Drug Resistance: Oncology 520 March 27, 2012

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Outline

- General principles
  - Impact of malignancy and drug resistance
  - Hallmarks of malignancy
  - Emerging appreciation of cancer complexity
- Types and mechanisms of drug resistance
- Rational approach to progress
  - Appropriate combinations of drugs
  - Understanding the target
  - Predictive assays

The impact of malignancy

- **Despite advances in cancer diagnosis, prevention, and treatment, still 50% five year mortality in the developed world**
- **second most common cause of mortality the prosperous world: 1 in 5**
- **optimal therapy is curative in only 50% of patients presenting with cancer**

Concepts

- **Approximately half of cancers will have spread beyond the reach of local or regional treatments, where patients may benefit from systemic treatments**

Systemic therapy

- **Chemotherapy**
  - **systemic administration of cytotoxic drugs**
  - **intended to deal with non-localized disease**
- **Hormonal therapy**
  - **manipulation of the hormonal environment to suppress malignant cells**

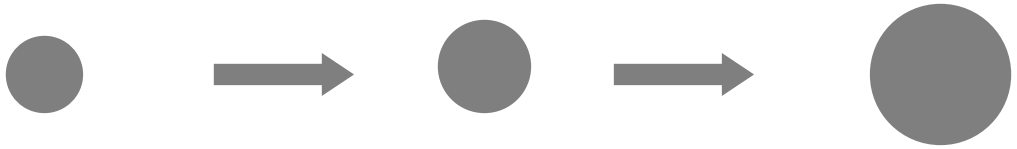
Concepts

- **Chemotherapy and hormone therapy are systemic treatments.**
- **Chemotherapy is usually given in repeated doses.**
- **Chemotherapy is usually given as combinations of drugs.**
- **Resistance to chemotherapy is a major clinical problem.**

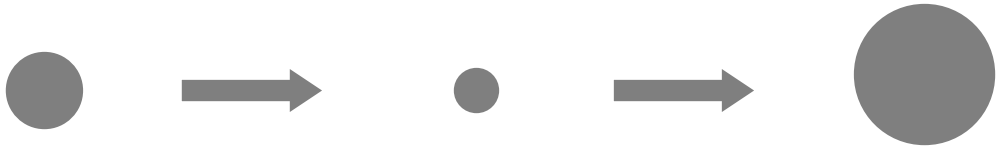
What does drug resistance mean to the patient?

Clinical resistance in solid tumours

- *de novo* **resistance (progressive disease)**

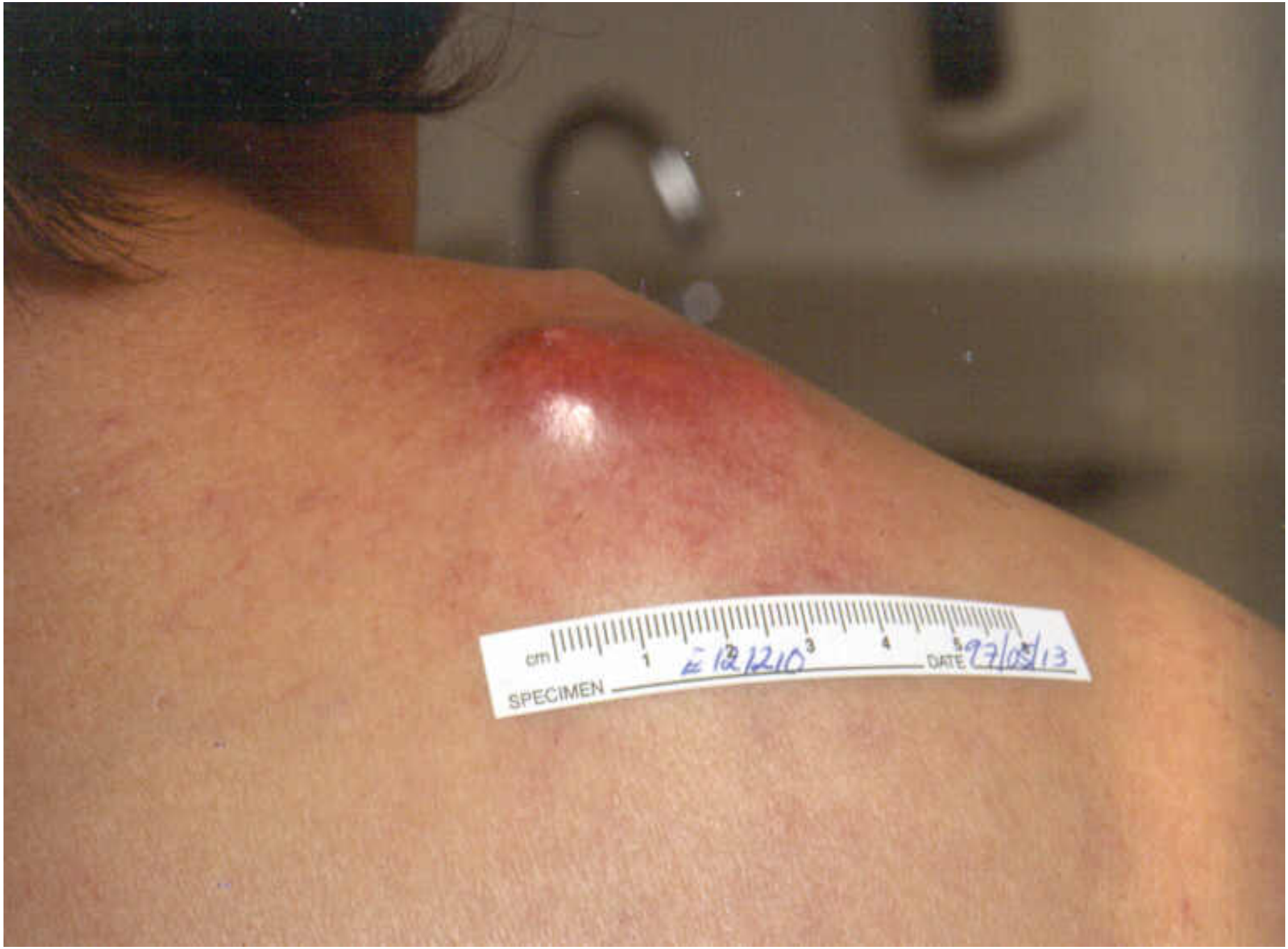


- **resistance develops after response (PR -> PD)**



- **stable disease**

- **major response**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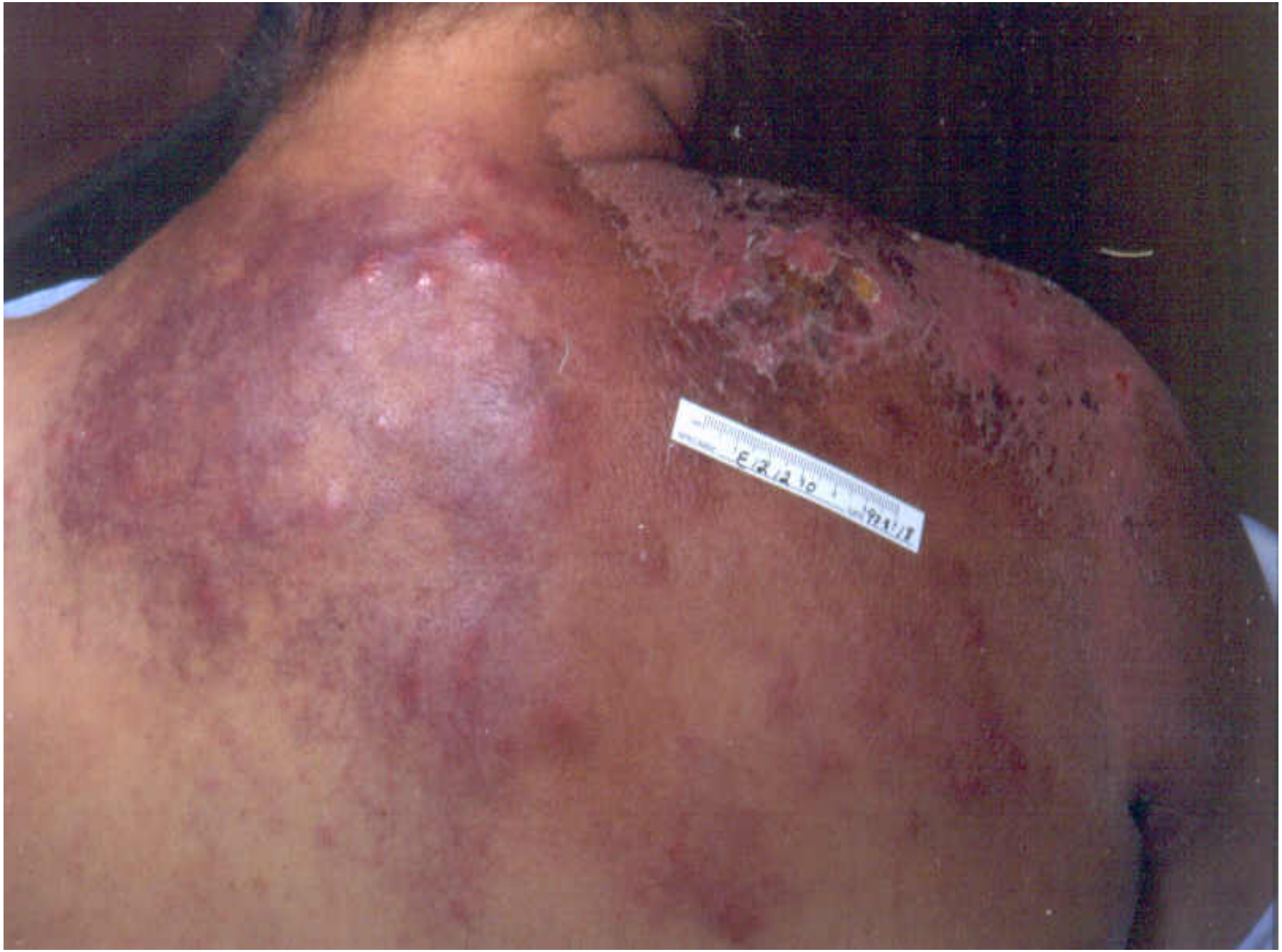
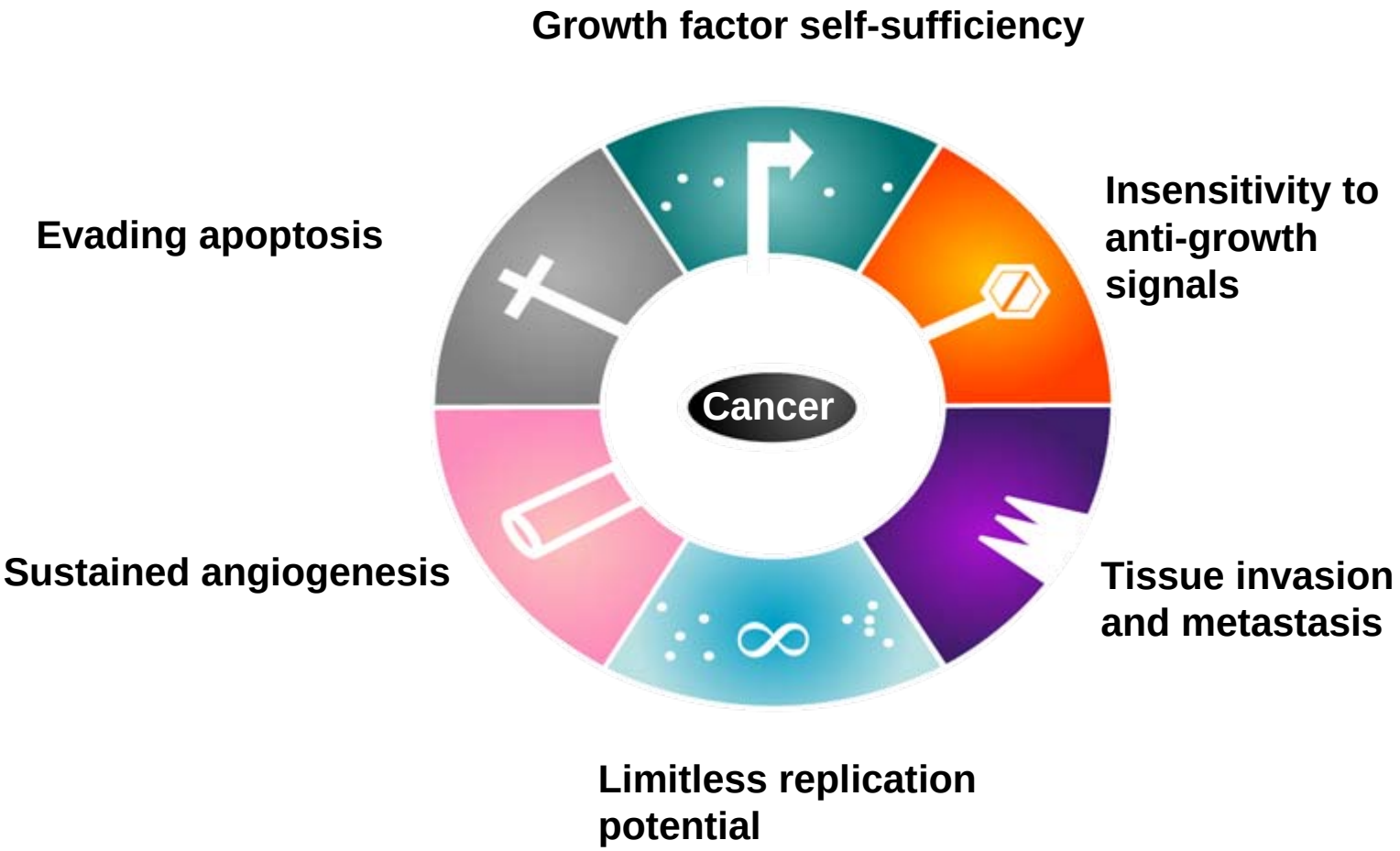


What underlies drug resistance?

Hallmarks of Malignancy



Hanahan D, et al. Cell. 2000;100:57-70.

Several of these defining characteristics of malignancy contribute to drug resistance

- **Evade / resistance to apoptosis**
- **Limitless replication potential**
- **Insensitivity to anti-growth signals**
- **Growth factor self-sufficiency**

Defining the problem

- **Some cancers are simple**
- **Some cancers are much more complex than we feared ….**

Simple, Stupid Cancers

- Single dominant mutation
- Monotherapy is effective
- Resistance is rare and late
  - Chronic myelogenous leukemia
    - BCR-ABL fusion gene due to translocation
  - Gastrointestinal stromal tumors
    - c-kit mutation

Complex, Smart Cancers

- **Multiple mutation drivers**
- **Large mutational burden**
- **Intratumor heterogeneity**
- **Multi-targeted therapy needed**
- **Resistance common and early**

adapted from G. Sledge ASCO 2010

Complex landscapes of somatic rearrangement in human breast cancer genomes

Philip J. Stephens[1], David J. McBride[1], Meng-Lay Lin[1], Ignacio Varela[1], Erin D. Pleasance[1], Jared T. Simpson[1], Lucy A. Stebbings[1], Catherine Leroy[1], Sarah Edkins[1], Laura J. Mudie[1], Chris D. Greenman[1], Mingming Jia[1], Calli Latimer[1], Jon W. Teague[1], King Wai Lau[1], John Burton[1], Michael A. Quail[1], Harold Swerdlow[1], Carol Churcher[1], Rachael Natrajan[2], Anieta M. Sieuwerts[3], John W. M. Martens[3], Daniel P. Silver[4], Anita Langerød[5], Hege E. G. Russnes[5], John A. Foekens[3], Jorge S. Reis-Filho[2], Laura van 't Veer[6], Andrea L. Richardson[4,7], Anne-Lise Børresen-Dale[5,8], Peter J. Campbell[1], P. Andrew Futreal[1] & Michael R. Stratton[1,9]

- **24 primary breast cancers each show unique pattern of DNA rearrangements**
- **No recurrent rearrangement identified**

Nature, 2010

Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution

Sohrab P. Shah[1,2]*, Ryan D. Morin[3]*, Jaswinder Khattra[1], Leah Prentice[1], Trevor Pugh[3], Angela Burleigh[1], Allen Delaney[3], Karen Gelmon[4], Ryan Guliany[1], Janine Senz[2], Christian Steidl[2,5], Robert A. Holt[3], Steven Jones[3], Mark Sun[1], Gillian Leung[1], Richard Moore[3], Tesa Severson[3], Greg A. Taylor[3], Andrew E. Teschendorff[6], Kane Tse[1], Gulisa Turashvili[1], Richard Varhol[3], René L. Warren[3], Peter Watson[7], Yongjun Zhao[3], Carlos Caldas[6], David Huntsman[2,5], Martin Hirst[3], Marco A. Marra[3] & Samuel Aparicio[1,2,5]

- **Primary breast cancer had 5 mutations (and subpopulations with an additional 6 mutations)**
- **At relapse 9 years later, this cancer had 32 mutations**
- **None of these 32 mutations were seen in a panel of 192 breast cancers (ie every cancer a most mutations are unique)**

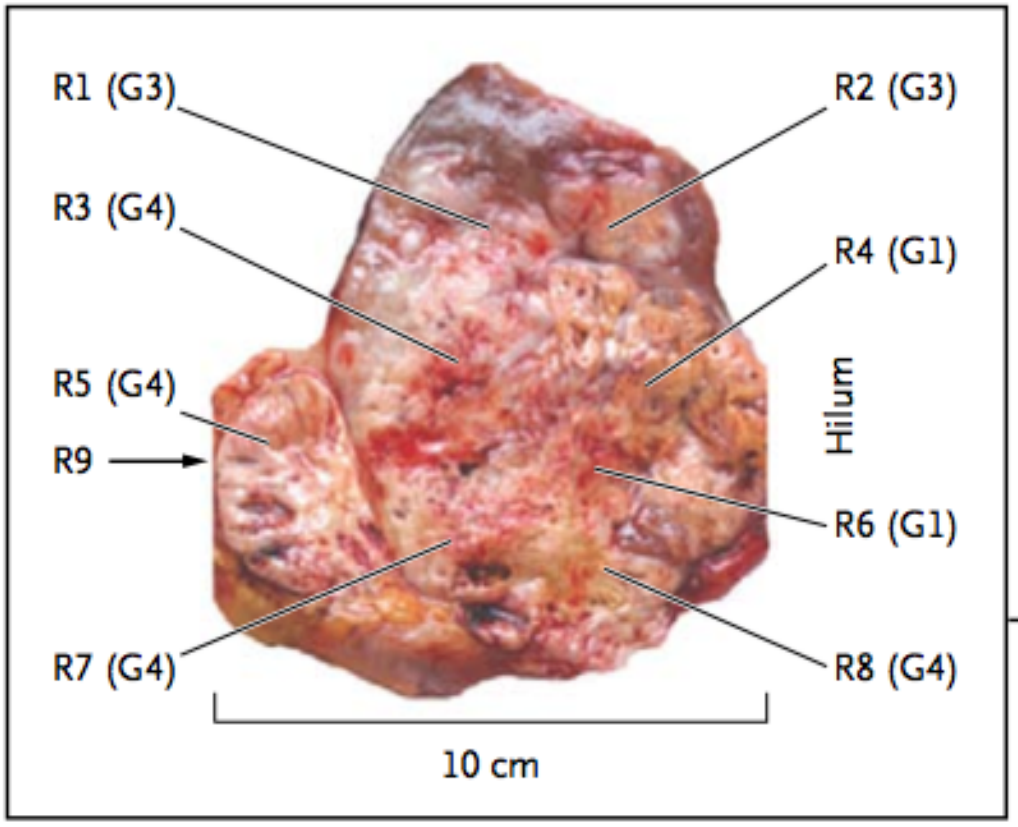
Nature, 2009

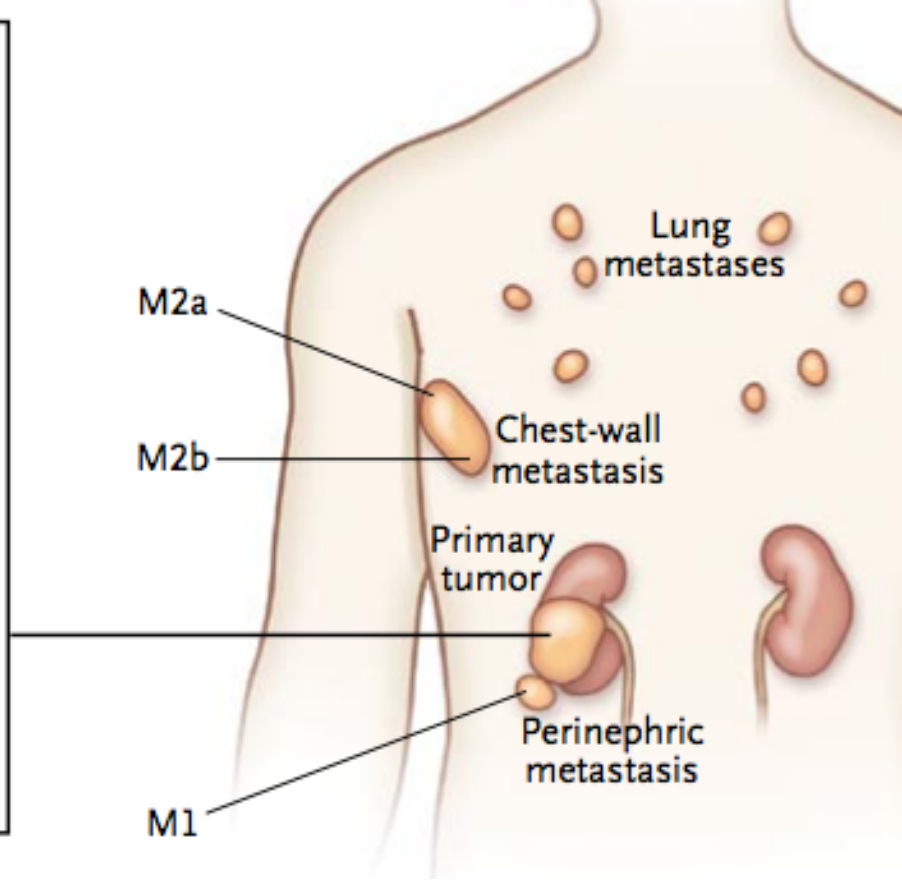
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 8, 2012 VOL. 366 NO. 10

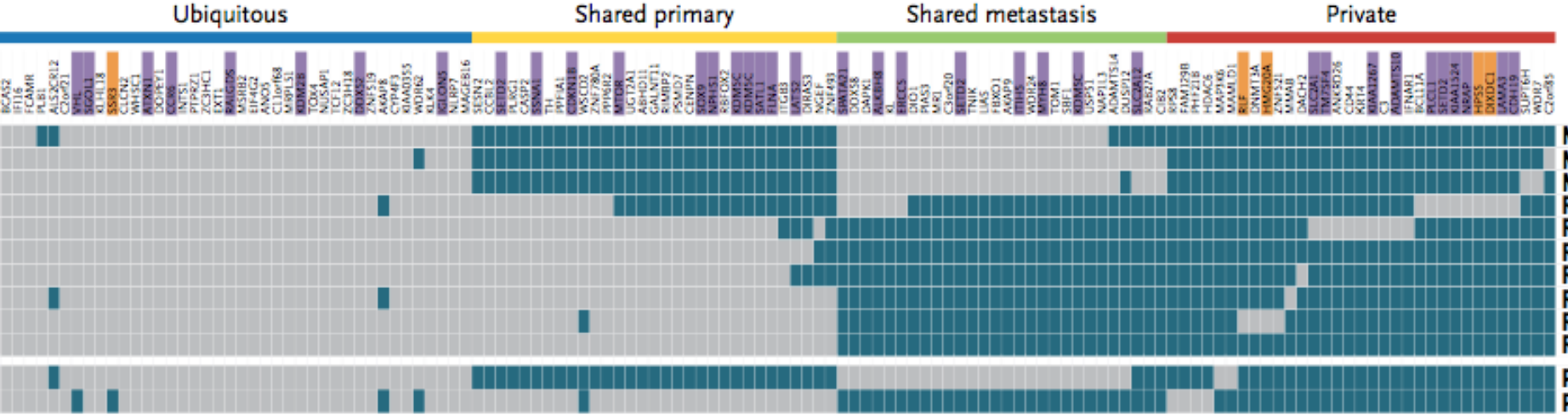
Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

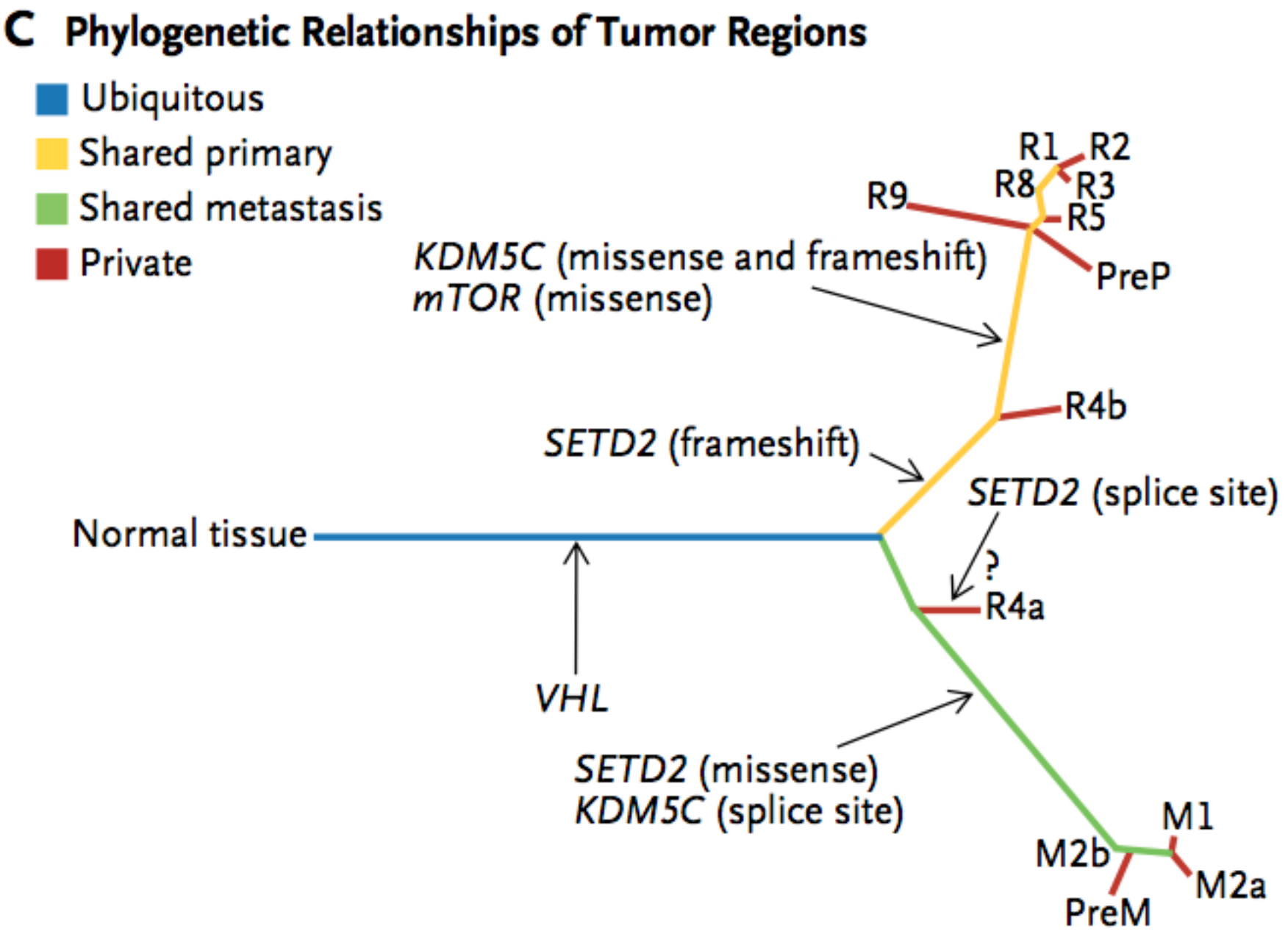
Marco Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D., David Endesfelder, Dip.Math., Eva Gronroos, Ph.D., Pierre Martinez, Ph.D., Nicholas Matthews, B.Sc., Aengus Stewart, M.Sc., Patrick Tarpey, Ph.D., Ignacio Varela, Ph.D., Benjamin Phillimore, B.Sc., Sharmin Begum, M.Sc., Neil Q. McDonald, Ph.D., Adam Butler, B.Sc., David Jones, M.Sc., Keiran Raine, M.Sc., Calli Latimer, B.Sc., Claudio R. Santos, Ph.D., Mahrokh Nohadani, H.N.C., Aron C. Eklund, Ph.D., Bradley Spencer-Dene, Ph.D., Graham Clark, B.Sc., Lisa Pickering, M.D., Ph.D., Gordon Stamp, M.D., Martin Gore, M.D., Ph.D., Zoltan Szallasi, M.D., Julian Downward, Ph.D., P. Andrew Futreal, Ph.D., and Charles Swanton, M.D., Ph.D.





Regional Distribution of Mutations




C Phylogenetic Relationships of Tumor Regions
Ubiquitous
Shared primary
Shared metastasis
Private
R1
R2
R8
R3
R9
R5
PreP
KDM5C (missense and frameshift)
mTOR (missense)
R4b
SETD2 (frameshift)
SETD2 (splice site)
Normal tissue
?
R4a
VHL
SETD2 (missense)
KDM5C (splice site)
M1
M2b
M2a
PreM

The problem

- **Genetic changes driving cancer are more complex than previously appreciated**
- **Standard drug treatments for early stage breast cancer frequently fail**
  - **molecular determinants driving treatment failure are largely unknown**
- **We need to reduce this complexity!**

Key questions

- **Can transcriptome analysis identify the pathways that are associated with early relapse despite state of the art therapy?**

- **Can we identify upregulated key genes and pathways in treatment refractory early breast cancers that might serve as new drug targets?**

BREAD Study

Beast cancer Relapsing EArly Determinants

BREAD Study Background

- **Samples and patients specifically selected from > 4000 primary tumors in the Alberta Research Tissue Bank**

- **Nonmetastatic breast cancers treated with curative intent**
  - **Standardized Alberta Breast Cancer Program therapy**
    - **Surgical resection**
    - **Chemotherapy – anthracycline and taxane**
    - **Trastuzumab**
    - **Hormonal therapy**
    - **Radiotherapy**

BREAD Samples

- **176 women consenting for analysis of frozen primary cancers**
  - **88 cancers have relapsed despite standard treatment**
  - **88 clinically identical control tumours have not relapsed despite substantially longer followup**

- **The two groups are matched for**
  - **ER status**
  - **HER2 status**
  - **Time to Relapse / minimum Time Free of Relapse**
  - **Stage**

Transcriptome analysis to identify drug targets

- Upregulated transcripts in known genes
- Looked within specific breast cancer subgroups
  - ER positive HER2 negative
  - ER negative HER2 negative
  - HER-2 negative (ER any)
- Pathway Analysis
- Machine learning predictor modeling
- Examine Disease-Free Survival curves dichotomized at median for individual genes

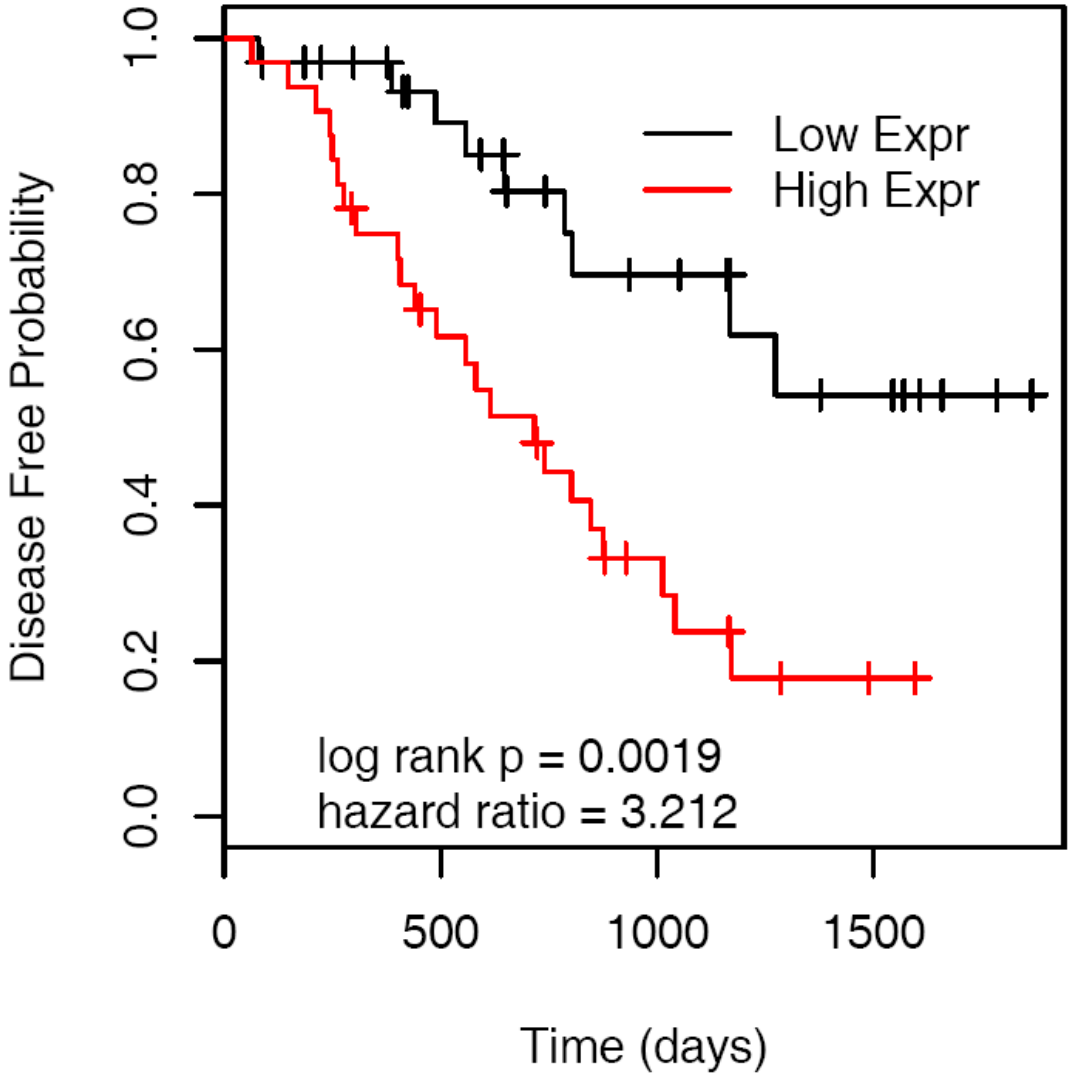
Genes and proteins of therapeutic interest

- **Upregulated in relapsed cases, highly statistically significant, large variation in transcription level on scatter plot, significant prognostic impact when dichotomized at median expression value, protein determinations prognostic**

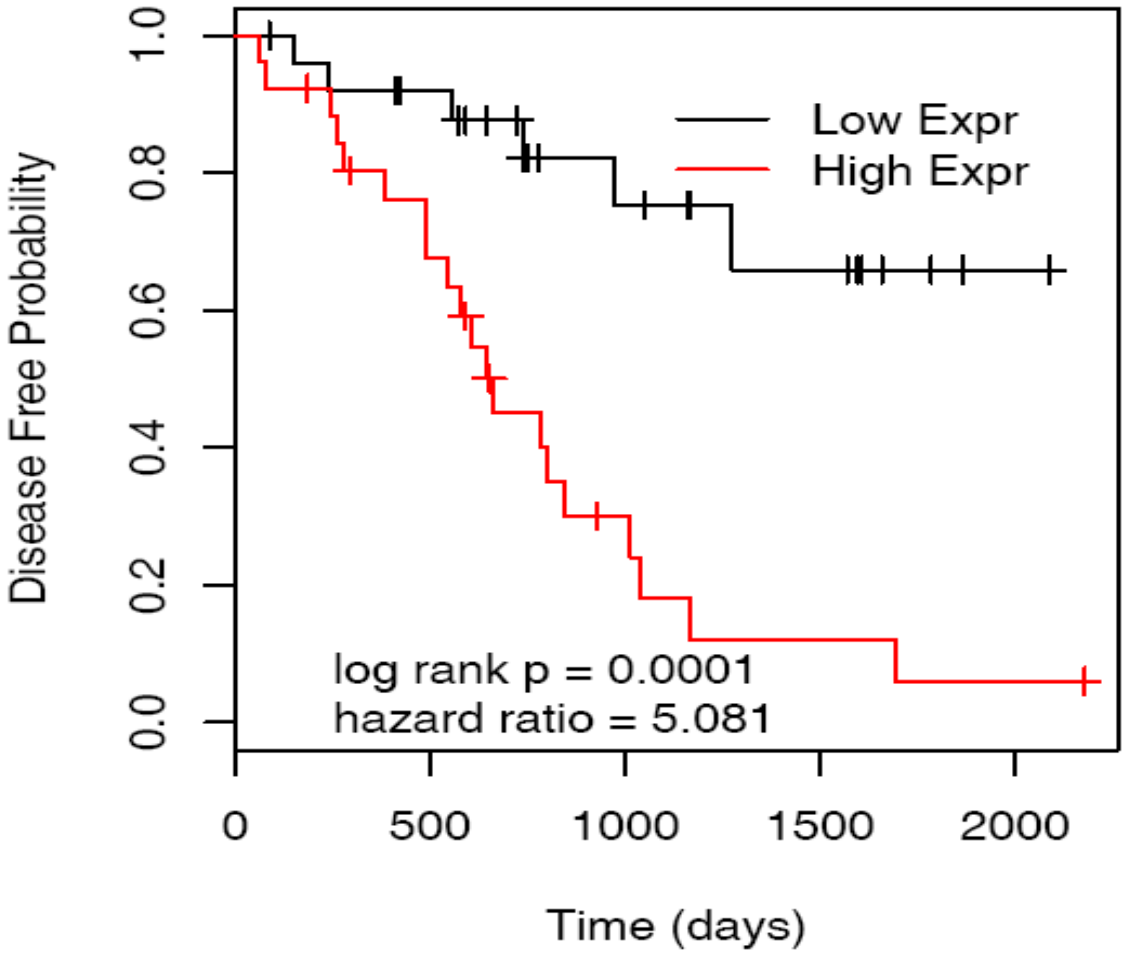
Protein validation

- **Drugs target proteins, not genes …**
- **Immunohistochemical validation**
  - **Does protein abundance / cellular localization:**
    - **Correlate with expression analysis?**
    - **Replicate the prognostic significance in the BREAD cohort?**
    - **Replicate the prognostic significance in an independent cohort with known ER and HER2 status (n=7300)?**

HER-2 negative ER negative cohort – dichotomized at median single gene with a reported small molecule inhibitor



HER-2 negative ER positive cohort – dichotomized at median



BREAD Study Findings
9 Validated Targets

++

Growth factor self-sufficiency

Evading apoptosis

++

Insensitivity to anti-growth signals

Sustained angiogenesis

++++

Tissue invasion and metastasis

+

Limitless replication potential

Hanahan D, et al. Cell. 2000;100:57-70.

Implications of BREAD study

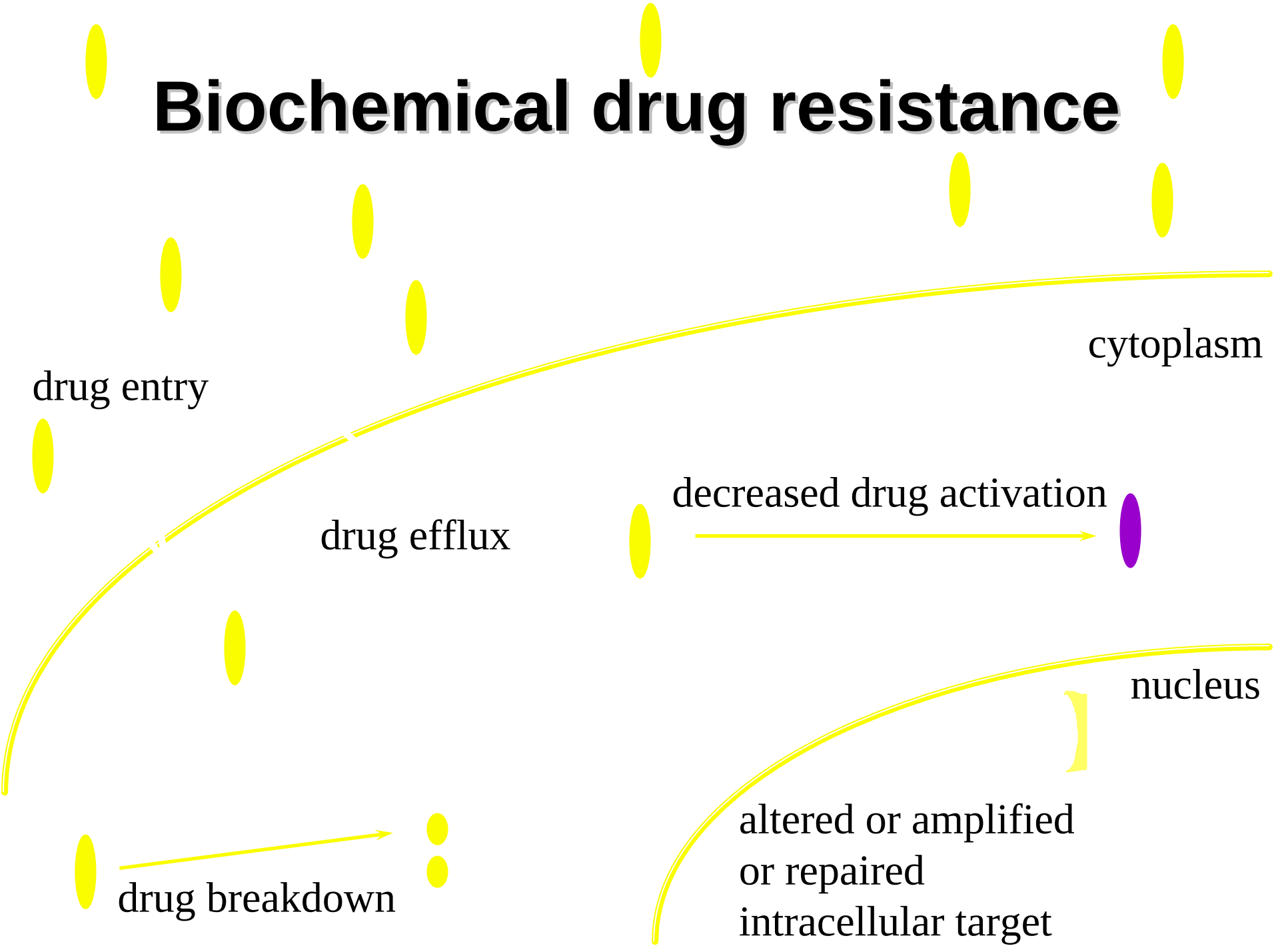
- **We have identified key pathways associated with treatment failure / relapse**
- **We may identify new drug targets and related predictive markers**
- **There are common pathways that appear to drive relapse despite standard adjuvant therapy**

Specific mechanisms of drug resistance

“Intrinsic” Resistance

- **A clinical definition**
  - **Initial insensitivity to therapy**
- **Reasons**
  - **Lack of selectivity for the malignant cells**
  - **Inadequate scheduling**
  - **Biochemical insensitivity**
  - **Inadequate drug delivery**

Biochemical drug resistance



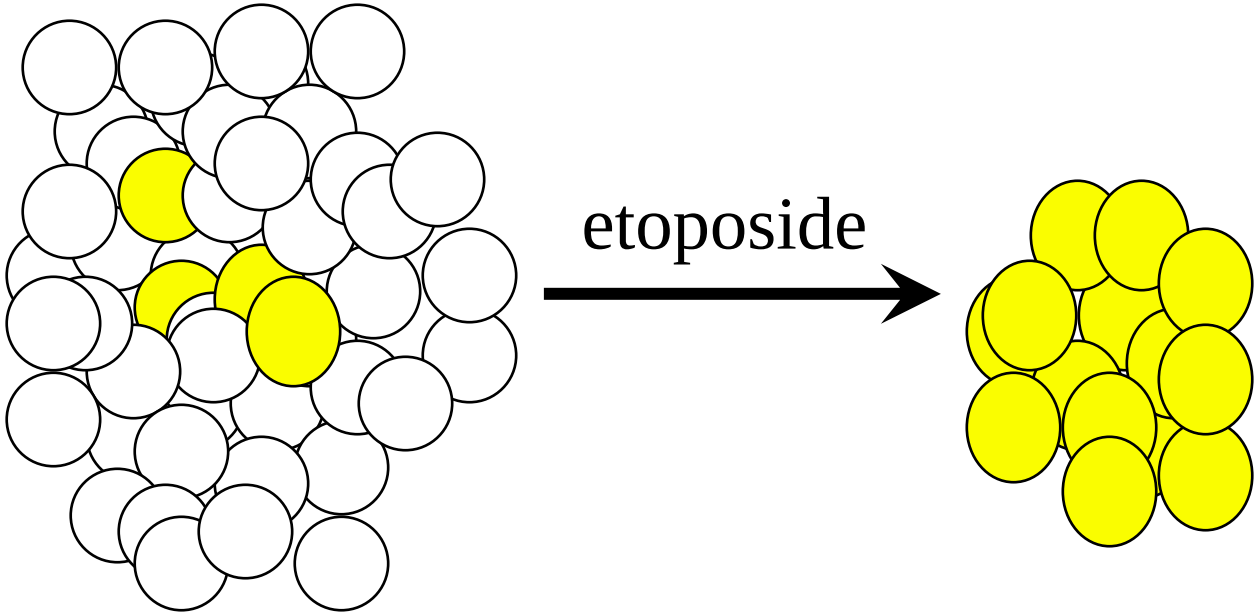
Circumventing biochemical resistance

- Use more than one drug
- Use combinations of agents that enhance drug activation / accumulation / efficacy
- Use agents that inhibit drug inactivation or target repair

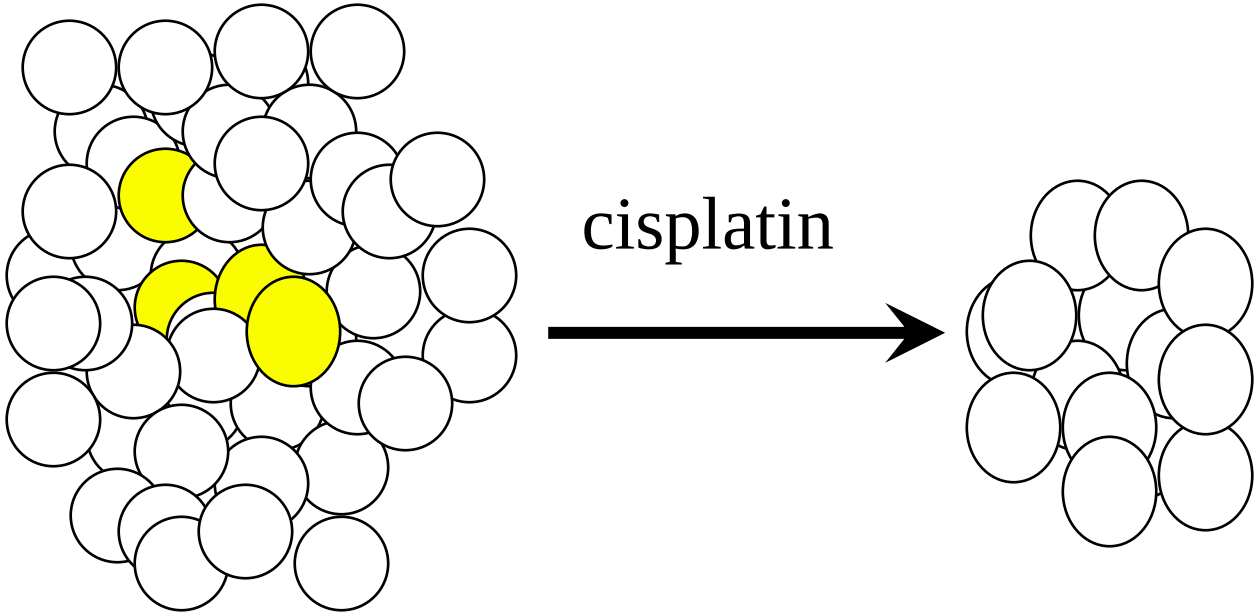
“Acquired” drug resistance

- A clinical definition
  - Insensitivity to therapy that develops during the course of treatment
- Reasons
  - Host changes that lead to inadequate drug delivery
  - Tumour changes that lead to inadequate drug delivery
  - Selection of initially resistant subclone
  - Genetic and epigenetic changes that results in insensitivity to drug
    - Upregulation of anti-apoptotic mechanisms

Therapy can select resistant clones



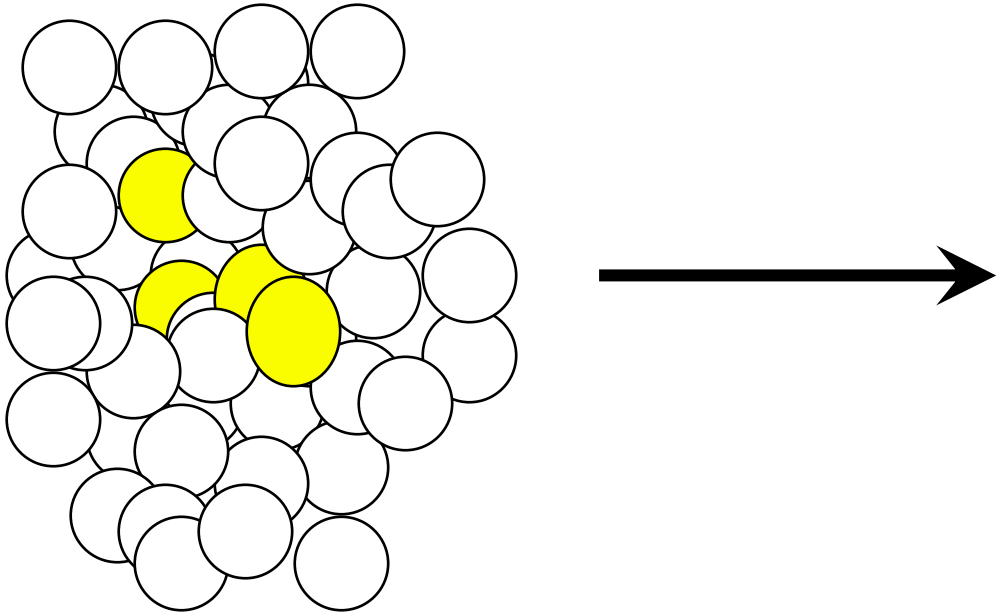
Different drugs may kill different clones



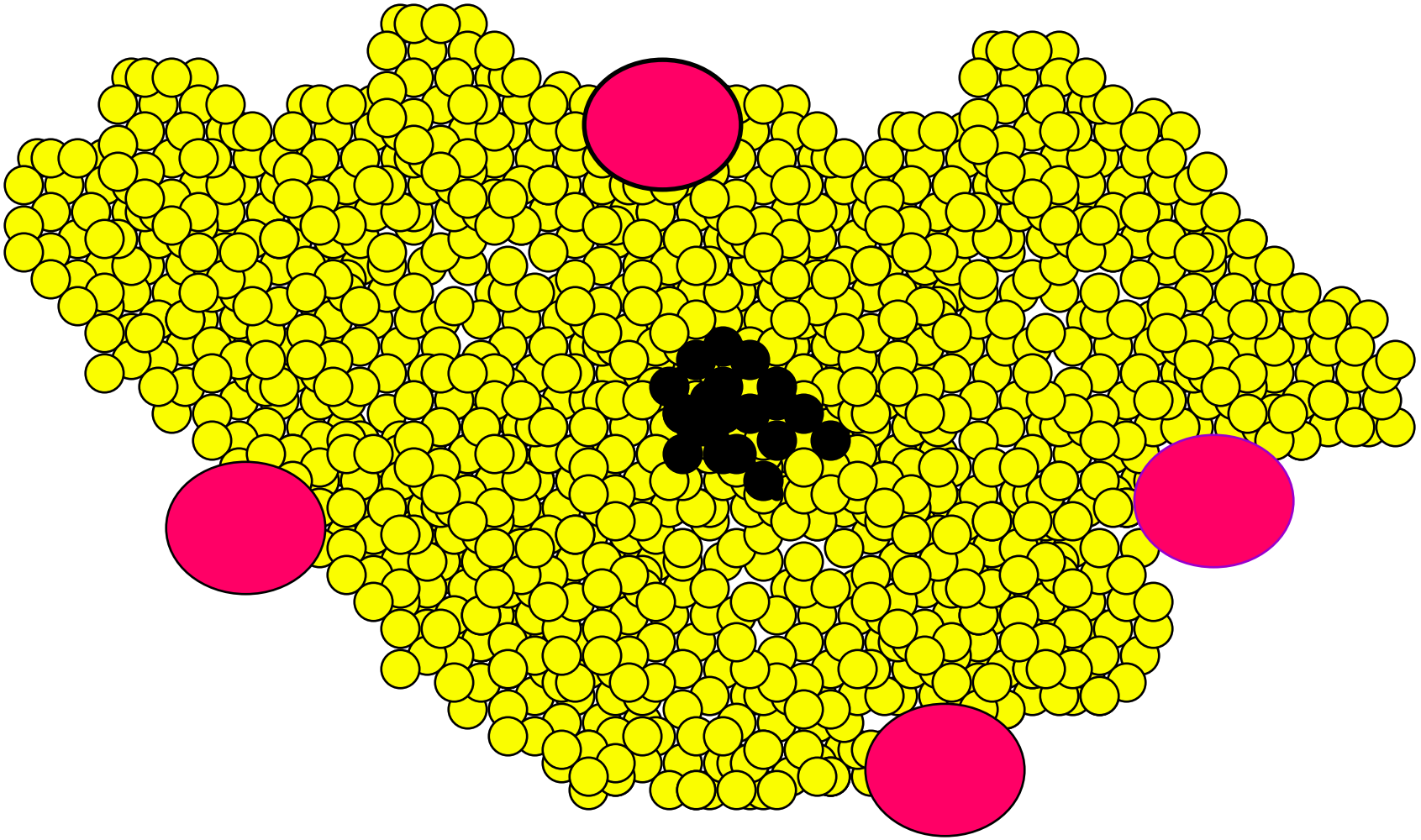
Principles of combination chemotherapy

- each agent should have single agent efficacy
- non-overlapping toxicities
- different mechanisms of action
- no cross resistance

Combination therapy may overcome drug resistance



Drug resistance due to physical factors



Physical barriers to drug delivery

- Tumour interstitial pressure
  - Cancers are hard – Why?
  - No lymphatic drainage
- Poor oxygenation / hypoxia
  - Neoangiogenesis
- Antiangiogenic therapy
  - Appears, in general, to augment effect of chemotherapy
  - Three FDA approved drugs (bevacizumab, sorafenib, sunitinib)

Concepts

- **Systemic therapy may be given for three reasons:**
  - **to cure**
  - **to prolong life**
  - **to reduce symptoms**
- **Knowing the treatment goal helps the doctor and patient decide on treatment and "justifiable" side effects.**

Some cancers respond poorly to chemotherapy

- Non-small cell lung cancer
- Colorectal cancer
- Pancreatic cancer
- Renal cell carcinoma
- Hepatocellular carcinoma
- Head and neck carcinoma
- metastatic melanoma

Some cancers respond to chemo but are not curable

- Advanced small cell lung cancer
- Metastatic breast cancer
- Bladder cancer
- Chronic leukemias
- Multiple myeloma

Some cancer that chemotherapy may cure at advanced stages

- Hodgkin's disease
- aggressive lymphomas
- acute lymphoblastic leukemia
- testicular cancer
- gestational trophoblastic neoplasia

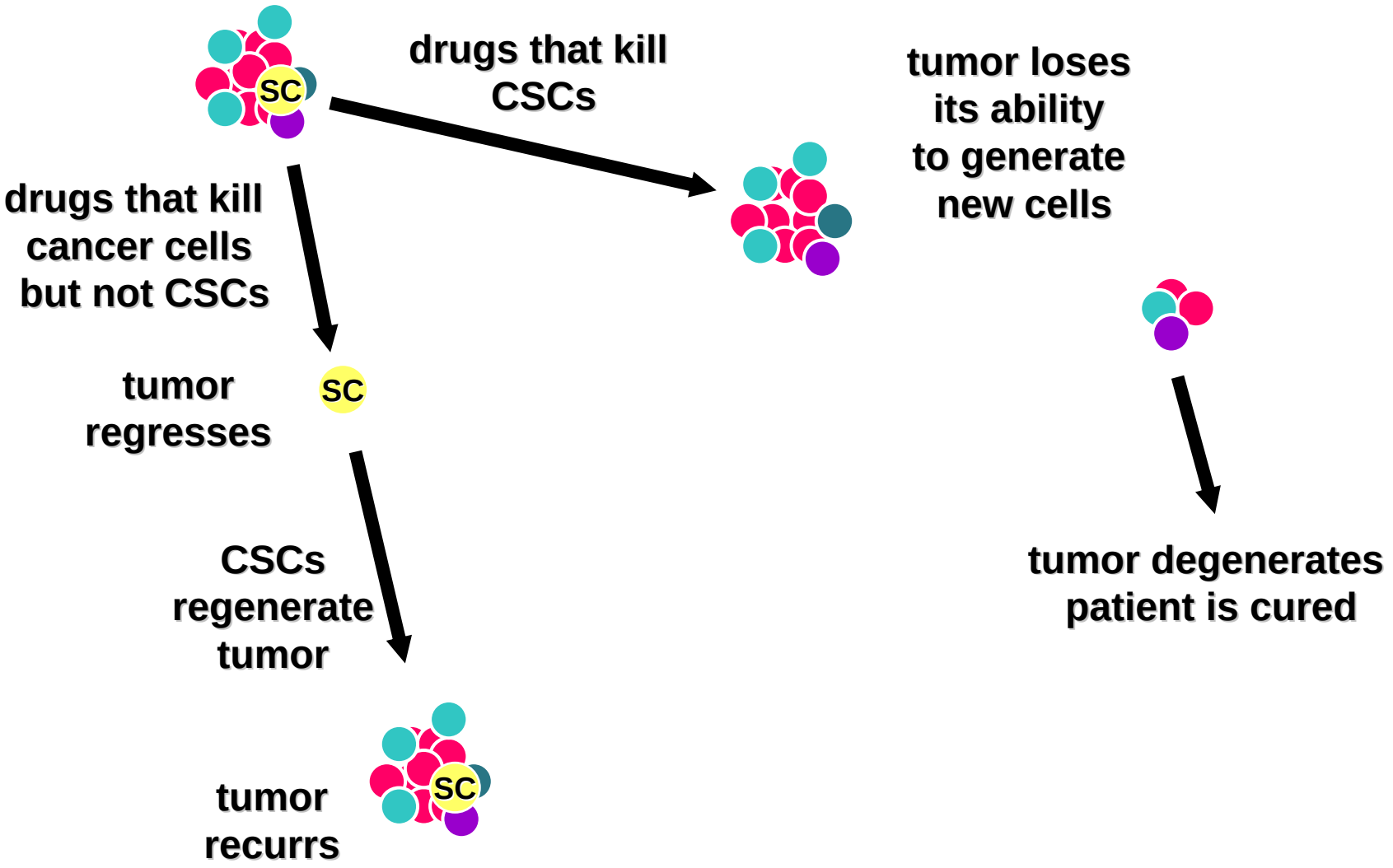
How do we improve systemic therapy?

- **Understand the target cell**
  - **Is the cancer stem cell the real target?**
- **Understand and exploit relevant mechanism of resistance**
  - **Molecular predictive assays**

Cancer Stem Cells

- **Identified in leukemia, breast, colon, and brain cancers**
- **Features**
  - **can differentiate into all the cell types of the parental tumor**
  - **activation of pluripotency genes (Oct4, Sox2, Nanog)**
  - **self renewal**
  - **tumorigenic**
  - **Multidrug resistance**

The Implications of Cancer Stem Cells (CSCs) for Treatment



Characteristics of malignant breast stem cells

- Able to exclude Hoechst dye (drug efflux pump)
- Less susceptible to apoptosis
- Don’t look much like “breast cancer” cells

What opportunities does this knowledge convey?

- New drugs should be tested for their ability to kill breast stem cells, not shrink big tumours
- Analysis of the entire tumour may be less helpful than analysis of the stem cell component

Never, never, think outside the box.

Anti-Cancer Stem Cell Therapies

Targets?

- **Self renewal pathways (wnt, Notch, Hedgehog)**
- **Epidermal – mesenchymal transition pathways**
- **Cytokine and inflammatory pathways**
- **CD-44 and integrins**

Stem cell drugs?

- **salinomycin, metformin, tesmilifene, sulforaphane, curcumin, piperine**

Stem Cell Take Home Messages

- **Inherently drug resistant and resistant to apoptosis**
- **May be a major contributor to clinical drug resistance**
- **Yet to be shown whether it is possible to kill malignant stem cells yet spare normal tissue stem cells …**

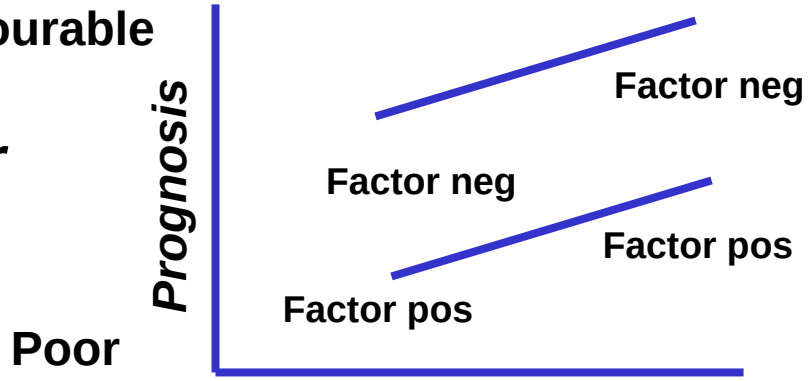
How do we improve systemic therapy?

- **Understand the target cell**
  - **? Stem cell – the real target**
- **Understand and exploit relevant mechanism of resistance**
  - **Molecular predictive assays**

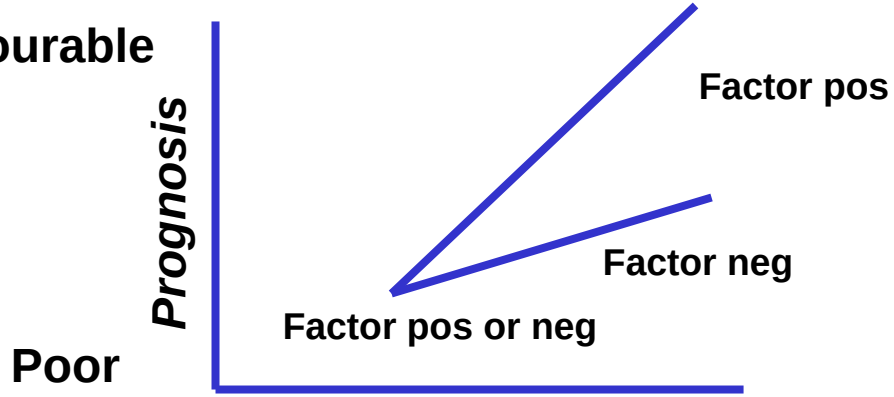
Two Questions

- **Prognostic assay**
  - **“How bad is my cancer, Doc?”**
- **Predictive assay**
  - **“What is the right way to treat my cancer, Doc?”**
  - **“Is this drug going to work?”**
  - **“Am I going to get severe side effects?”**

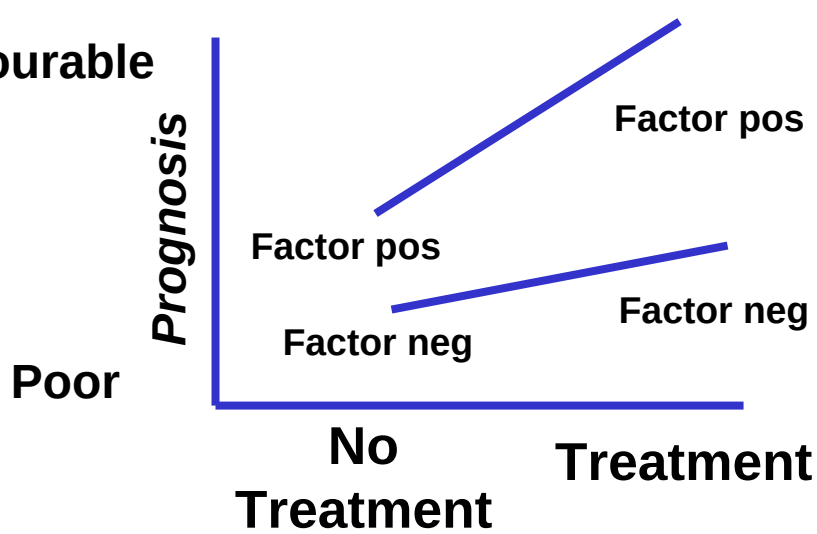
Pure prognostic factor



Pure predictive factor



Mixed predictive / prognostic factor



Why do we need predictive assays?

- Ineffective therapy is costly
  - patient time
  - patient toxicity
  - societal costs
- Predictive assays improve the risk: benefit ratio

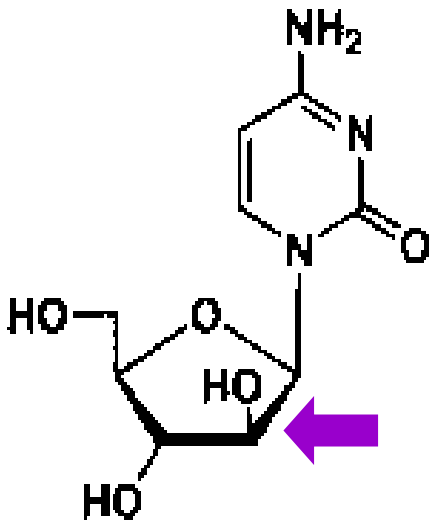
Validated predictive assays for systemic therapy

- ***Estrogen receptor*** **status for breast cancer benefit from hormonal therapy (42 years old)**
- ***HER-2*** **amplification for benefit from trastuzumab therapy for breast cancer (14 years old)**
- ***hENT1*** **overexpression for benefit from gemcitabine for advanced pancreatic cancer (validation underway)**

Predictive assays in development for nucleoside chemotherapy

- **Gemcitabine as an example**

Pyrimidine nucleoside analogs



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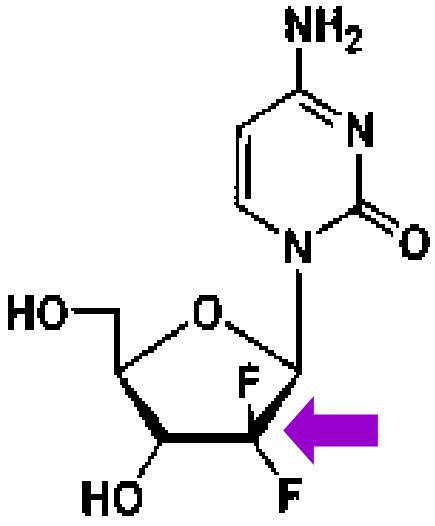
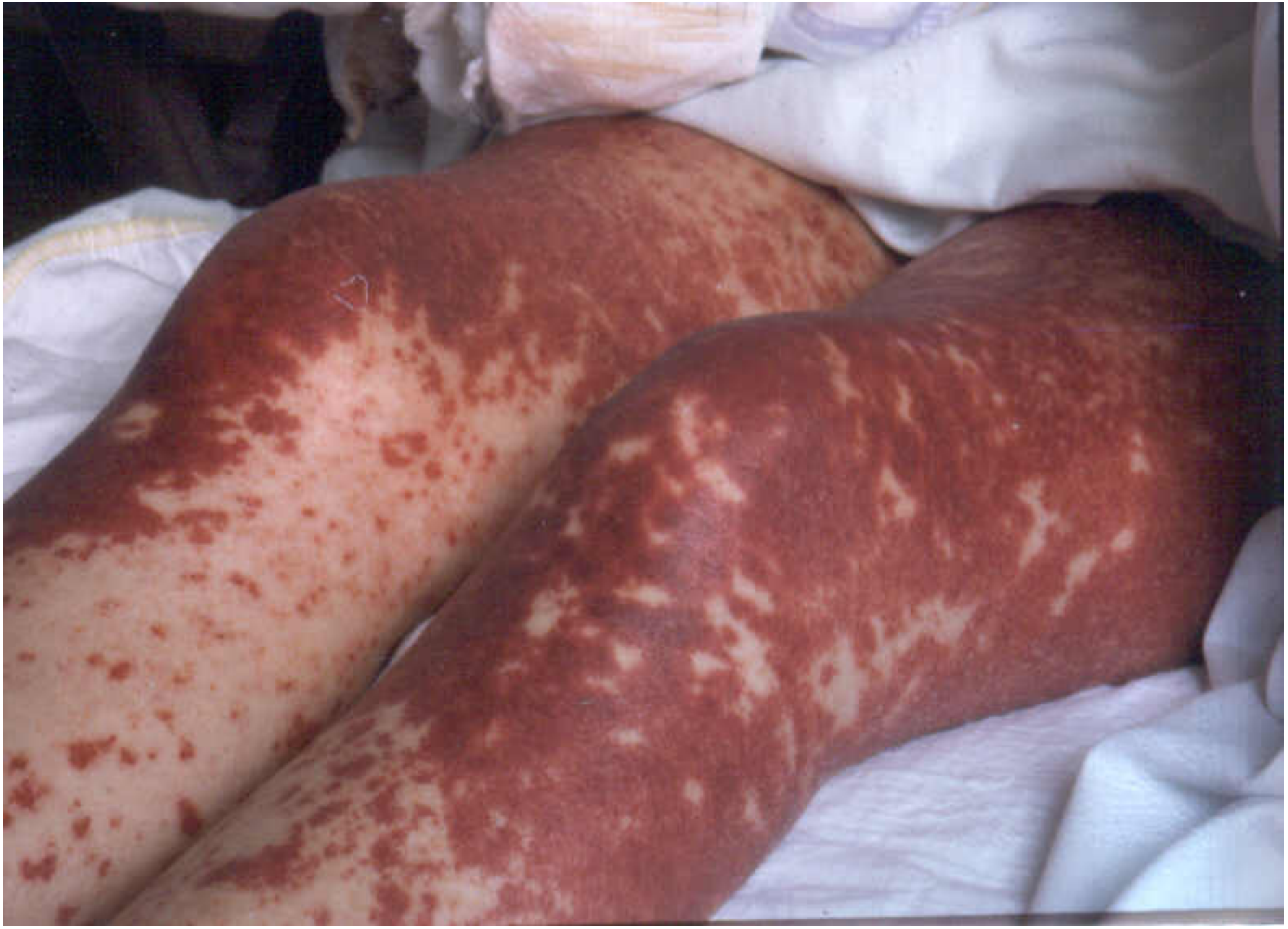


Gemcitabine

Toxicities of anticancer nucleosides

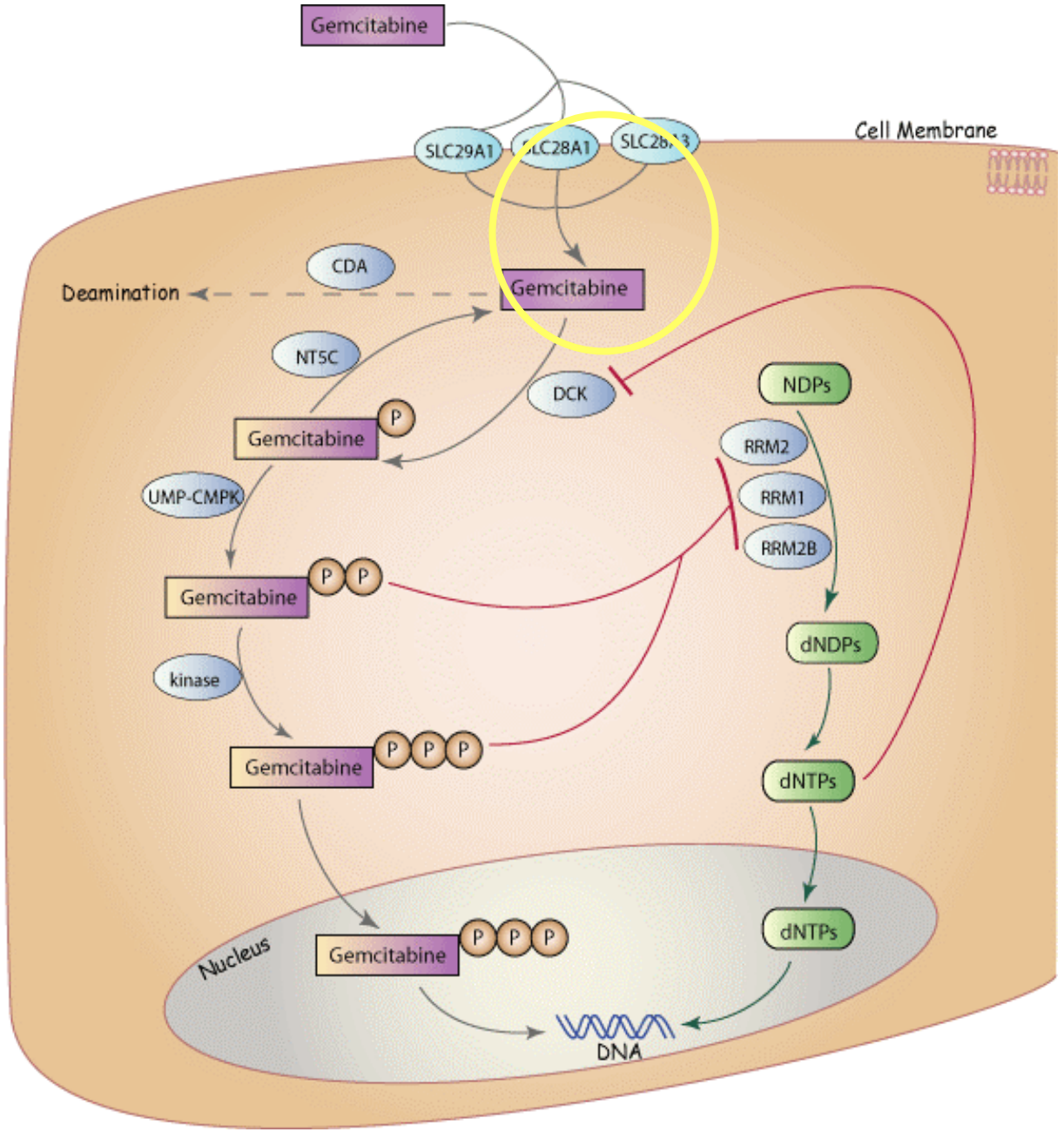
- Hematologic
  - neutropenia
  - thrombocytopenia
  - T cell depletion
  - anemia
- mucositis
- diarrhea
- skin toxicity



Anticancer nucleosides

- **Cytotoxicity and / or clinical response correlates with cellular accumulation of cytotoxic metabolites in target cells**
  - gemcitabine - in vitro
  - cytarabine (Ara-C) - in vitro and in vivo
  - **fludarabine - probably / variable results**
  - capecitabine - in vitro and in vivo

Gemcitabine Uptake and Metabolism



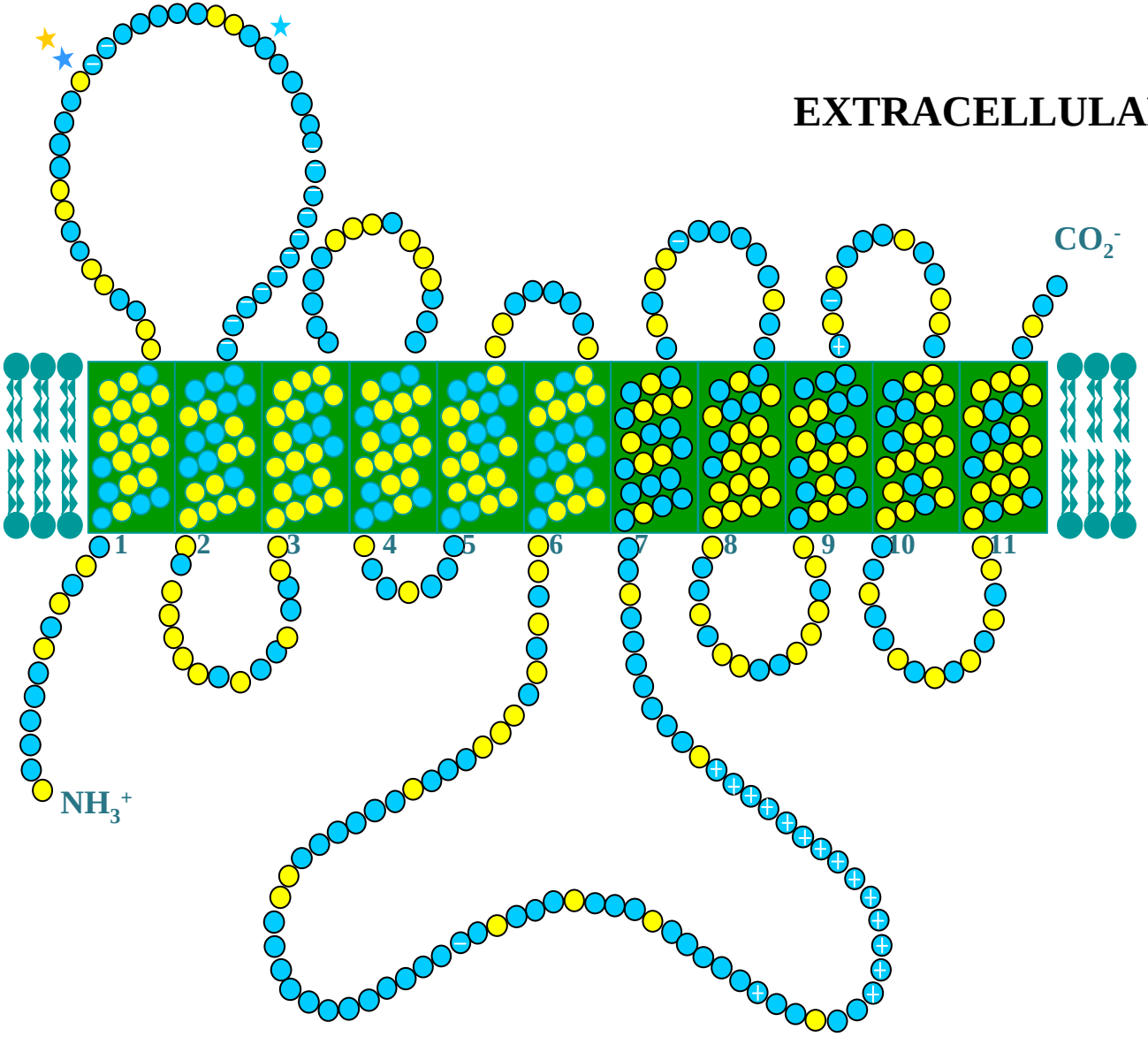
Hypothesis

- **Early steps of nucleoside transport and metabolism are important determinants of clinical nucleoside drug sensitivity**
- **Analysis of clinical samples for nucleoside transport and metabolic capacity will identify patients with drug-resistant disease**

hENT1 and pancreas cancer

- **Gemcitabine monotherapy is standard palliation for advanced pancreatic adenocarcinoma**
- ***In vitro* studies show hENT1 deficiency confers resistance to gemcitabine toxicity**
    - ***Mackey et. al. Cancer Res, 1998***

hENT1



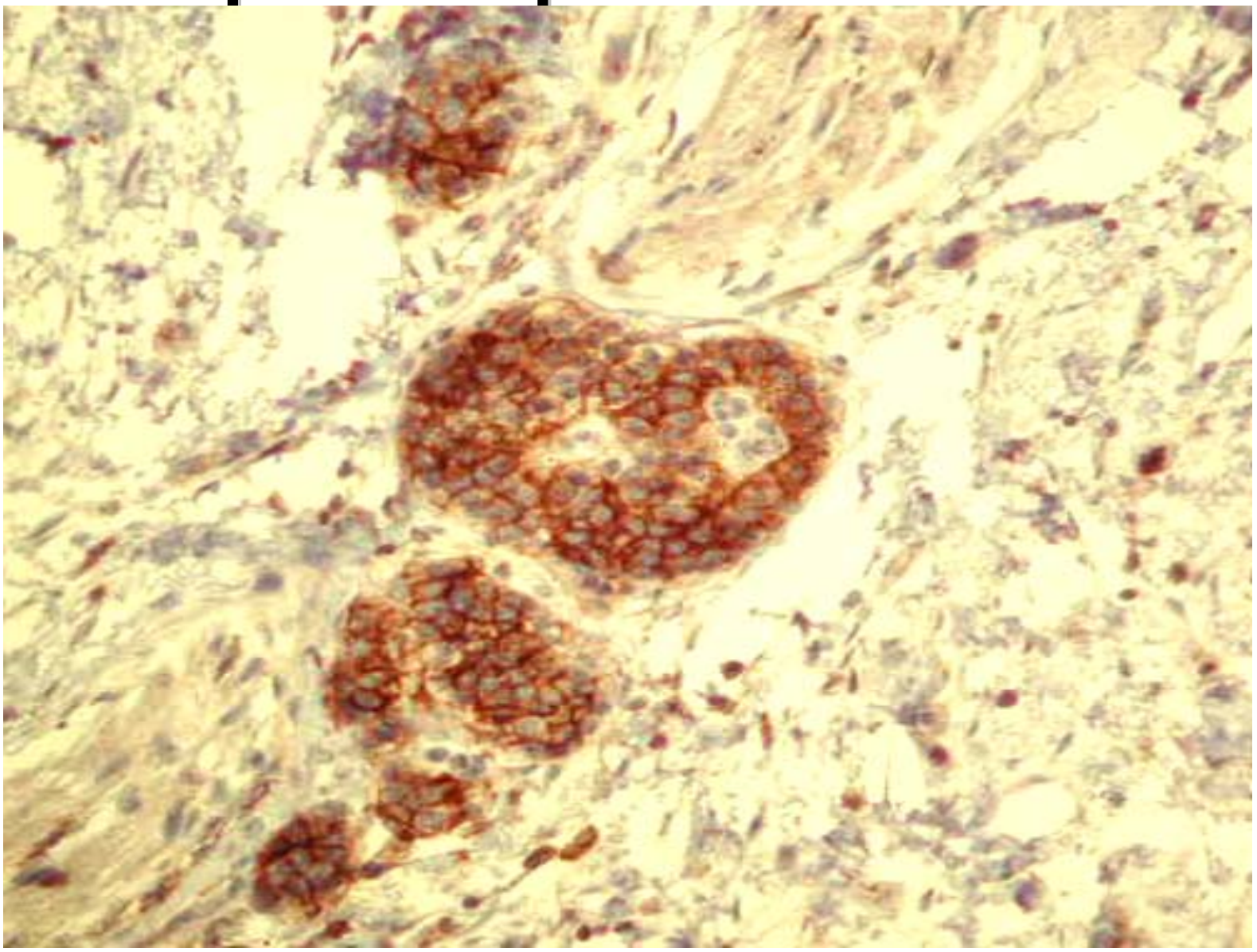
hENT1 immunohistochemistry

- murine monoclonal antibody raised against intracellular loop of hENT1
- antigen detection was performed using a goat-anti mouse antibody directly labeled with a polymer-peroxidase conjugate - BROWN stain
- hENT1 staining intensity on a 0-2 + scale
  - *Mackey et. al. Clin Cancer Research 2002, 2003*

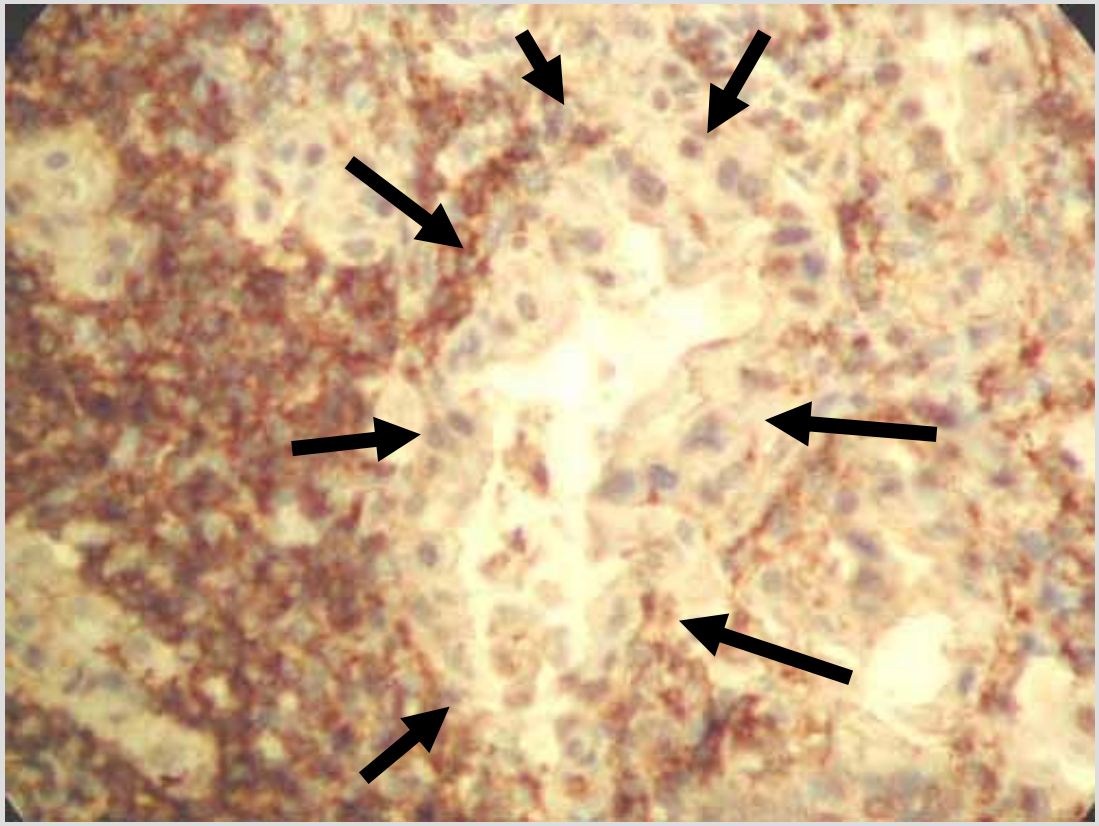
Pancreas CA patients

- Inclusion in this study required each of the following criteria
  - histologic diagnosis of pancreatic adenocarcinoma
  - Formalin-fixed paraffin-embedded tumor sample adequate for study
  - no gemcitabine or radiotherapy prior to the tissue sampling
  - treatment with gemcitabine at an Alberta Cancer Board facility between Sept 1998 and Dec 2002

hENT1 positive pancreatic cancer

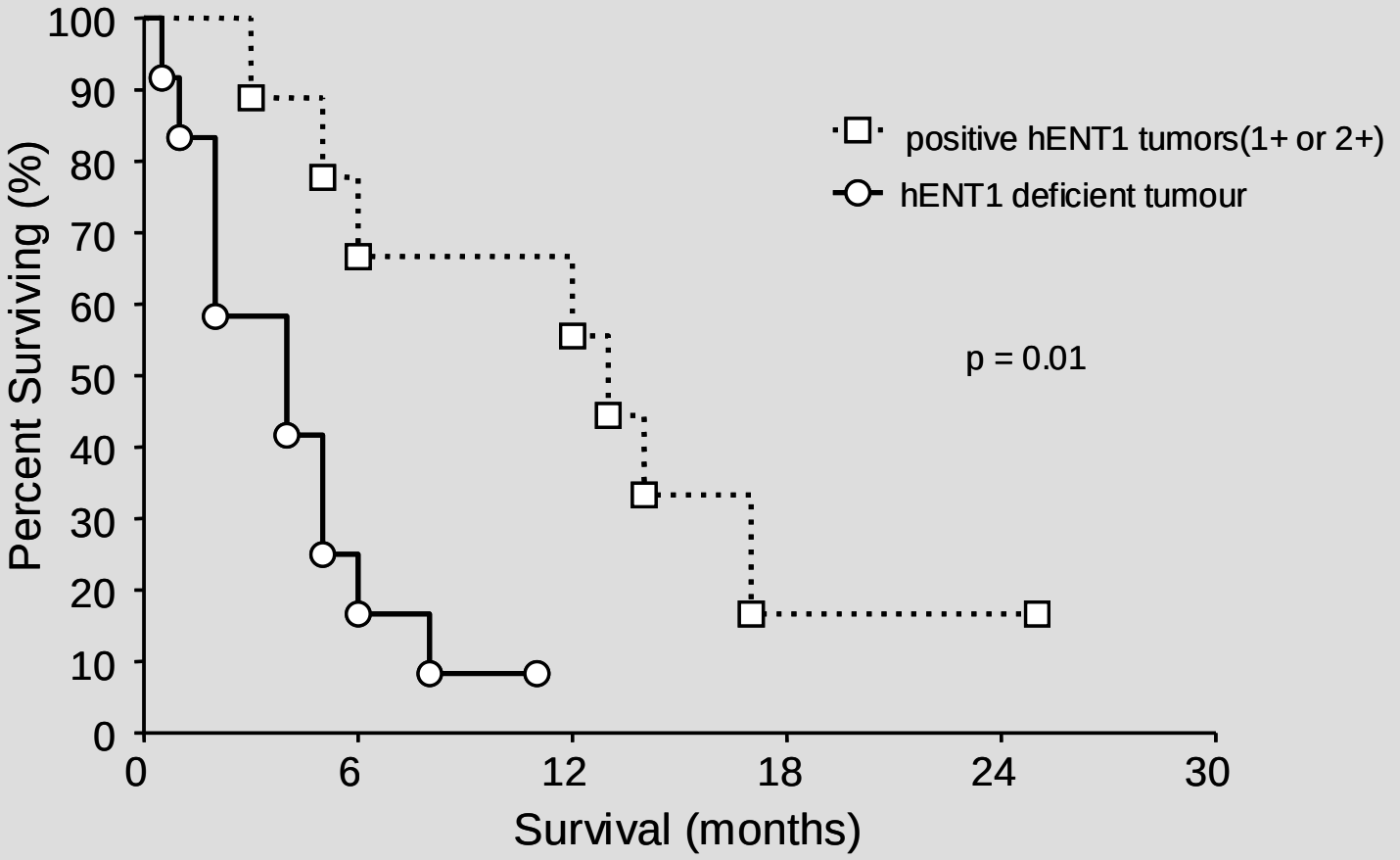


hENT1-negative Pancreatic Adenocarcinoma



Multiple arrows highlight a hENT1-negative gland. Lymphocytes (as positive internal controls) were strongly positive

Kaplin-Meier estimate of survival in gemcitabine-treated pancreatic cancer patients



Conclusions

- **patients with pancreatic adenocarcinomas with uniformly detectable hENT1 immunostaining have a significantly longer survival after gemcitabine chemotherapy**
- **hENT1 immunohistochemistry is candidate for a predictive assay to appropriately select patients for palliative gemcitabine therapy**
- **Is hENT1 predictive, or only prognostic?**
- **Requires confirmation in randomized study to distinguish predictive markers from prognostic markers!**

Human ENT1 is predictive of response in patients with pancreatic cancer treated with gemcitabine: Results from the RTOG 9704 Prospective Randomized Trial.

James Farrell, Miguel Garcia, Raymond Lai, Ali Ammar, W. Regine, R. Abrams, A. Bowen Benson, J. Macdonald, Carol E. Cass, Hany Elsaleh, John Mackey.

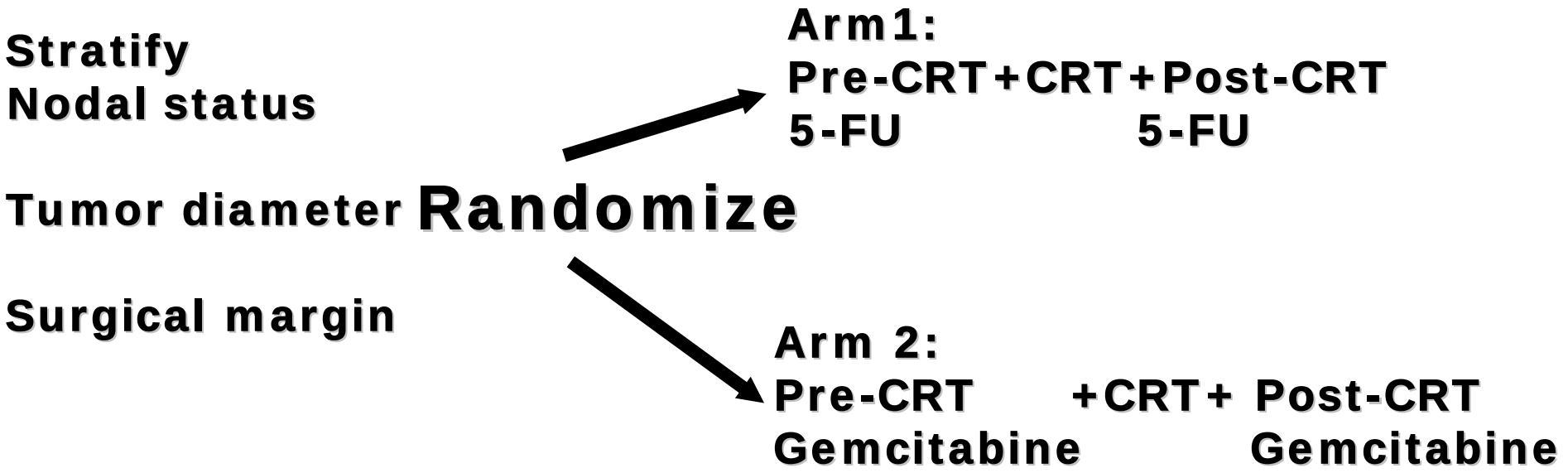
Gastroenterology, 2009

Methods

RTOG 9704

- Adjuvant treatment of resected pancreatic cancer
- A Phase III randomized study
  - Pre and post chemoradiation 5-FU

    vs
  - Pre and post chemoradiation 5FU and Gemcitabine



RTOG: Radiation Therapy Oncology Group

Methods
hENT1 Protein Expression : IHC

- **RTOG 9704 Tissue Microarray**
  - **220 patient tumors per TMA**
  - **3 separate TMAs**
- **hENT1 Immunohistochemistry (IHC)**
  - **Mouse anti hENT1 monoclonal antibody**
  - **Score in triplicate**
  - **Blinded score, unaware of clinical outcomes data**

Methods
Statistical Analysis

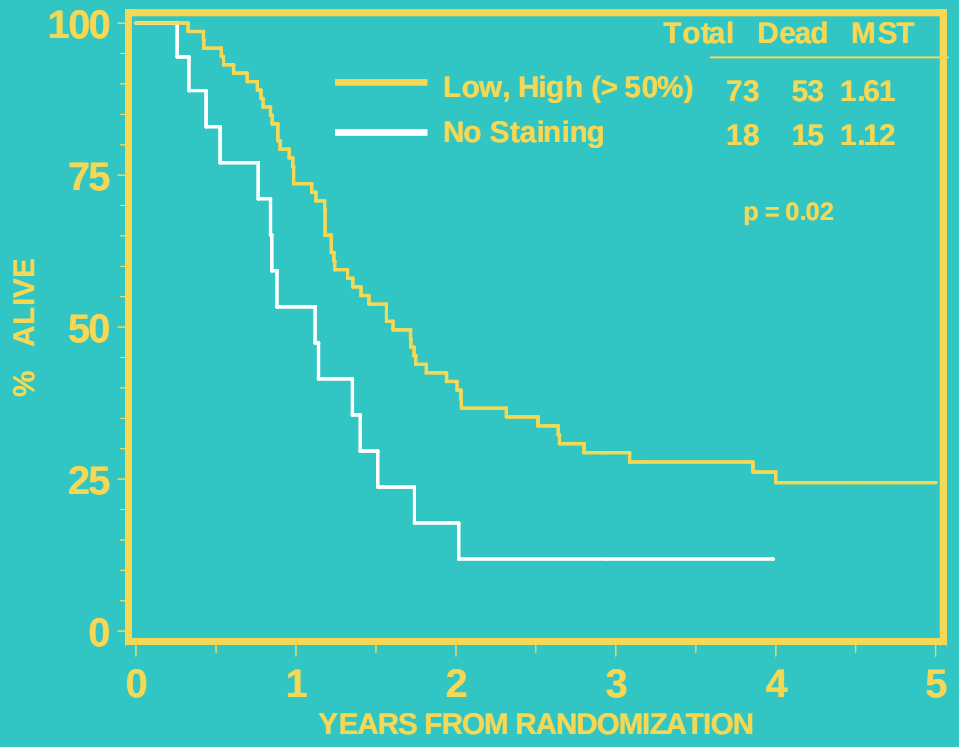
- **hENT1 score was correlated**
  - **Treatment Group**
    - **Overall**
    - **5-FU vs Gemcitabine**
  - **Treatment outcome**
    - **Overall survival**
    - **Disease free survival**
  - **Toxicity**

- **Unconditional logistic regression analysis using the Chi-square test and the Cox proportional hazards model.**

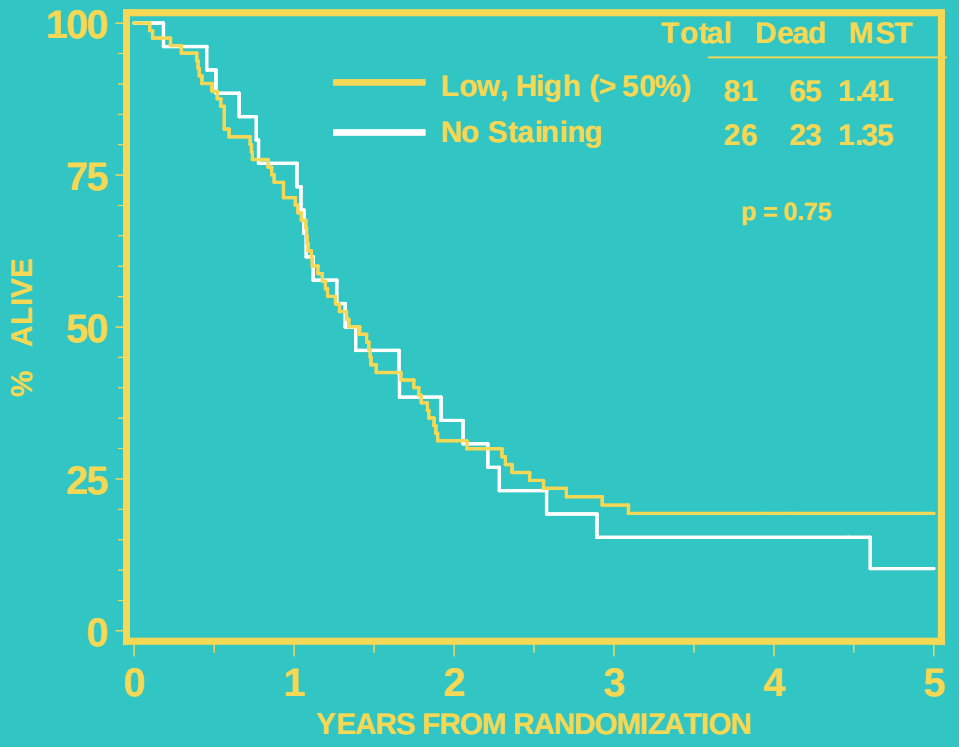
Results

hENT1: Overall Survival (univariate analysis)

Gemcitabine Arm

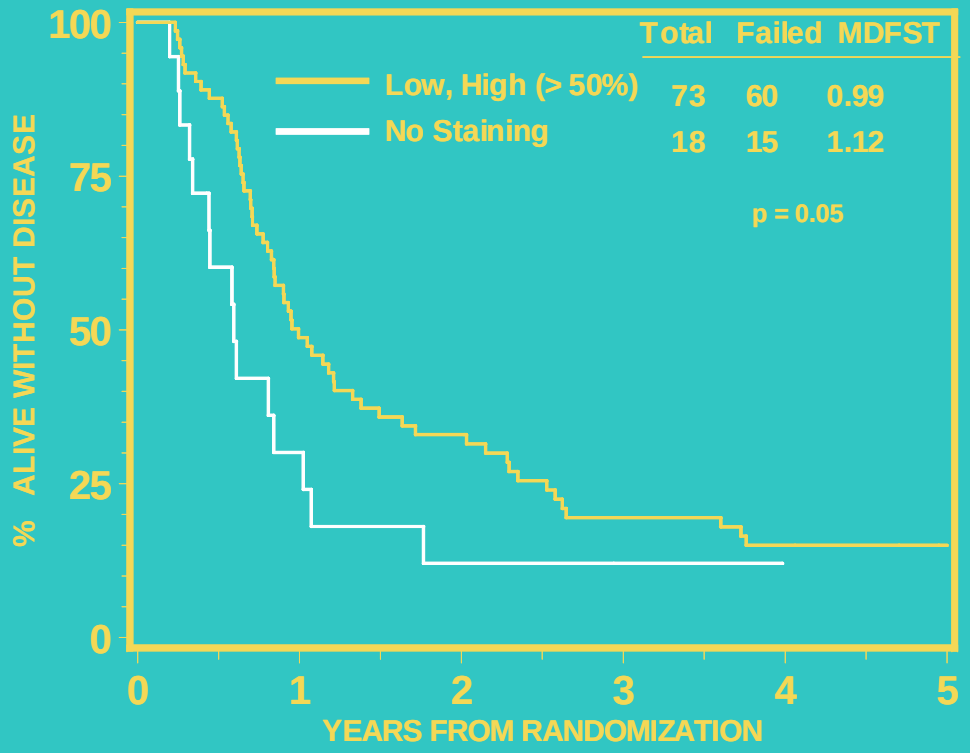


5-FU Arm

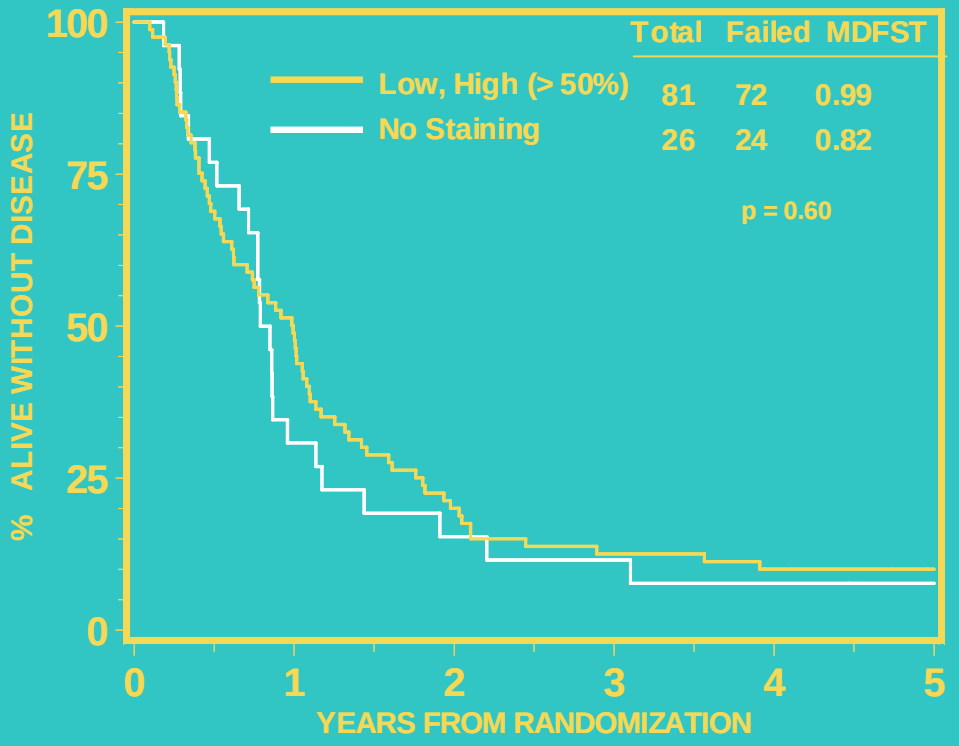


Results
Disease Free Survival (univariate analysis)

Gemcitabine Arm



5-FU Arm



Conclusion

- hENT1: RTOG 9704 Study
  - Improved Overall Survival
    - Gemcitabine, but not 5-FU Treatment Arm
  - Improved Disease Free Survival
    - Gemcitabine, but not 5-FU Treatment Arm
  - Univariate and Multivariate Analysis
  - Correlation between outcome and hENT1 Score

  - Has predictive value, not prognostic value

Conclusions

- **Predictive assays can pick out patients unlikely to benefit from treatment**
- **Predictive assays can improve risk: benefit ratio of treatment**

Drug resistance take home messages

Drug resistance is the main barrier to cure of advanced cancers

Multiple mechanisms contribute to drug resistance

An understanding of these mechanisms is leading to improvements in anticancer drug treatment

- **rational combinations**
- **molecularly targeted therapy**
- **stem-cell targeted approaches**
- **predictive assays**