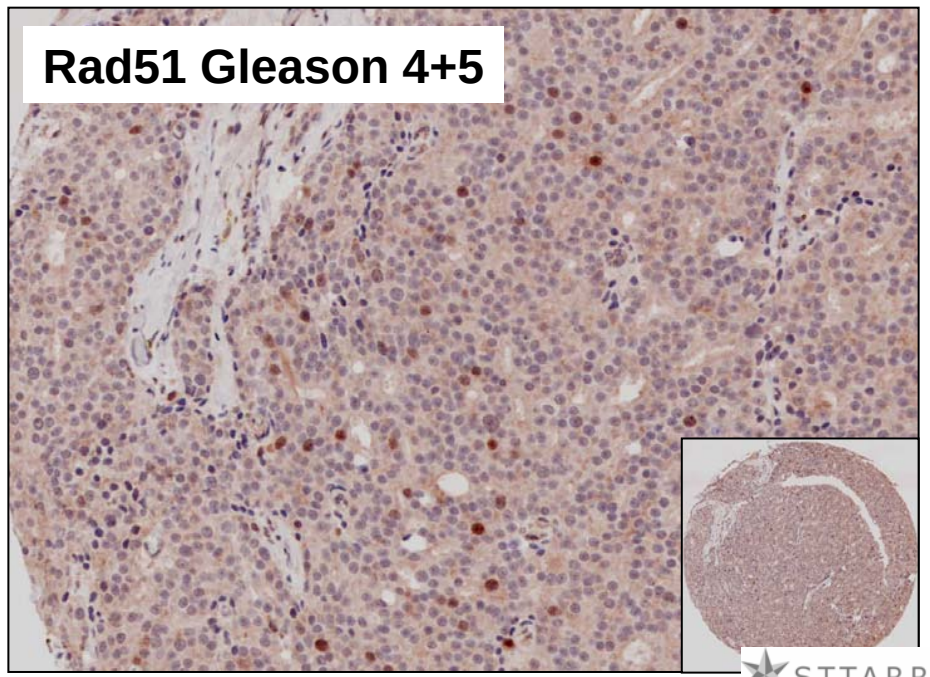

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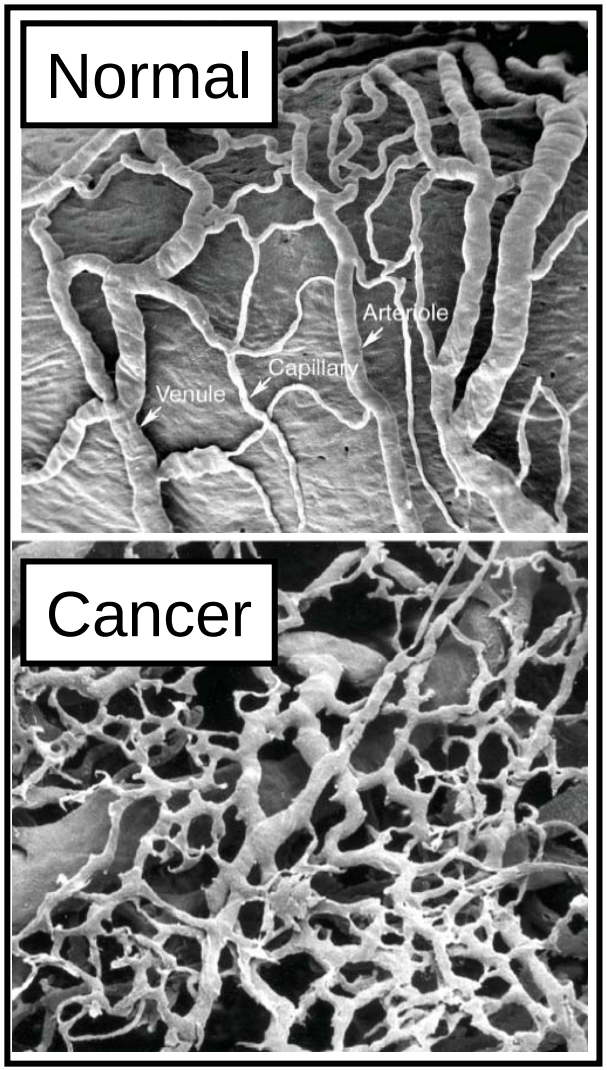
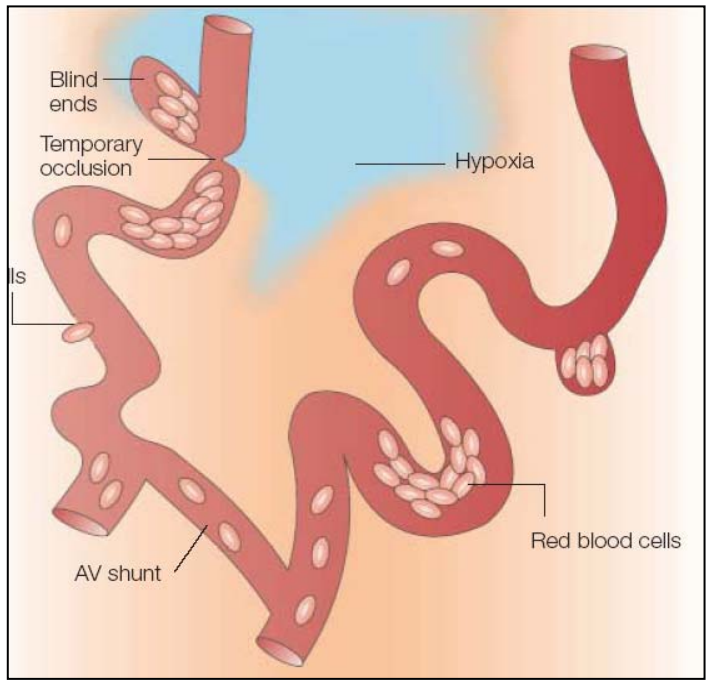
Aggressive Cells Develop in Poorly-Oxygenated (Hypoxic) Prostate Cancer Cells: Why ?

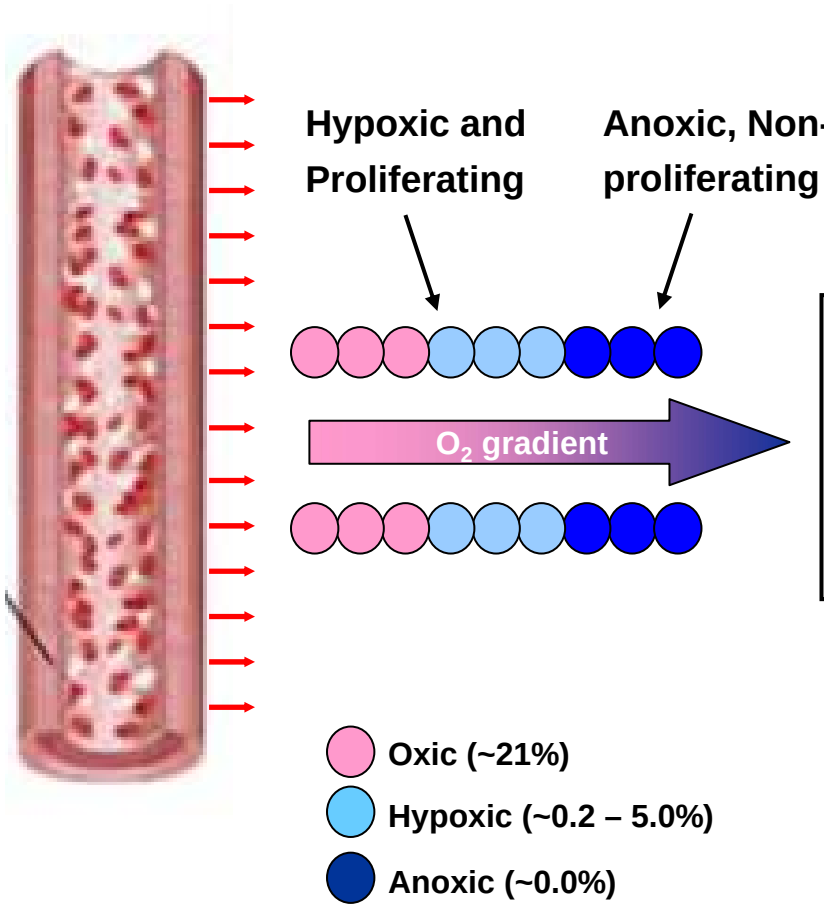


Cancer Hypoxia is linked to increased metastatic spread, chromosomal instability and resistance to chemo- and radiotherapy.

How/Why?



Hypoxic Cells Can Be Aggressive



- Increased genetic instability and mutation
- Increased resistance to cell death
- Increased resistance to radiotherapy
- Increased resistance to chemotherapy
- Increased metastases/spread

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Hypoxia Program Integration: " Decade of Clinic To Lab"

Project 5: Fyles& Milosevic

IFP
Gleevec and PDGF

Project 1: Hill

Molecular markers
Novel treatments

Project 2: Hedley

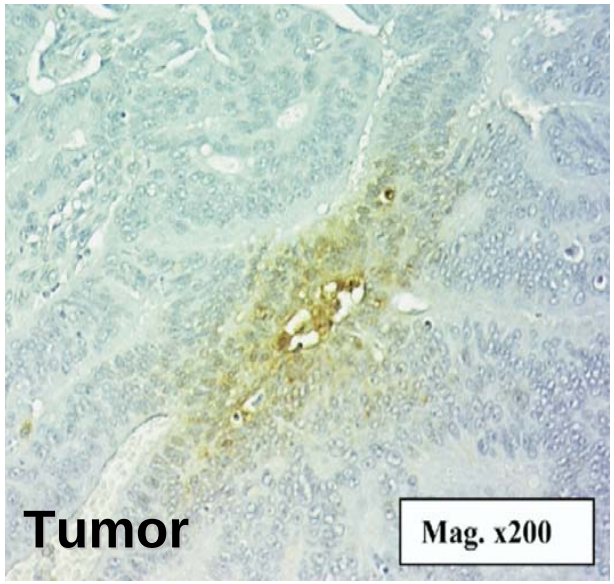
DNA repair
Gleevec and Rad51
Prostate molecular studies
Novel treatments

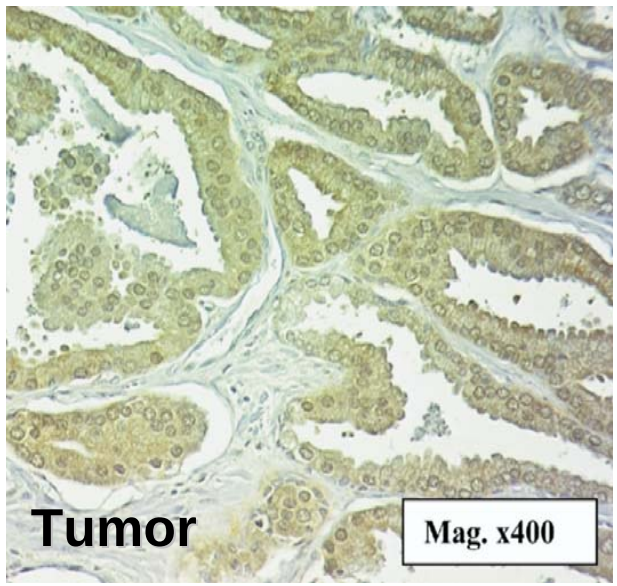
Project 3:Bristow

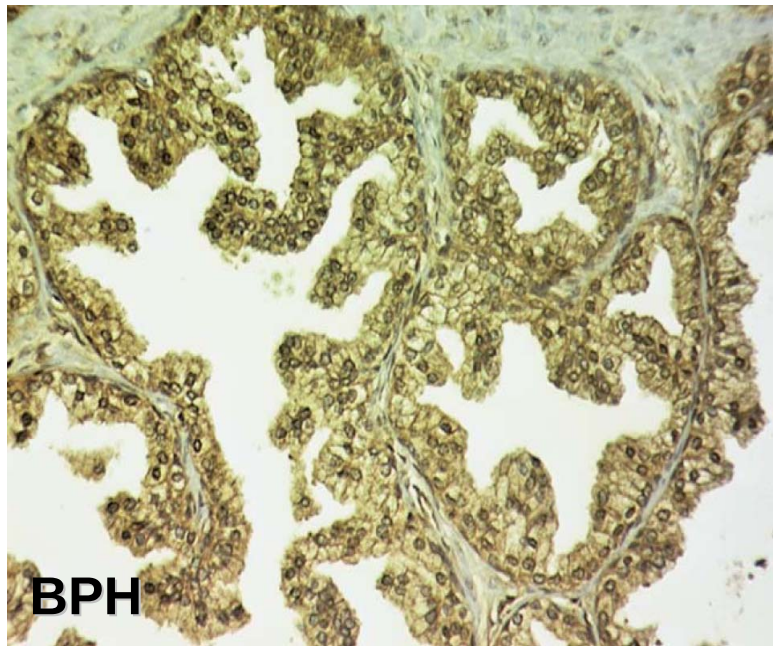
Clinical imaging
Vascular complexity
IFP

Project 4: Yeung

Hypoxia in Cancer and BPH



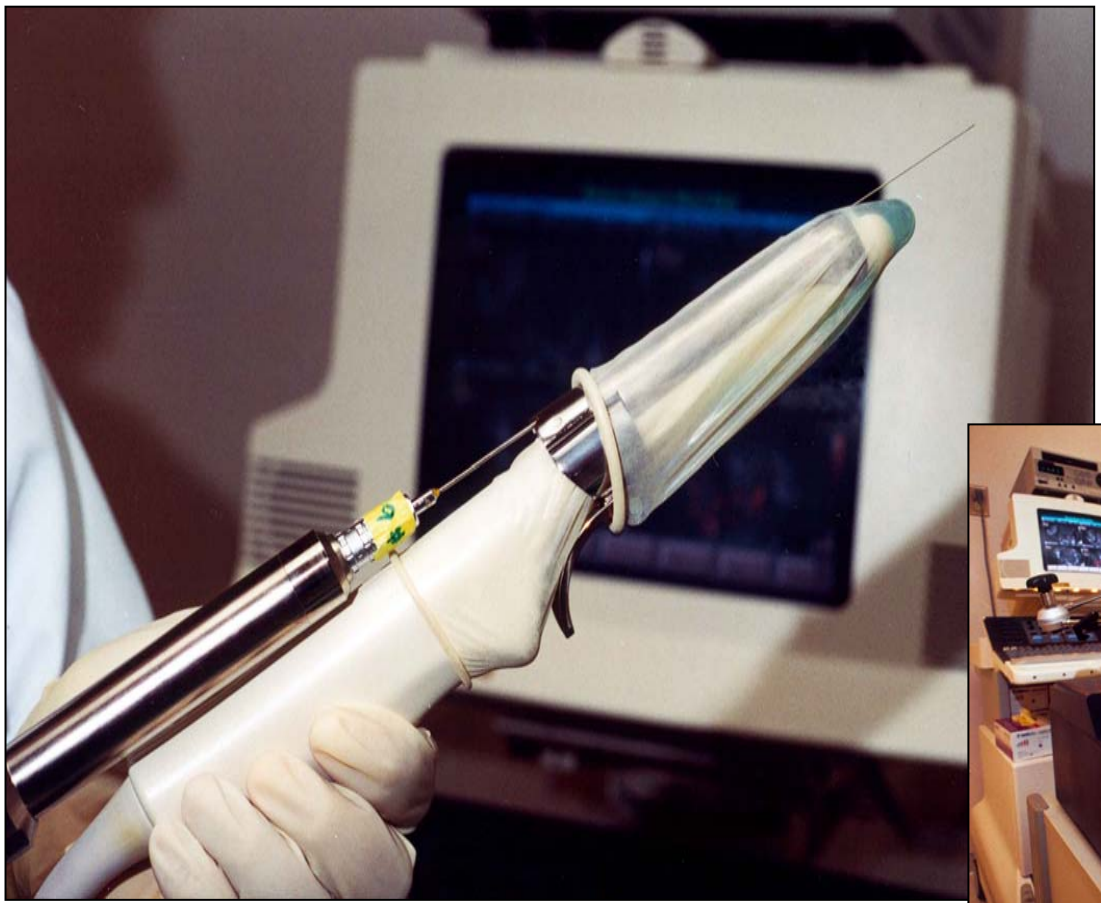


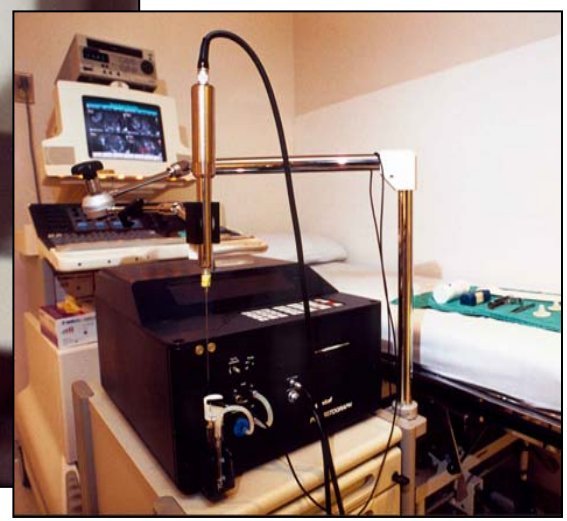


Pimonidazole uptake in 92% of tumors, and in BPH in 95% of patients

Carnell, 2006

Measuring Oxygenation: Eppendorf pO2 Probe





Prostate: trans-rectal *Eppendorf*
Electrode & biopsies

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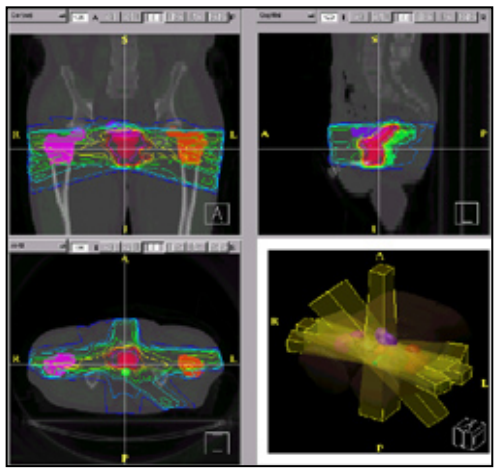
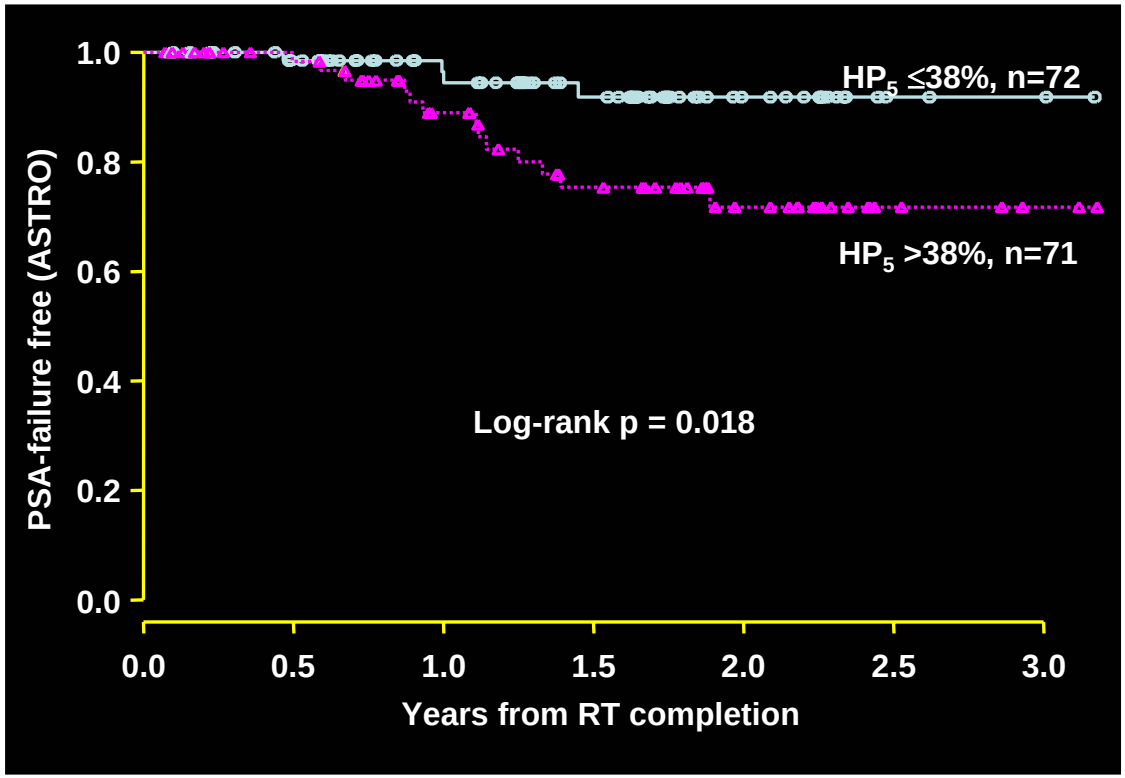
Hypoxia (Eppendorf pO_2) Predicts For Biochemical Relapse Post-Radiotherapy

-p02 taken when placing fiducial markers for plan
-Dose: 75.6-79.8 Gy
-Intermediate Risk
(T1/T2; GS7, PSA 10-20)





Milosevic and colleagues; PMH-2005

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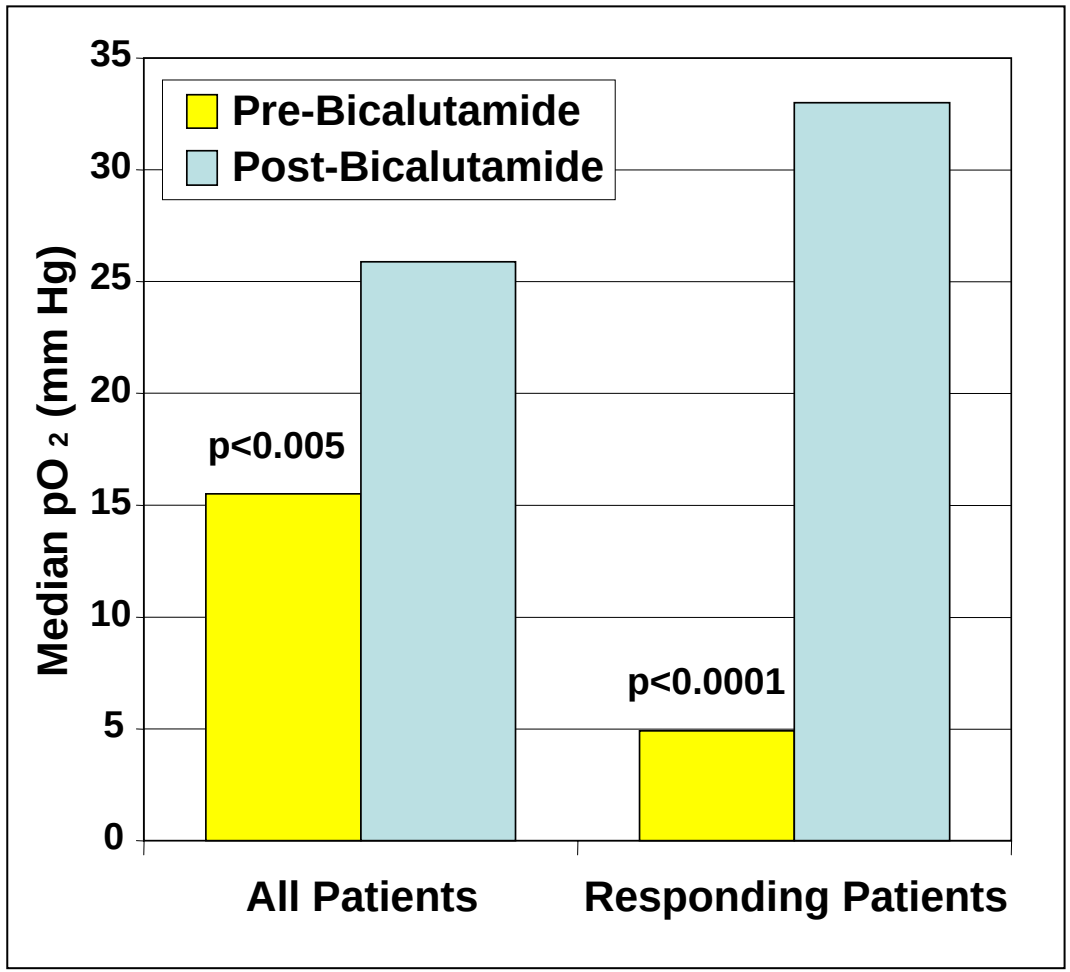
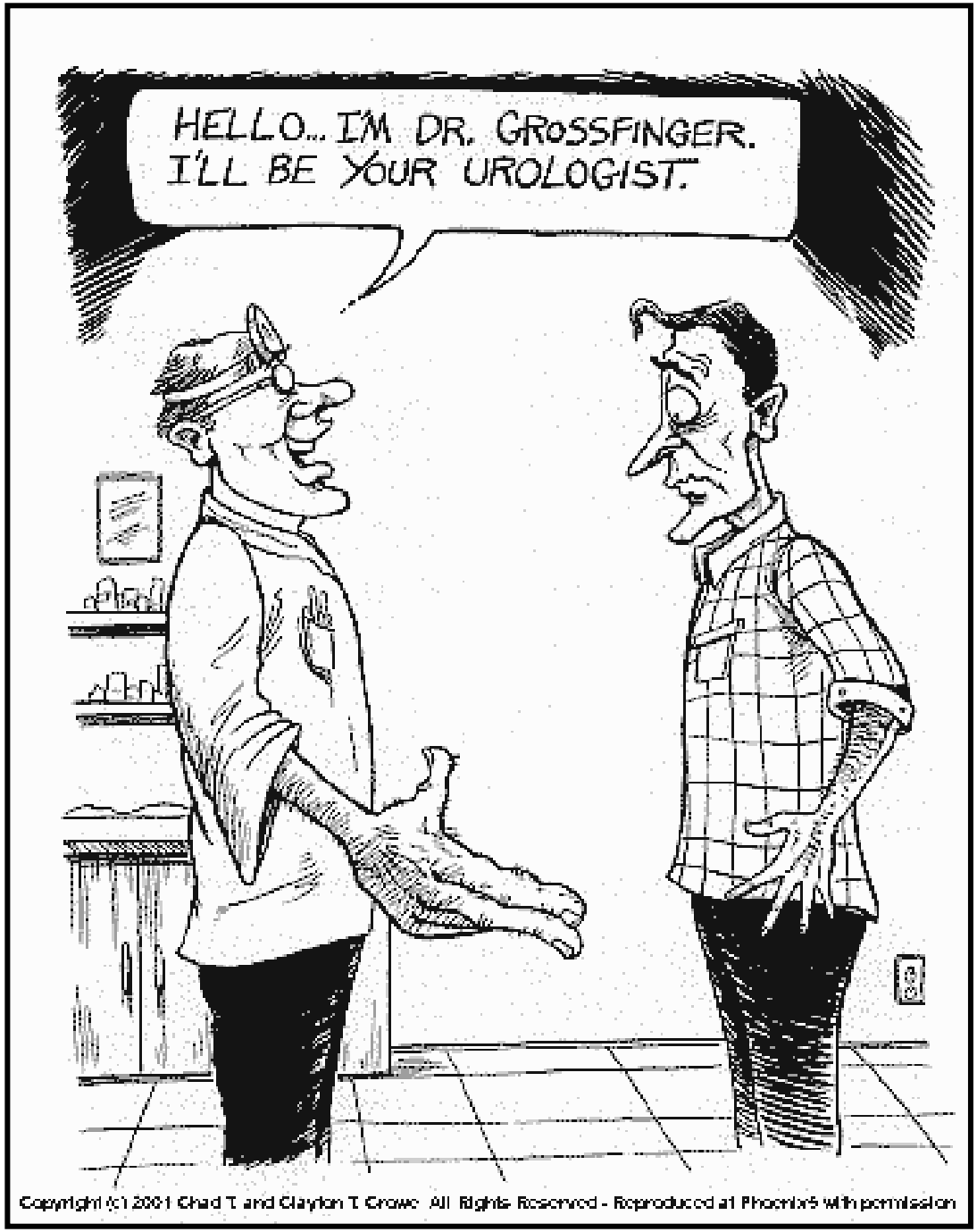
Hormone Therapy Improves Oxygen

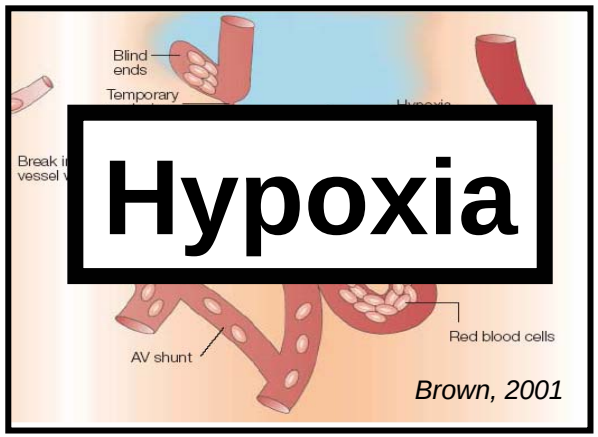
New marker that was not related to:

- **Cancer Stage**
- **Gleason score**
- **PSA**
- **Change in PSA**
- **Duration of bicalutamide (Casodex)**





THE BIG PICTURE



Local Control

DNA Repair ?

Increased Genetic Instability

Prostate Progression
& Metastasis

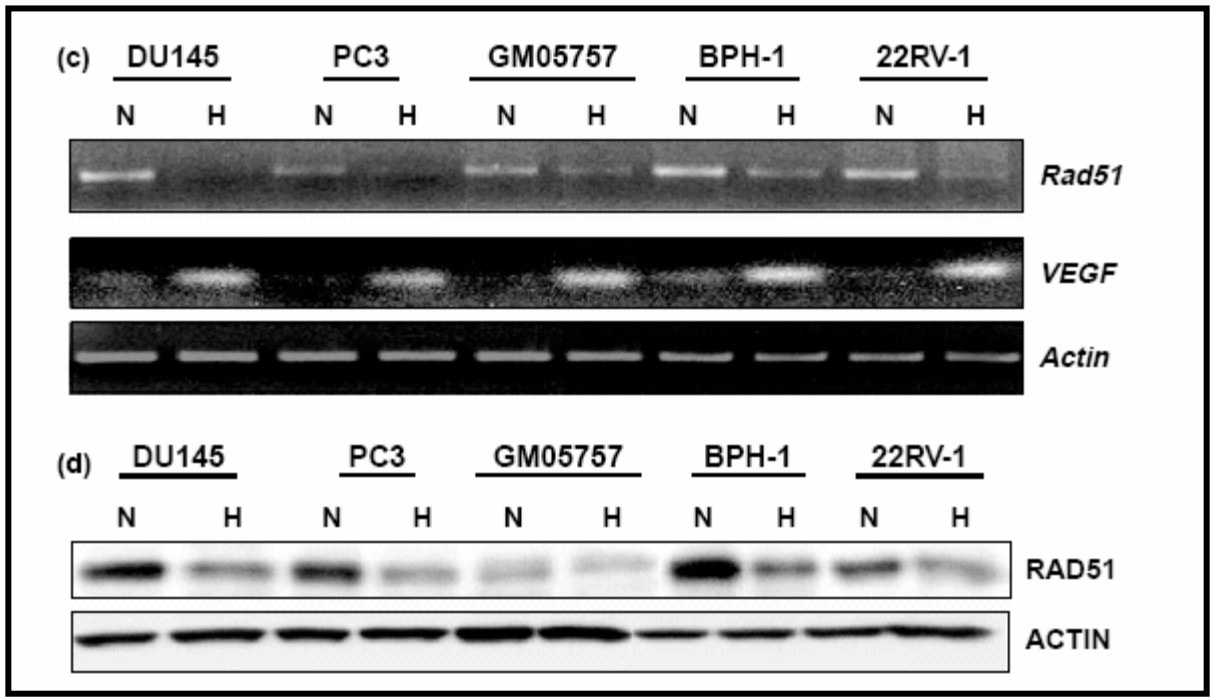
Radiotherapy and Oncology 76 (2005) 168–176
www.thegreenjournal.com

Molecular radiobiology

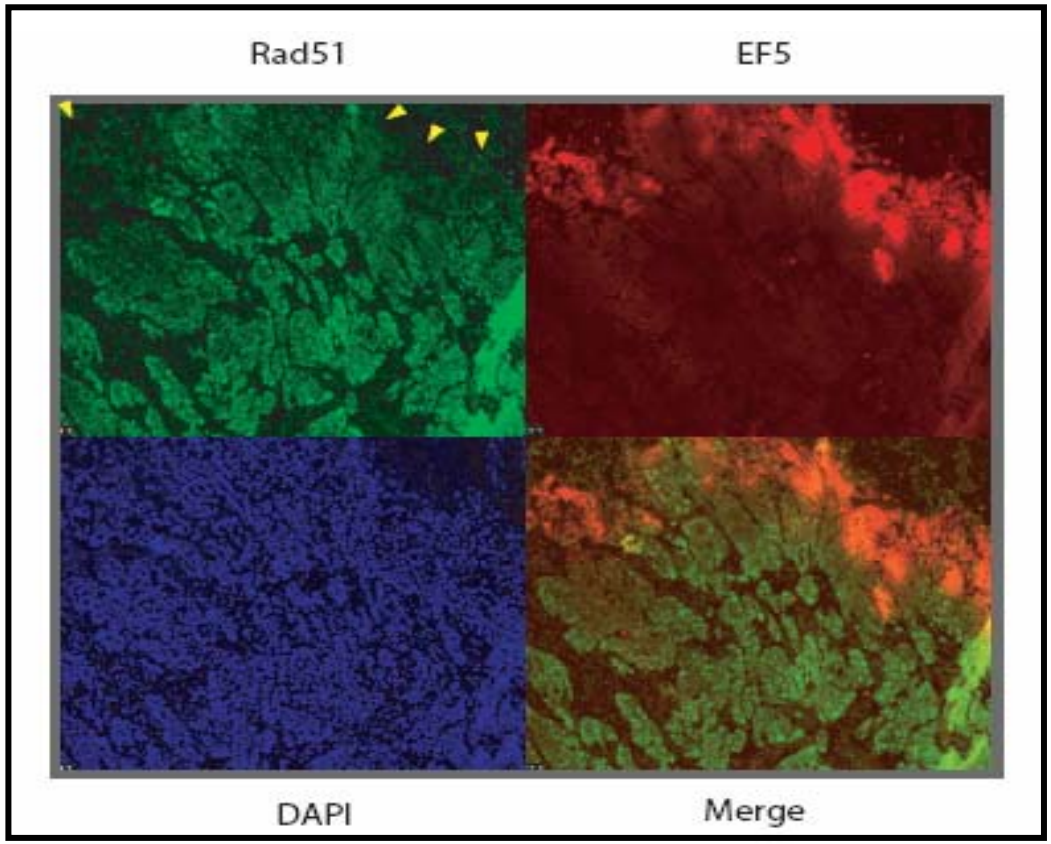
Hypoxia down-regulates DNA double strand break repair gene expression in prostate cancer cells

Alice X. Meng[a], Farid Jalali[a], Andrew Cuddihy[a], Norman Chan[a], Ranjit S. Bindra[b], Peter M. Glazer[b], Robert G. Bristow[a,b,*]

[a]*Ontario Cancer Institute and Princess Margaret Hospital (University Health Network), Toronto, Ont., Canada,* [b]*Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, USA,* [c]*Departments of Medical Biophysics and Radiation Oncology, University of Toronto, Canada*



Hypoxia And Repair In Vivo ?



HYPOXIA → **Decreased DNA Repair In Cells Adapted to Low Oxygen** → **Alter Therapy Sensitivity & Progression (Metastases)**

Bindra, Glazer, Bristow; MCB-2004; Meng,Bristow; Rad&Oncol-2005

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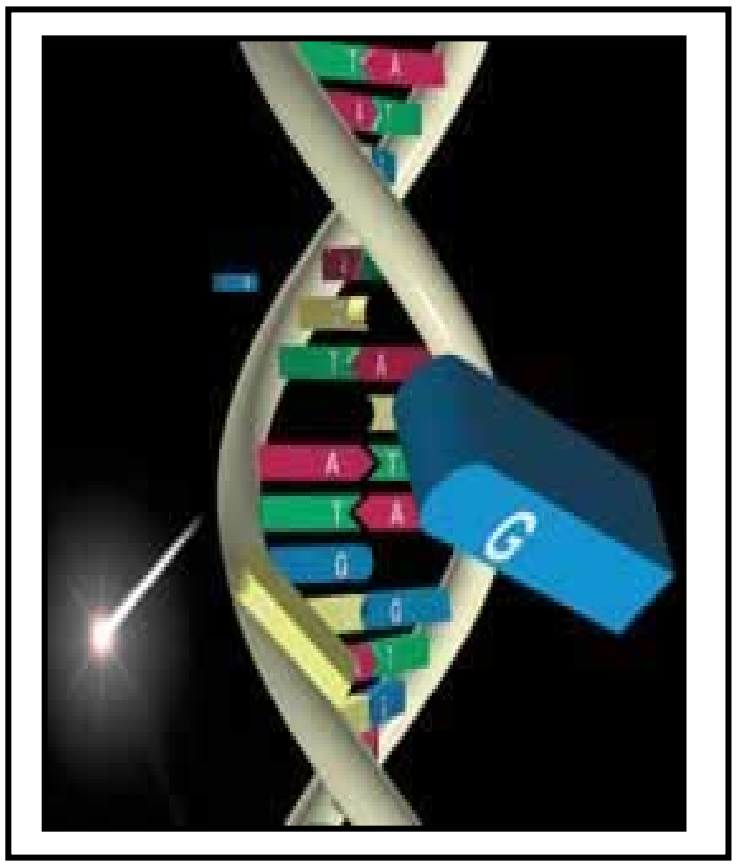
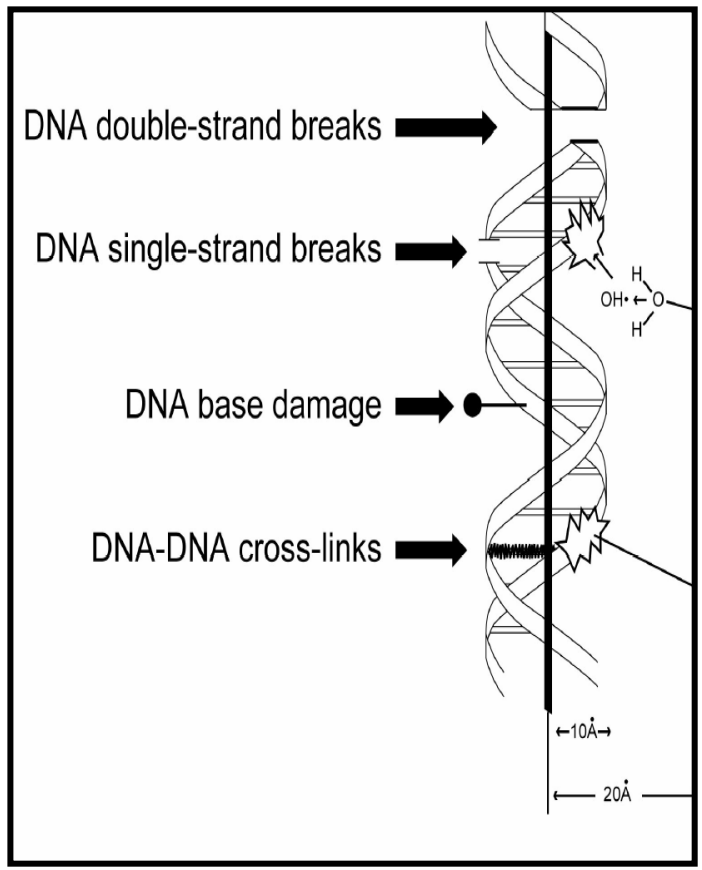
DNA Breaks: A Way To Kill Cancer Cells With Therapy





Number of Breaks Dependent on Oxygen !

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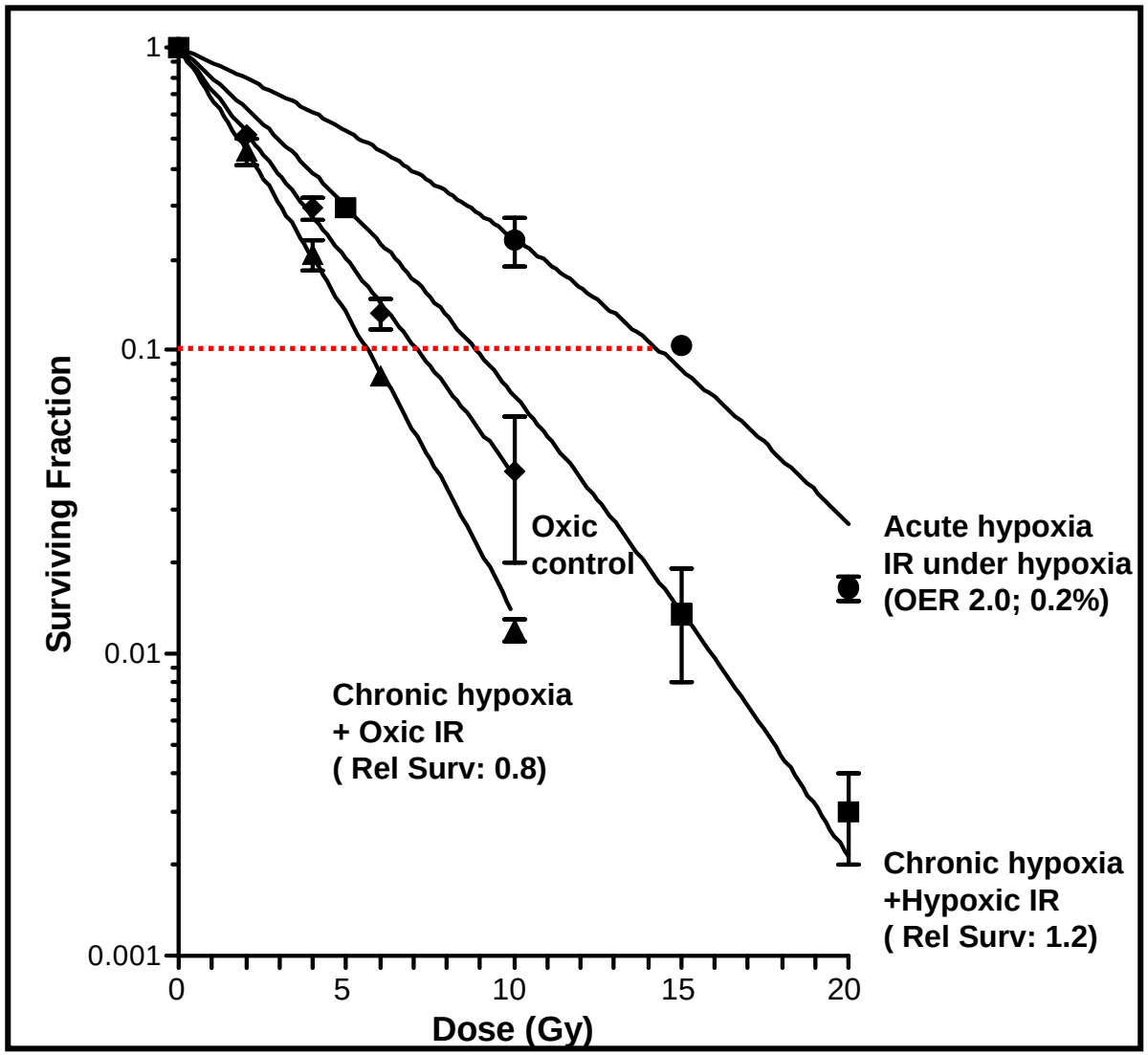
Chronic Hypoxia Decreases Functional HR and Decreases IR/MMC Survival



Gassing at 0.2%

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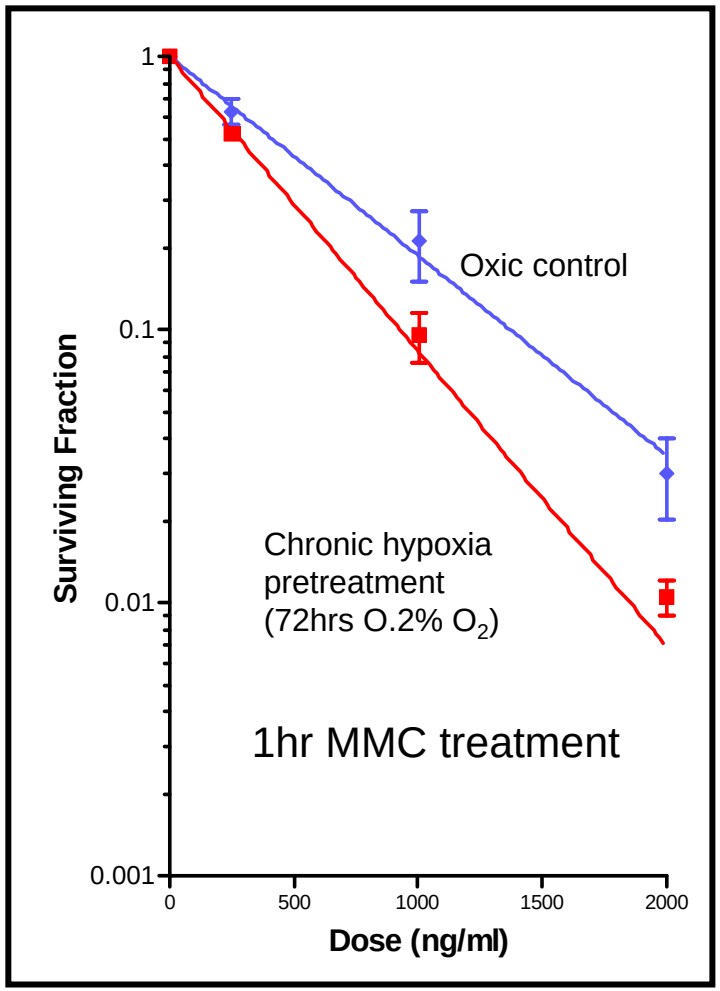
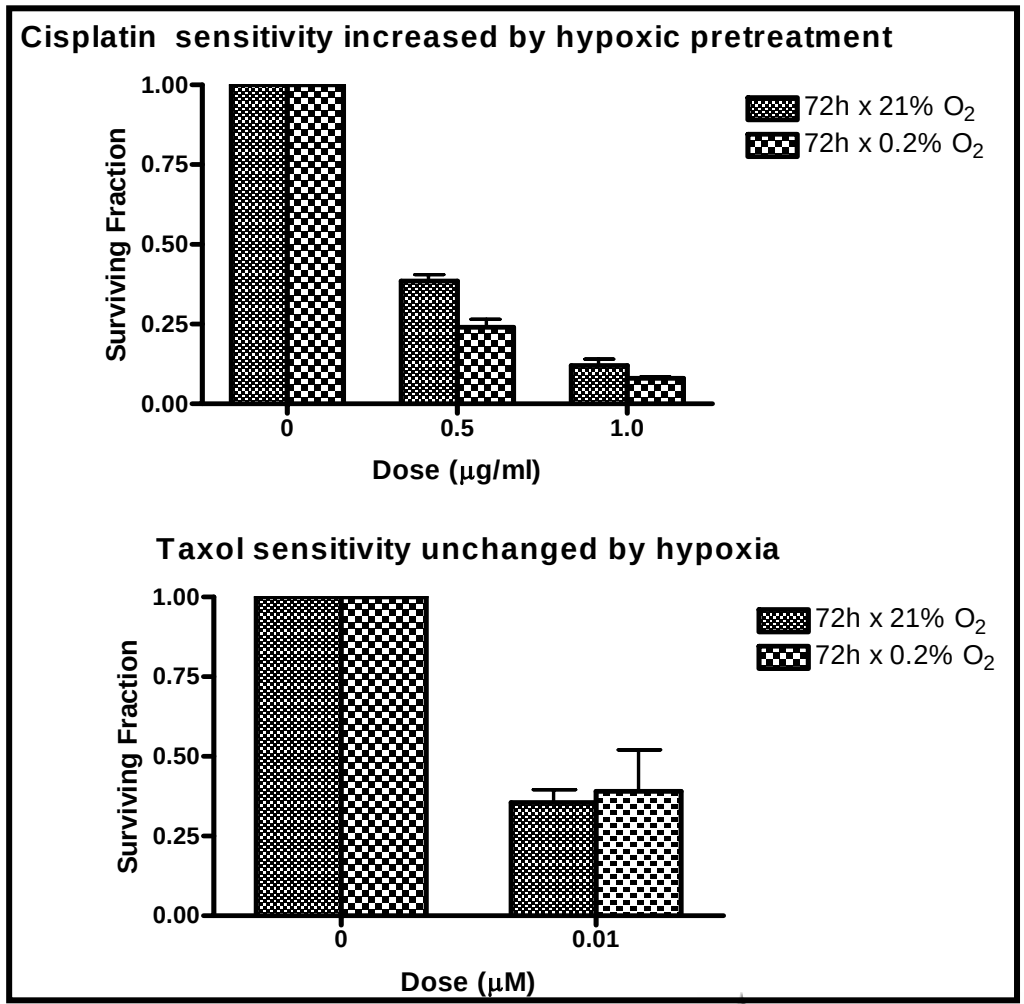
Pretreatment with hypoxia increases oxic MMC/CDDP (but not TAXOL) sensitivity





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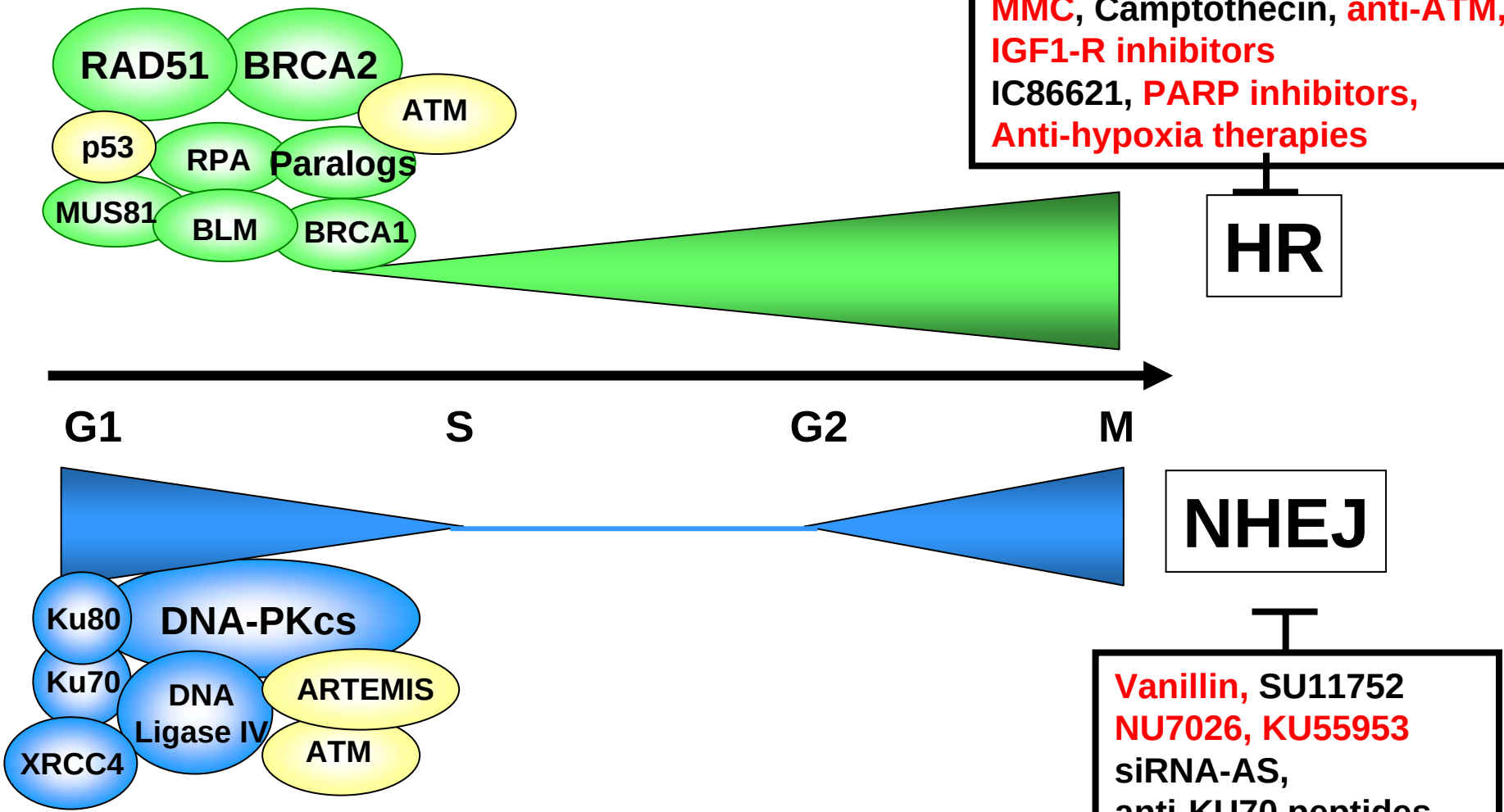
Targeting HR vs NHEJ



siRNA-AS, Imatinib-Gleevec
Gemcitabine, cDDP,
MMC, Camptothecin, anti-ATM,
IGF1-R inhibitors
IC86621, PARP inhibitors,
Anti-hypoxia therapies

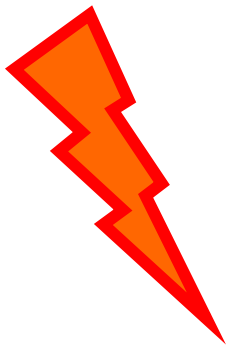
HR

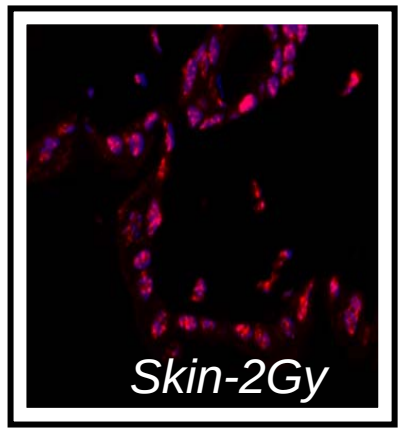
NHEJ

Vanillin, SU11752
NU7026, KU55953
siRNA-AS,
anti-KU70 peptides
Bleomycin, Etoposide

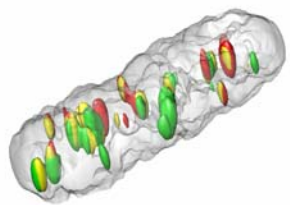
Tissue Specificity: Gene Expression and Tissue Repair Studies in Mice with Varying HR/NHEJ Defects

Miniature Cone-beam CT (~partial volume XRT)





Repair in:
Lung ?
Rectum ?
Bladder ?
Skin ?



Center for Biophysical Assessment and Risk Management Following Irradiation

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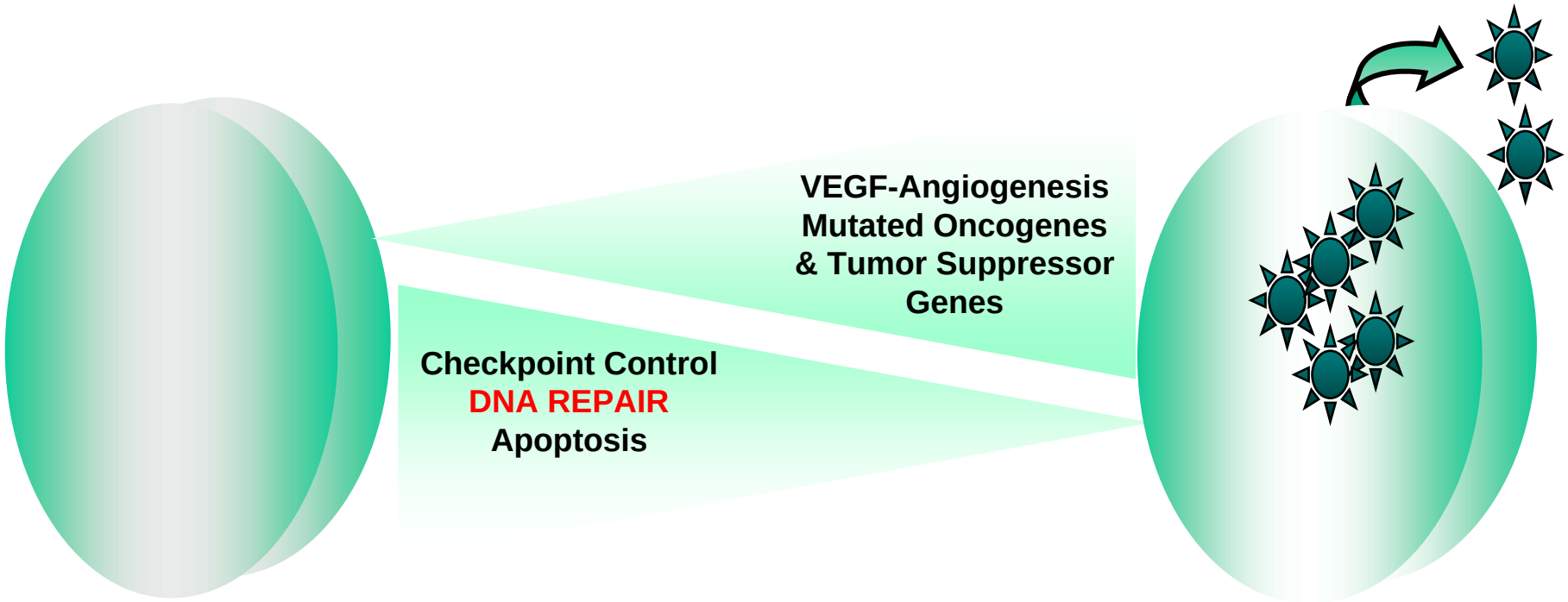
SUMMARY OF THE DNA REPAIR AND PROSTATE CANCER

- **Faulty DNA repair genes** may be involved in prostate cancer causation and progression
- Within the solid tumour, there are areas of low oxygenation (**hypoxia**) in which aggressive tumour cells develop
- Hypoxia can lead to decreased radiotherapy response and increased **metastases**
- This effect may involve decreased DNA repair in the hypoxic areas and explain increased **hypoxia-induced mutation** rates



Pre-Cancer
(Increasing Mutations)

Increasing Hypoxia

- **Anti-VEGF/HIF1α therapies**
- **Precision Radiotherapy to hypoxic areas**
- **Hormone Therapy**
- **Target Repair-deficient cells**
- **Hypoxic cell toxins (e.g. tirapazamine)**

Aggressive Cancer
(Fast growing; Hormone-Independent; Radioresistant and Metastatic)

Chan, Milosevic and Bristow, Future Oncology, 2007

POTENTIAL CLINICAL IMPLICATIONS & THE LEGEND PROGRAM

- **New biomarker for prostate cancer risk or progression to predict tumour behaviour ?**
  - In the next 5 years, we are going to measure DNA repair in normal, pre-malignant and malignant prostate cancer to try and predict the risk of prostate cancer (Familial Prostate Clinic)
  - We are also attempting to measure relative DNA repair within individual men to predict their response to cancer therapy

- **New treatments:**
  - We are developing ways to reduce the level of hypoxia within tumours to improve therapy response
  - We are developing new drugs and therapies to target abnormal DNA repair in cancer cells as a means to specifically kill these cells and not normal cells

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The Future:

- Different kinds of scientists: informatics, genomics, proteomics
- Greater patient participation in clinical trials
- More translational researchers
- More drugs in the pipeline
- Genetically personalized medicine
- More emphasis on prevention and early detection

The Future:

- Molecular profiling common in academic centers based on non-invasive imaging (MRI +/- novel PET tracers)
- Low risk disease is defined as a state in which no treatment is required
  - save for possible use of molecular medications deigned to prevent low-> intermediate risk progression (chronic disease)
  - Surgery and radiotherapy are saved for patients who fail new molecular agents
- Hormone-resistant disease largely made chronic disease by new molecular agents
- Genes involved in familial prostate cancer discovered with diagnostic tests available in family doctor's office
  - patients placed on prevention medications

30 RadOncs:
10 CS

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CFI: 20 Million

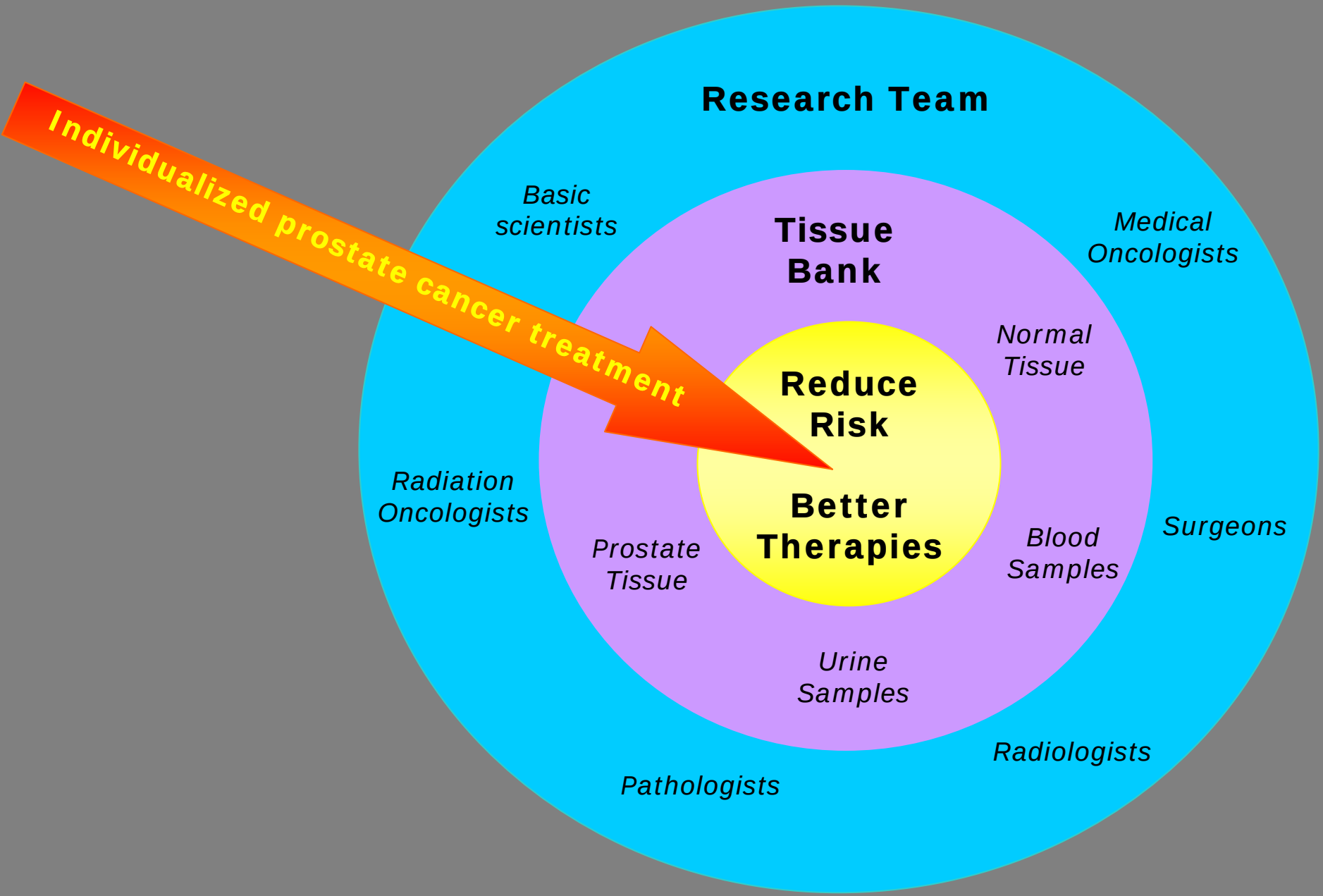
Small Animal Imaging/IGTx

-micro CT
-micro PET
-micro MRI
-gene imaging
-micro Cone Beam CT

LEGEND

-SNPs, microarrays
-Tissue Arrays
-Intracellular DNA Repair
-Biopsies & Biomarkers (Hypoxia)

The PMH Prostate Program



The Bristow Lab

THE Prostate CENTRE
University Health Network • Princess Margaret Hospital

PMH-Terry Fox Hypoxia PPG Team
(Hill, Hedley (DDP), Milosevic, Yeung, Fyles)
Radiation Medicine Program &
Prostate CRP (Sweet, van der Kwast, Evans, Squire, Fleshner, Jongstra)
Bristow Lab: Farid Jalali, Alice Meng, Rong Fan (former), Shahnaz Al-Rashid, TS Kumaravel (former), Oliver Faulhaber, Helen Zhao,, Ananya Choudhury, Carla Coackley, Tien Phan, Jamil Sawani, Norman Chan, Ramya Kumareswaran

Canadian Cancer Society / Société canadienne du cancer

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HP5

Yale: Peter Glazer, Ranjit Gupta; WASH Univ: Simon Powell
LEEDs: Ananya Choudury and Anne Kiltie; UBC: Peggy Olive

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LET'S JUST BE FRIENDS
IT'S MY DNA... ISN'T IT?
HUMAN GENOME REPORT