Metabolism, Metabolomics and Cancer

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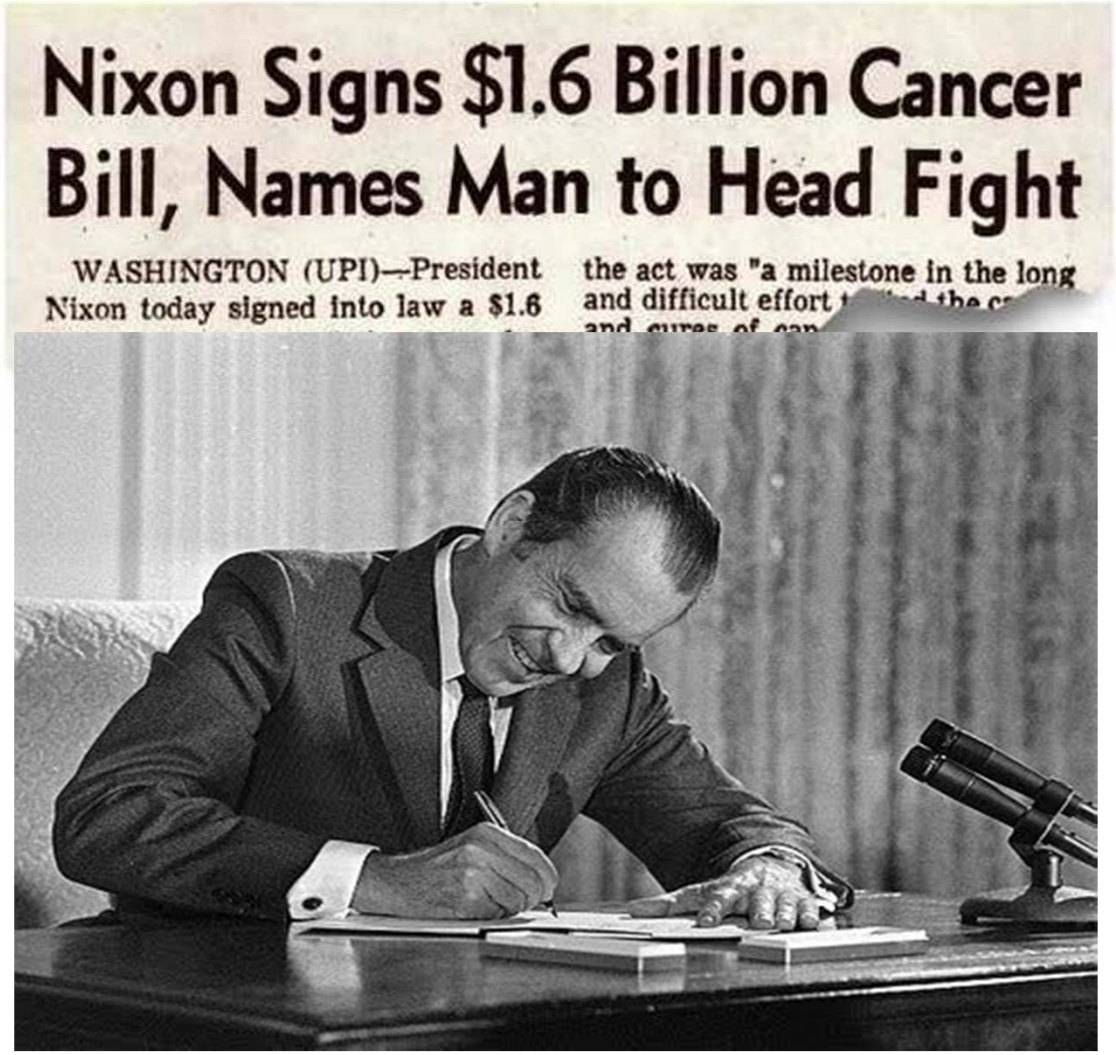
Birmingham, AL, June 19, 2015

Cancer

- A disease caused by an uncontrolled division of abnormal cells in a part of the body
- "The Emperor of All Maladies"
- 41% of us will develop cancer at some point in our lives
- 2nd leading cause of death in US
- Leading cause of death in Canada, UK, New Zealand, Australia, Denmark

44 Years Ago



Nixon Signs $1.6 Billion Cancer Bill, Names Man to Head Fight

WASHINGTON (UPI)—President Nixon today signed into law a $1.6

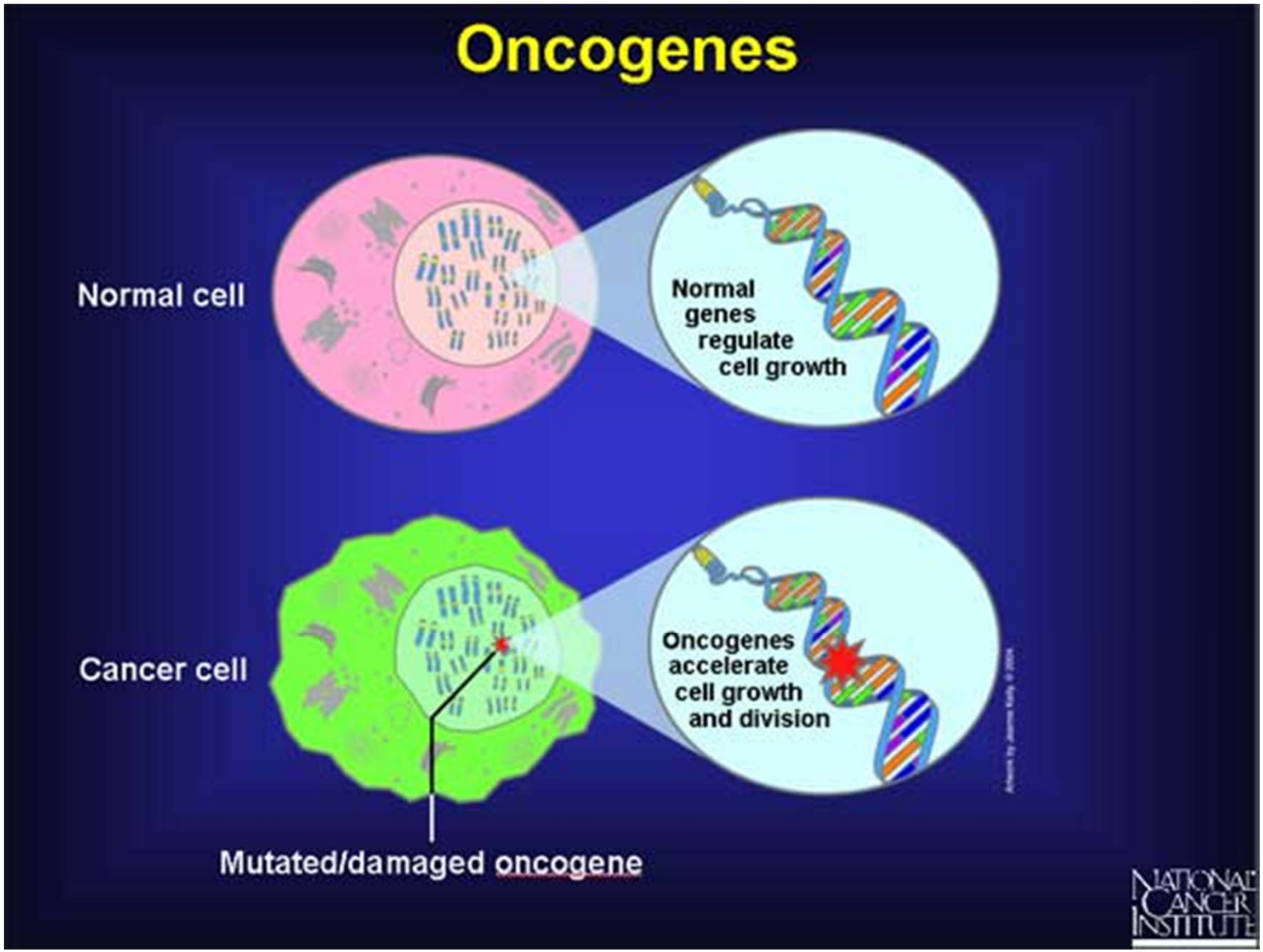
the act was "a milestone in the long and difficult effort

- Nixon declared war on cancer on Dec 23, 1971
- Since then >$200 billion has been spent on cancer research

39 Years Ago

The Discovery of Oncogenes (Varmus & Bishop ~1976)

Cancer as a Genetic Disease



Cancer as a Genetic Disease

- Every cancer cell has mutations leading to over-expression or perturbations to oncogenes, proto-oncogenes or tumor suppressor genes
- An **oncogene** is a gene that has the potential to cause cancer
- A **proto-oncogene** is a normal gene that can become an oncogene due to mutations or increased expression
- A **tumor suppressor gene (TSG)** is a normal gene that prevents tumor development
- Examples of oncogenes include: Ras, Myc, Raf, Src, EGFR, HER2/neu, HIF-1α, Wnt, Erk, Trk, Bcr-Abl
- Examples of TSGs include: BRCA1, p53, PTEN

15 Years Ago

June 26, 2000 – 1st Draft of Human Genome Completed

15 Years Ago

The Hallmarks Of Cancer

The Hallmarks Of Cancer

Hanahan D & Wienberg RA, (2000) Cell, Jan 100(1): 57-20

Unbounded Optimism

Time Magazine
April 1, 2003

New Cancer Therapies

- **Gene therapy**
- **T-cell therapy**
- **Stem cell transplant**
- **Monoclonal antibody therapy**
  - Rituximab
  - Campath
- **Mitotic inhibitors**
  - Paclitaxel
  - Vinblastine
- **Topoisomerase inhibitors**
  - Irinotecan
  - Etopiside
- **Anti-hormone therapy**
  - Tamoxifen
- **Targeted wonder drugs**
  - Gleevec

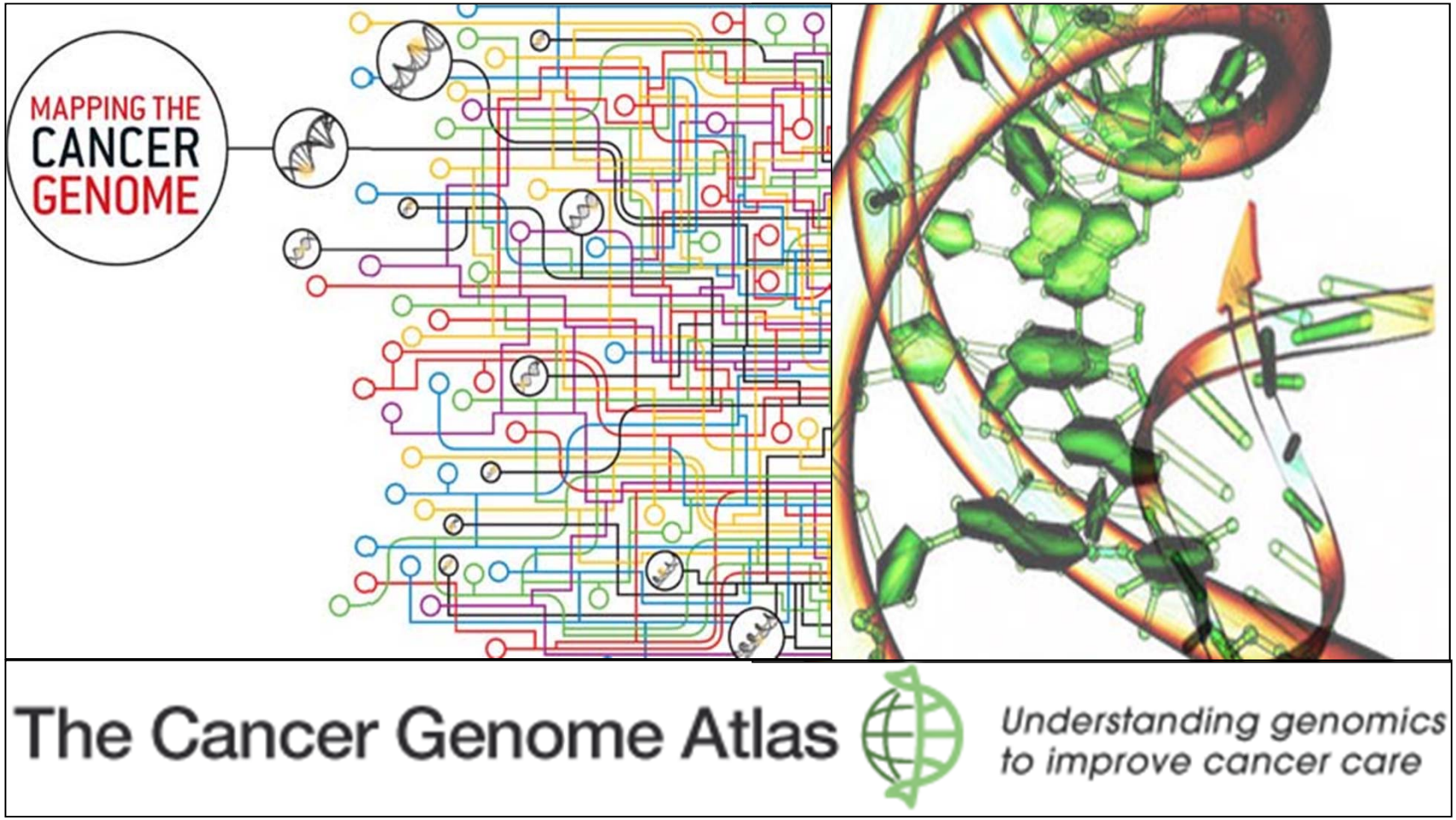
5 Years Ago

Next Generation DNA Sequencing

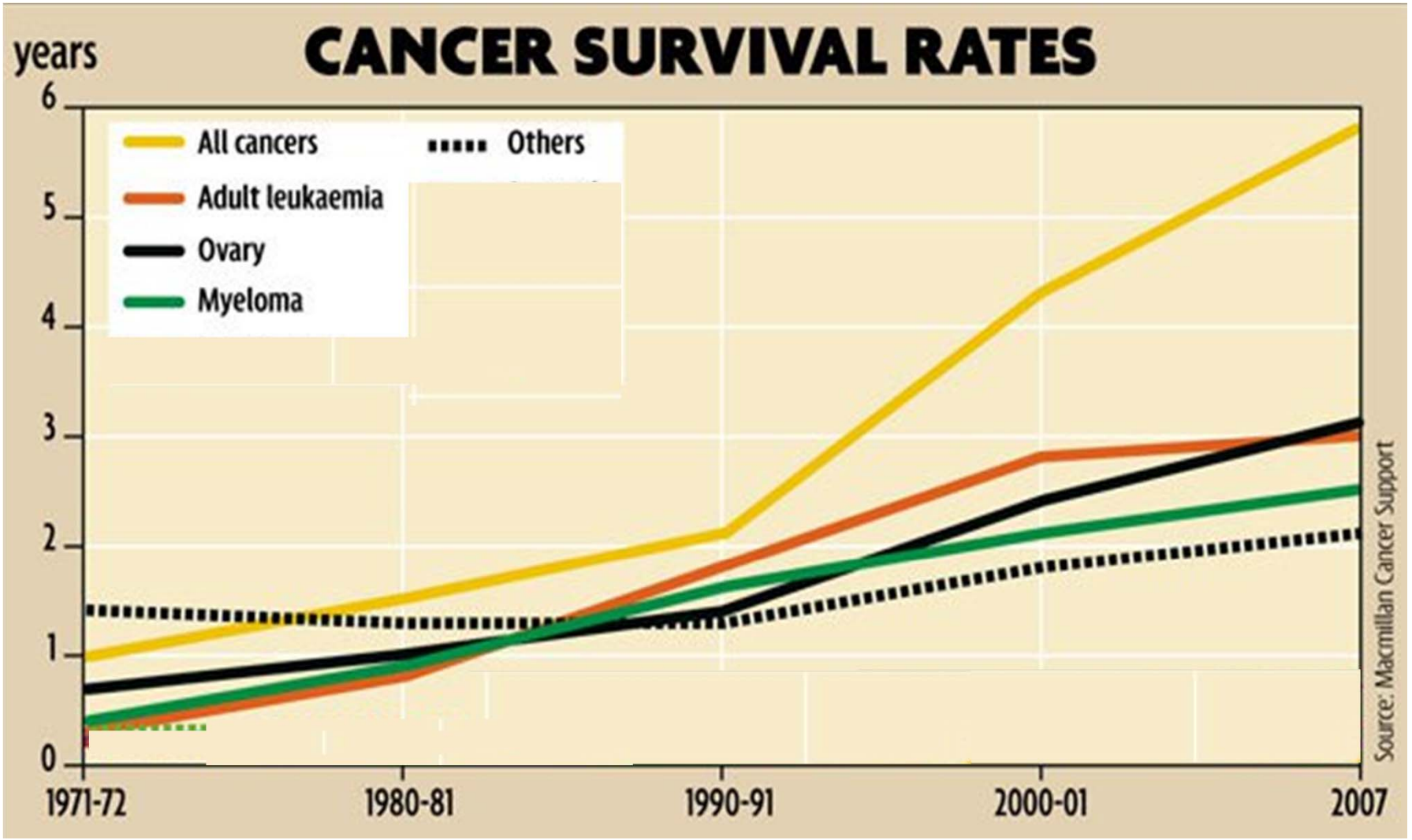
ABI SOLiD - 20 billion bases/run
Sequencing by ligation

Illumina/Solexa 15 billion bases/run
Sequencing by dye termination

The Cancer Genome Atlas



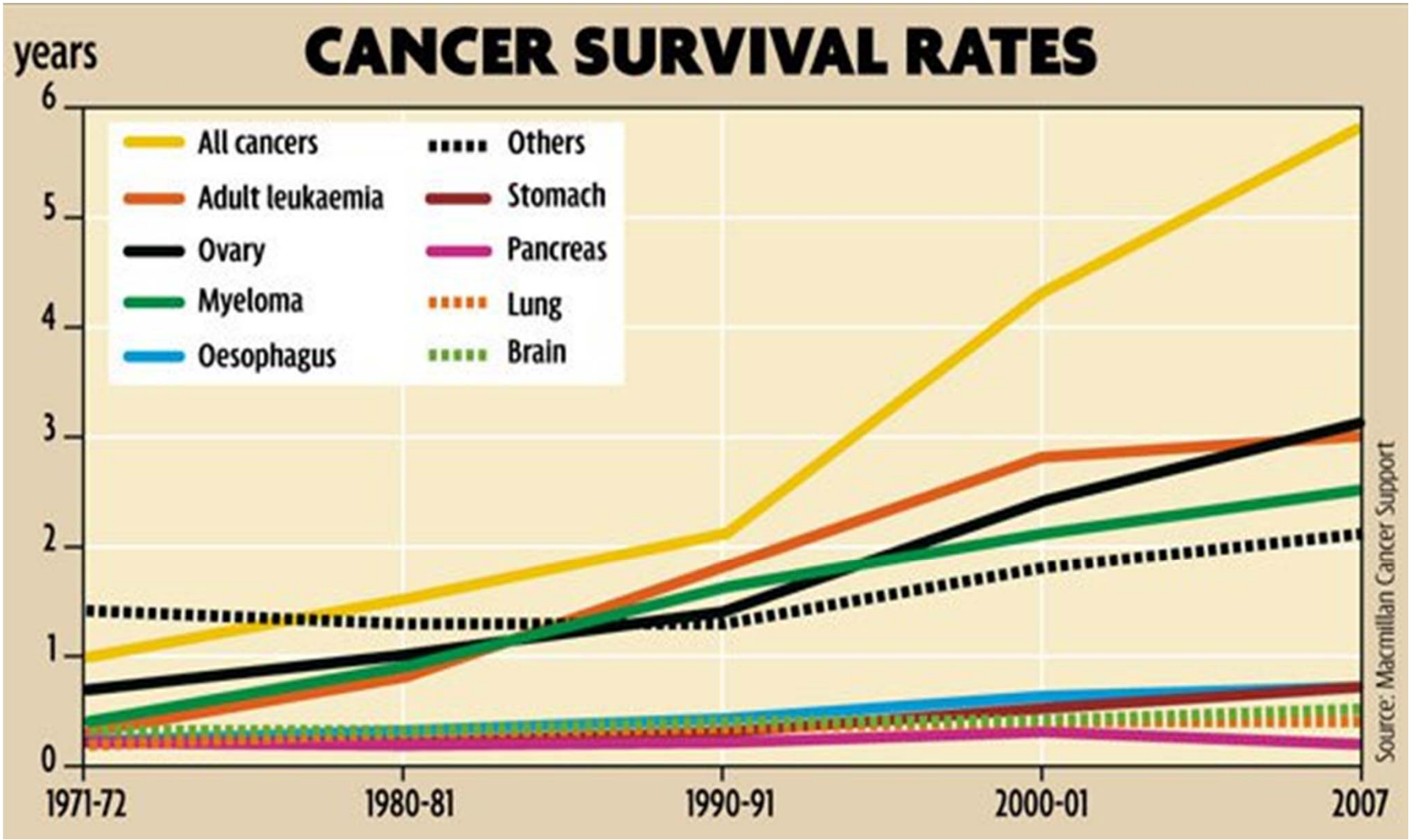
The Good News



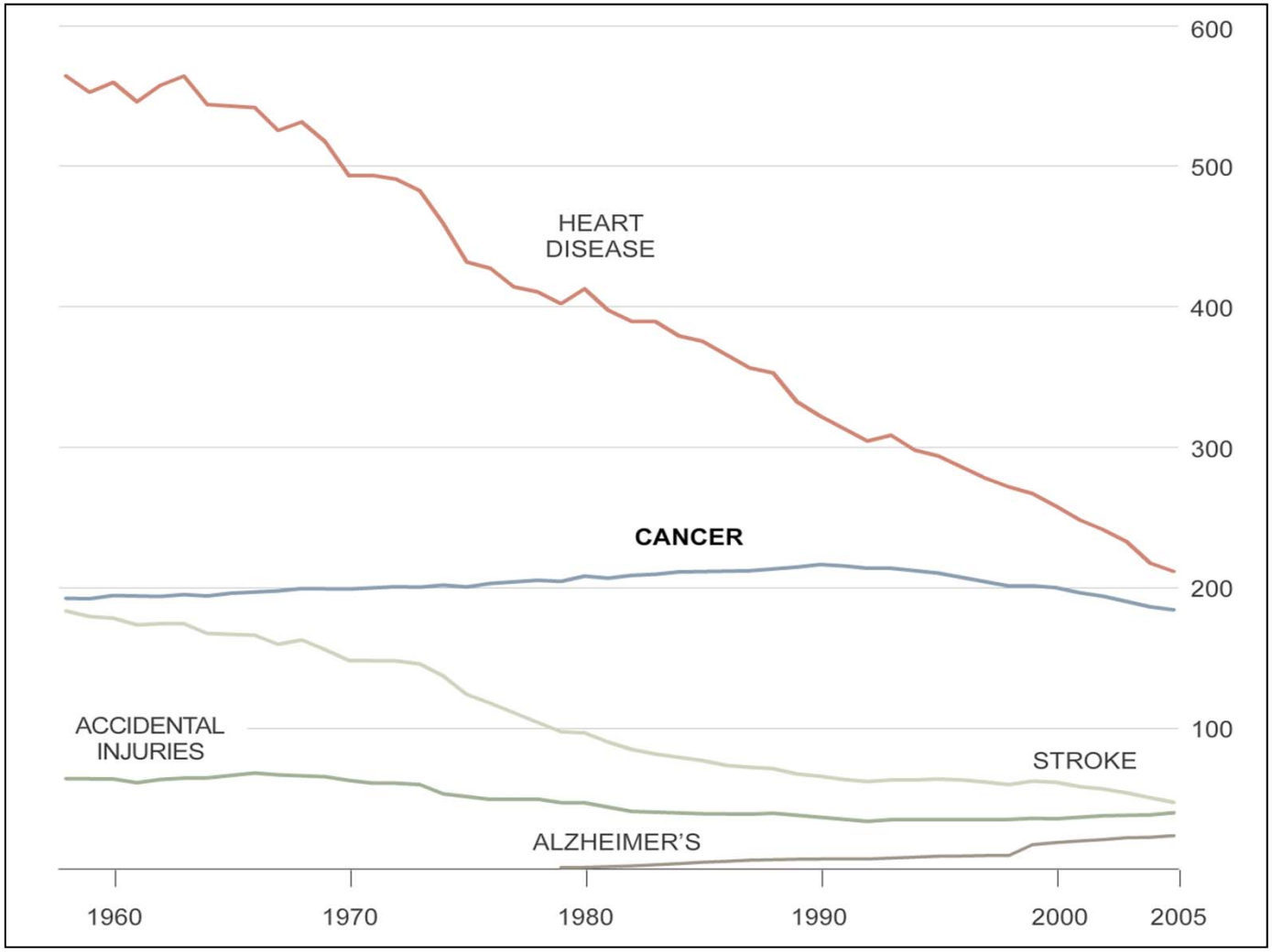
However…

- Most improvements in cancer survival are due to better screening, which leads to earlier detection (stage I or II), which leads to statistically longer survival times
- Most advances in “curing” cancer have been seen in relatively rare cancers (childhood leukemia, certain types of lymphomas)

Not So Good News



The Bad News



Age-Adjusted Death Rates (US)

The Really Bad News

- Cancers are caused by 2-3 “founder” mutations (to oncogenes/TSPs)
- ~250 oncogenes, ~700 tumor suppressor genes identified so far
- Cancer is 1,000,000+ different diseases
- Cancer cells accumulate ~10,000-50,000 mutations/CNVs after conversion (genetic noise)
- Cancer cells are a “genetic train wreck”

Where To Next?

How Was Cancer Viewed Prior to 1970?

- Prevailing opinion among most oncologists was that cancer was a "metabolic disease"
- Cancer cells were metabolically dysregulated (cause of the metabolic dysregulation was unknown)
- Cancer drugs were called "anti-metabolites" and cancer chemotherapy was call anti-metabolite therapy

Anti-Metabolite Cancer Drugs

Anti-metabolite	Metabolite equivalent
5-Fluorouracil (5-FU) - 1957	Uracil
Gemcitabine (Ara-C) - 1981	Cytosine
6-Mercaptopurine - 1951	Adenine/Guanine
Fludarapine (Ara-A) - 1968	Adenine
Methotrexate - 1956	Folate
Aminopterin - 1947	Folate
Megestrol acetate - 1956	Progesterone
Asparaginase* - 1963	Asparagine/Glutamine*

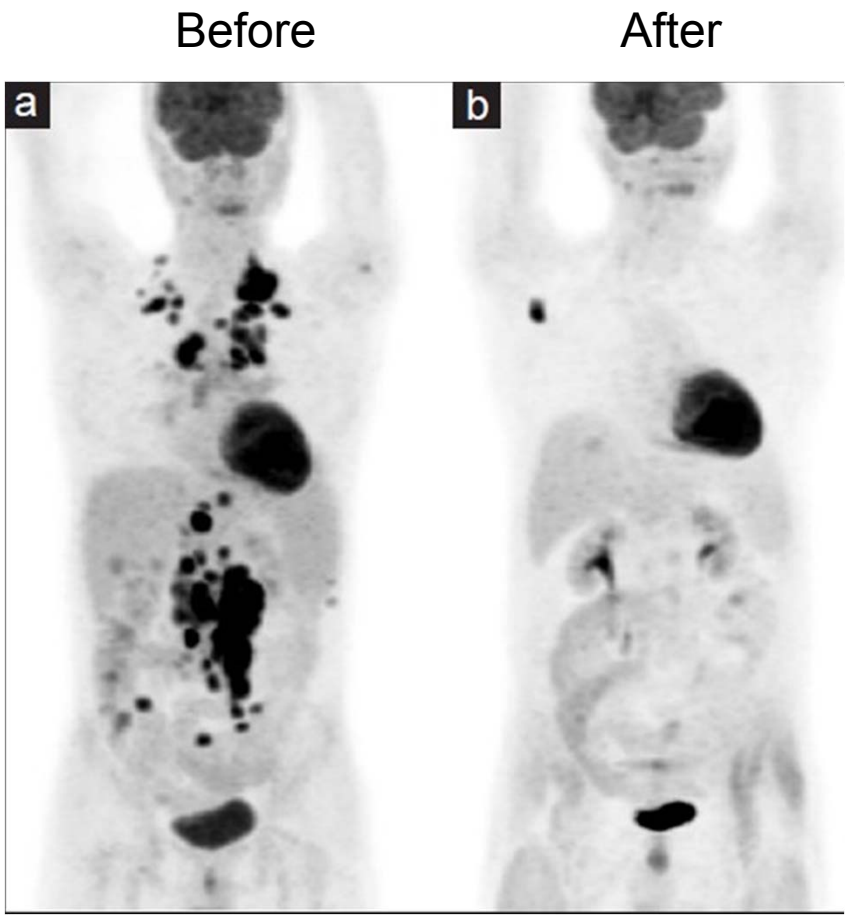
Who Came Up With This Crazy Idea?

Otto Warburg

- Observed in 1924 that cancer cells use aerobic glycolysis to fuel growth instead of oxidative phosphorylation
- Won the Nobel Prize in 1931
- Advocated that: "replacement of oxygen-respiration by fermentation is the prime cause of cancer"
- The metabolic view of cancer predominated thinking from 1920's up to Warburg's death in 1970

Cancer is a Metabolic Disease

- Cancer cells consume 100-200X more glucose that other cells in the body
- This unique metabolism is the basis to PET (positron emission tomography) scans for cancer using fluorinated deoxyglucose
- This metabolic shift is called the **Warburg effect** or cytosolic aerobic glycolysis



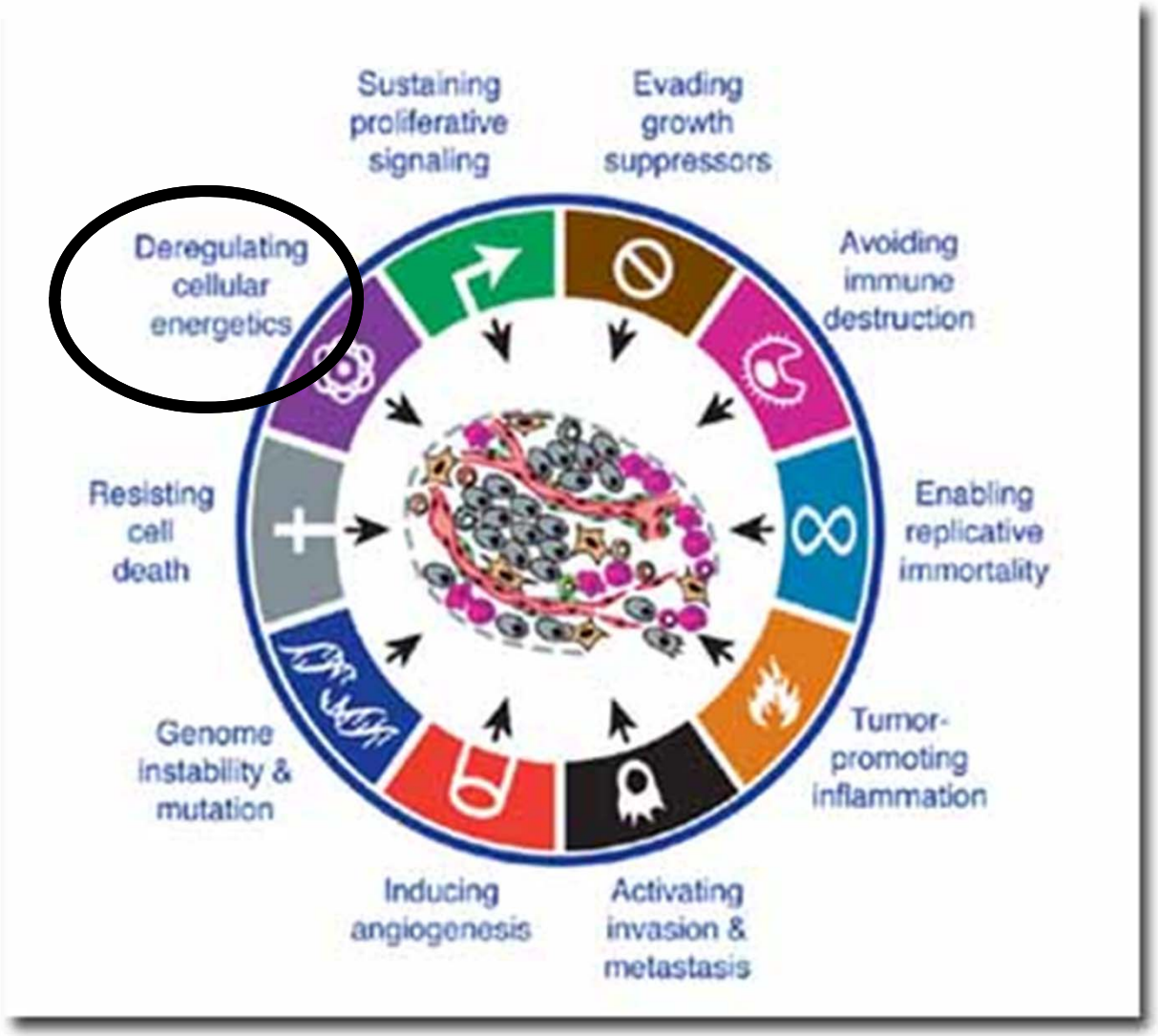
Tumors are marked in black in this PET image (lots of glucose)

How Is A Metabolic View of Cancer Compatible With the Genetic View?

Oncogenes are Metabolic Hubs

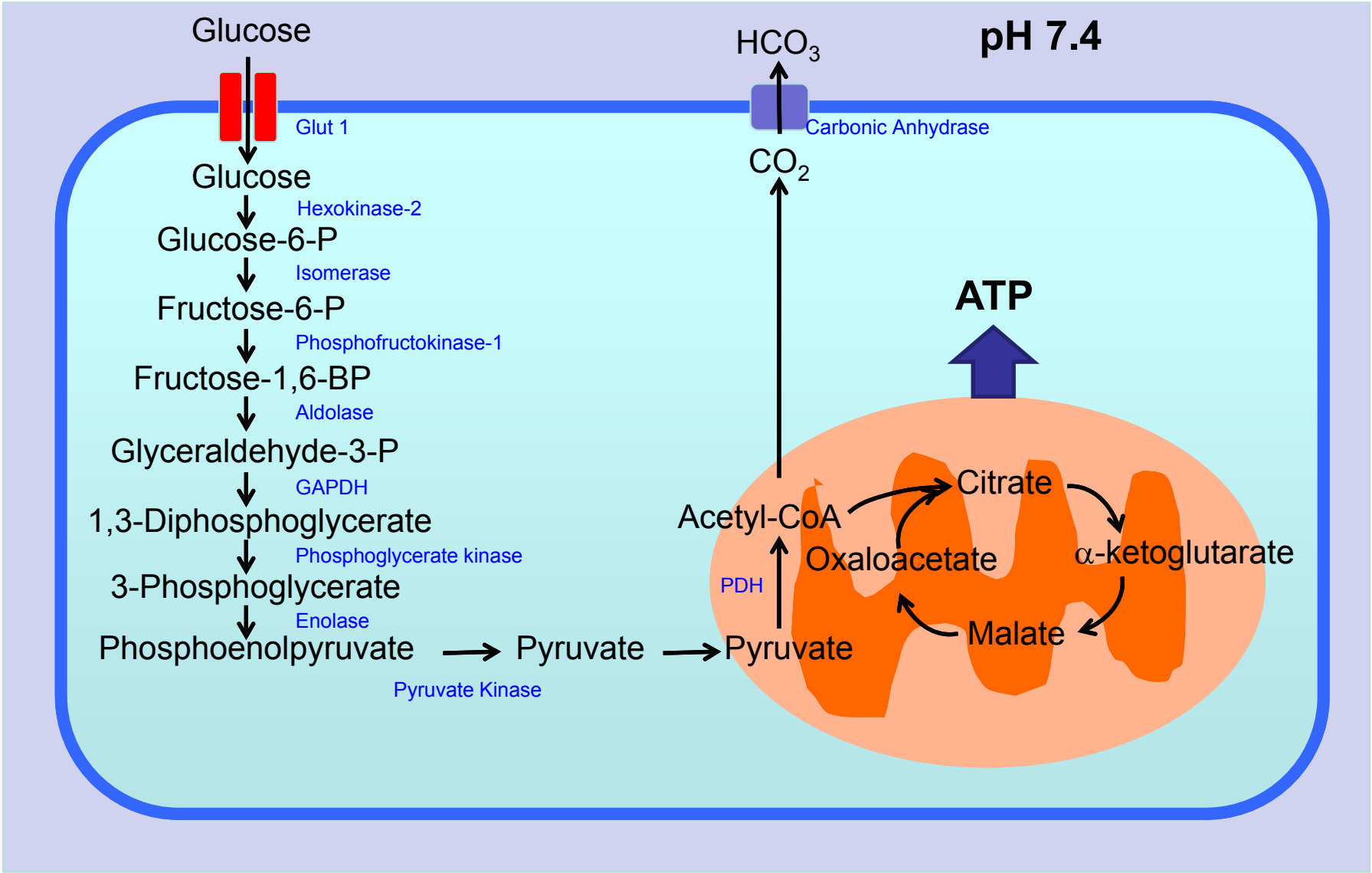
Oncogene or Tumor Suppressor	Metabolic Effect
Akt	Enhances glucose uptake, activates hexokinase II
c-Myc	Enhances glycolysis, activates LDH-A
h-Ras, k-Ras	Enhances glycolysis, activates complex II
Src	Phosphorylates PKM2, upregulates c-Myc
Brc-abl	Enhances glucose uptake, activates G6PD & HK II
Her2/neu	Enhances glycolysis, activates LDH and HSF1
Succinate dehydrogenase	Sustains TCA cycle, loss leads to HIF activation
Fumarate hydratase	Sustains TCA cycle, loss leads to HIF activation
Isocitrate dehydrogenase	Sustains TCA cycle, loss leads to DNA methylation
p53	Promotes OXPHOS, loss leads to glycolysis

Updated Hallmarks of Cancer

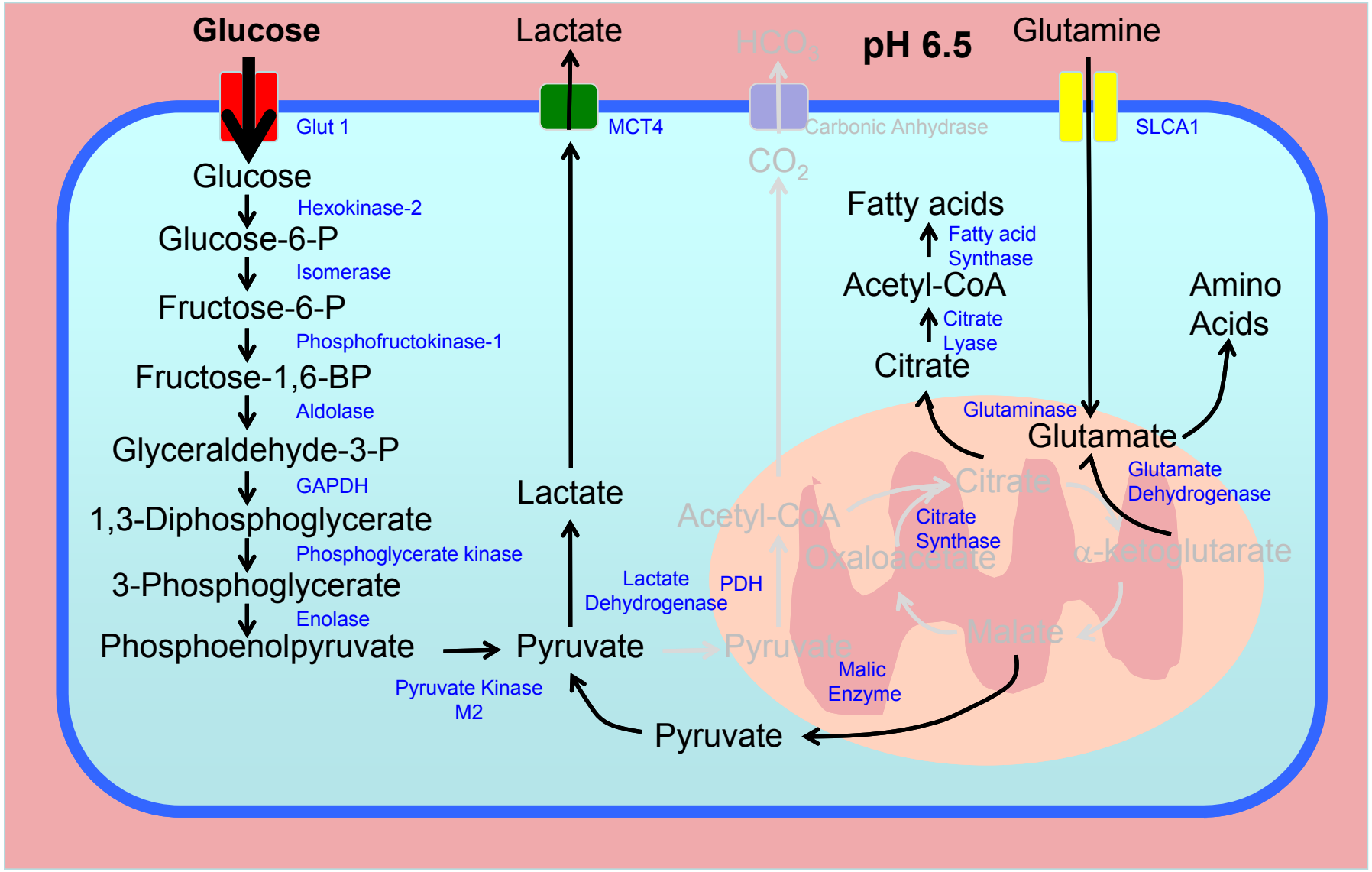


Hanahan D, Wienberg RA (2011) Cell, 144:646-674.

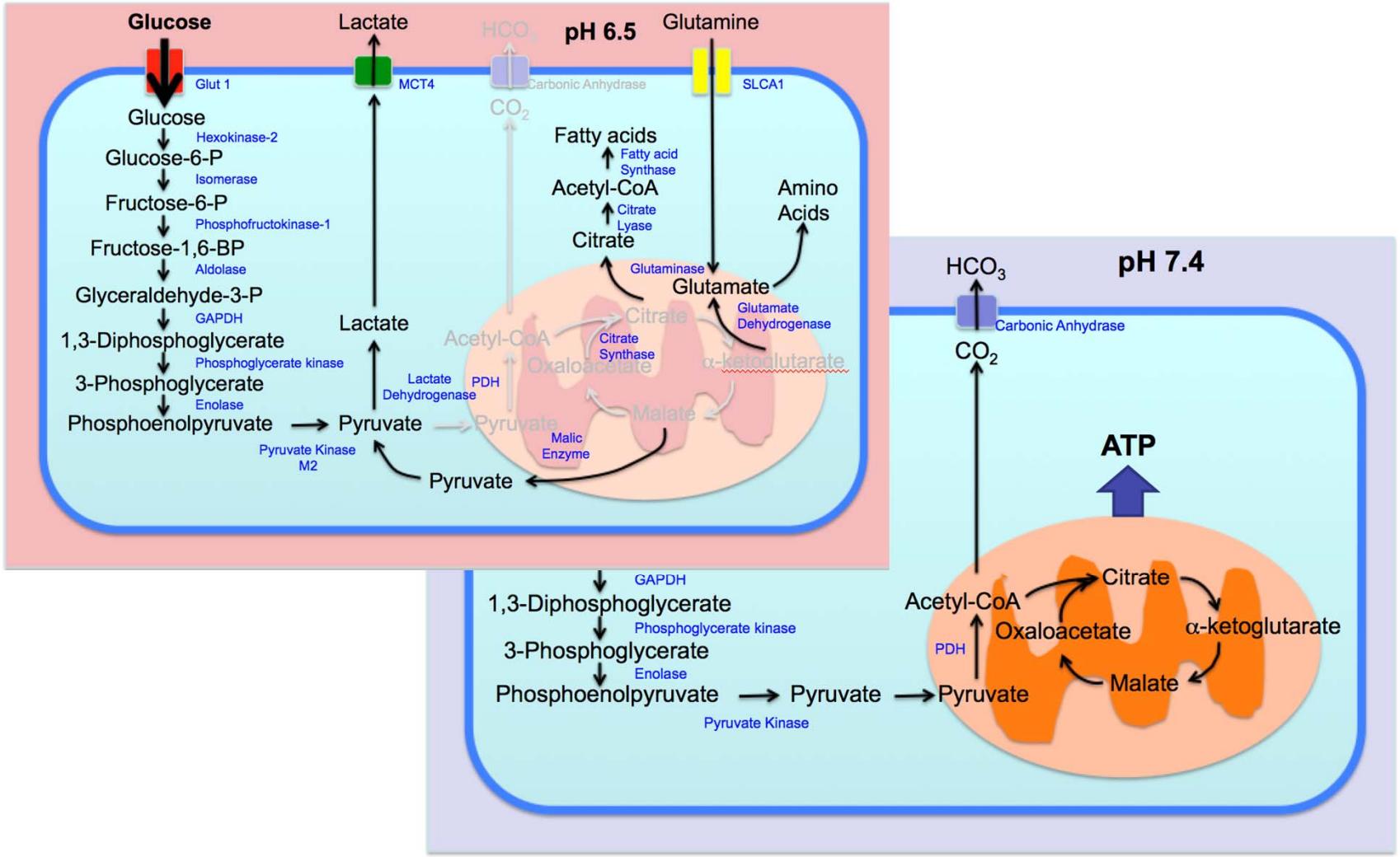
Normal Cell Metabolism



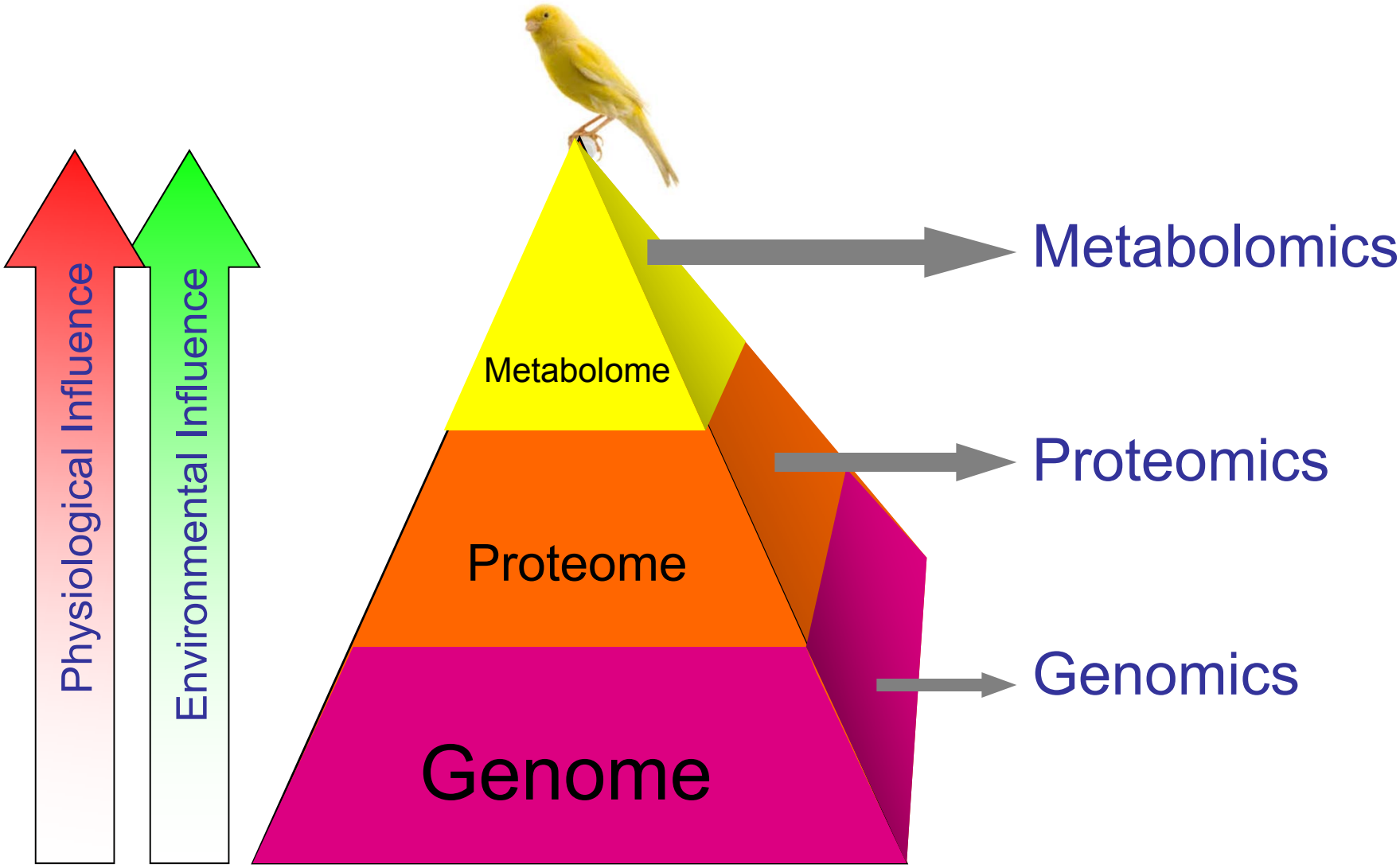
Cancer Cell Metabolism



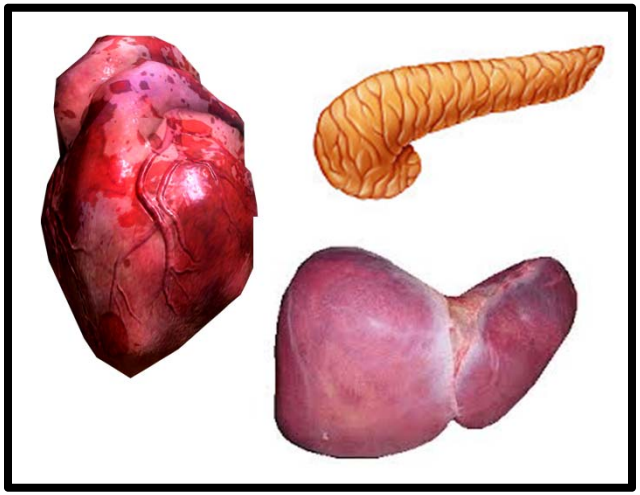
How To Measure All These Metabolic Changes?



Answer: Metabolomics



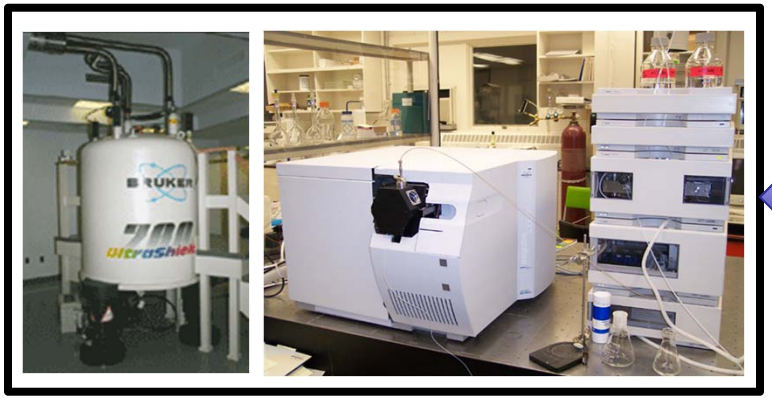
Measuring Metabolism with Metabolomics



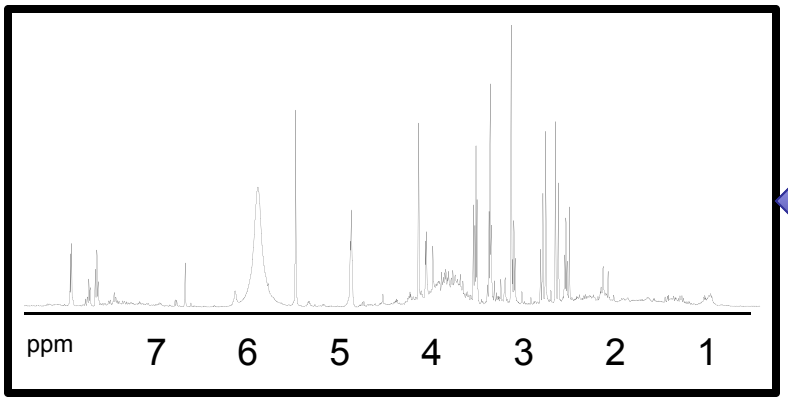
Biological or Tissue Samples

Extraction

Biofluids or Extracts

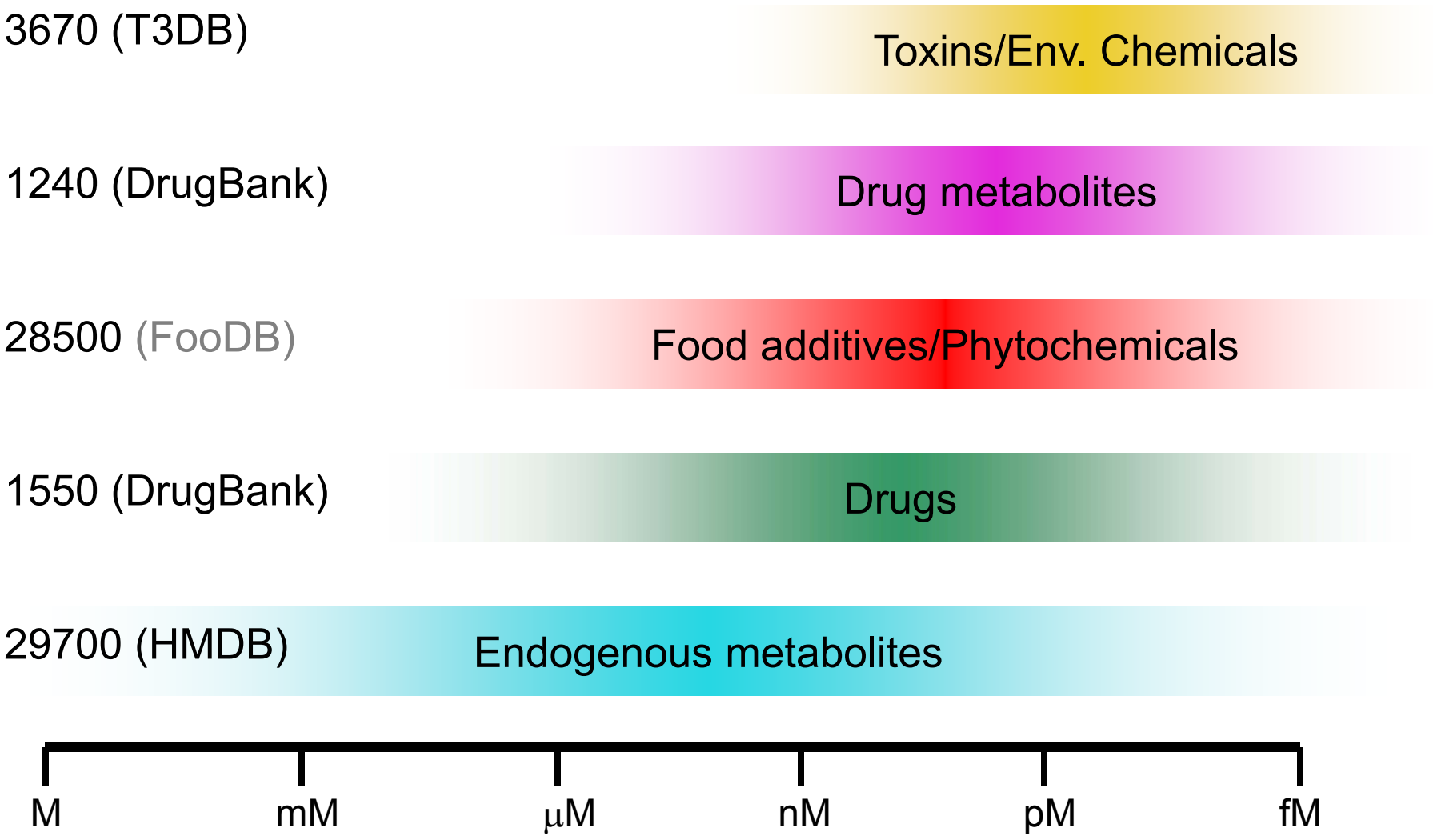


Chemical Analysis

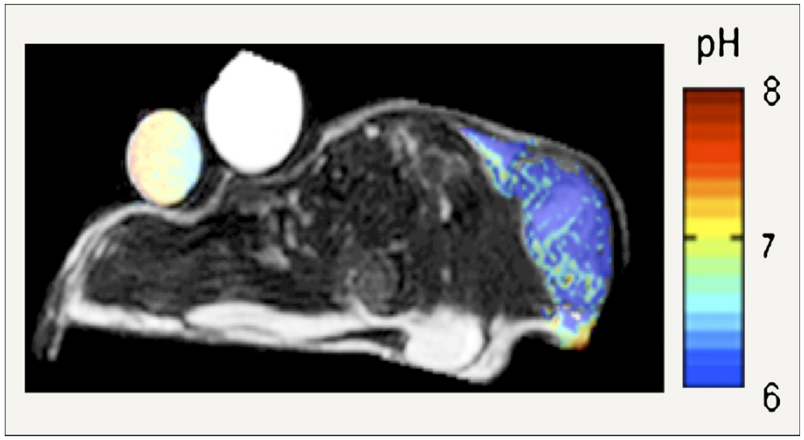


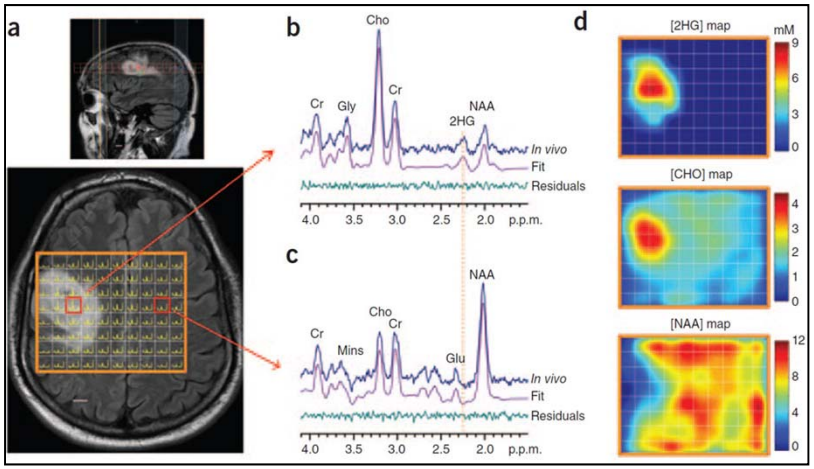
Data Analysis

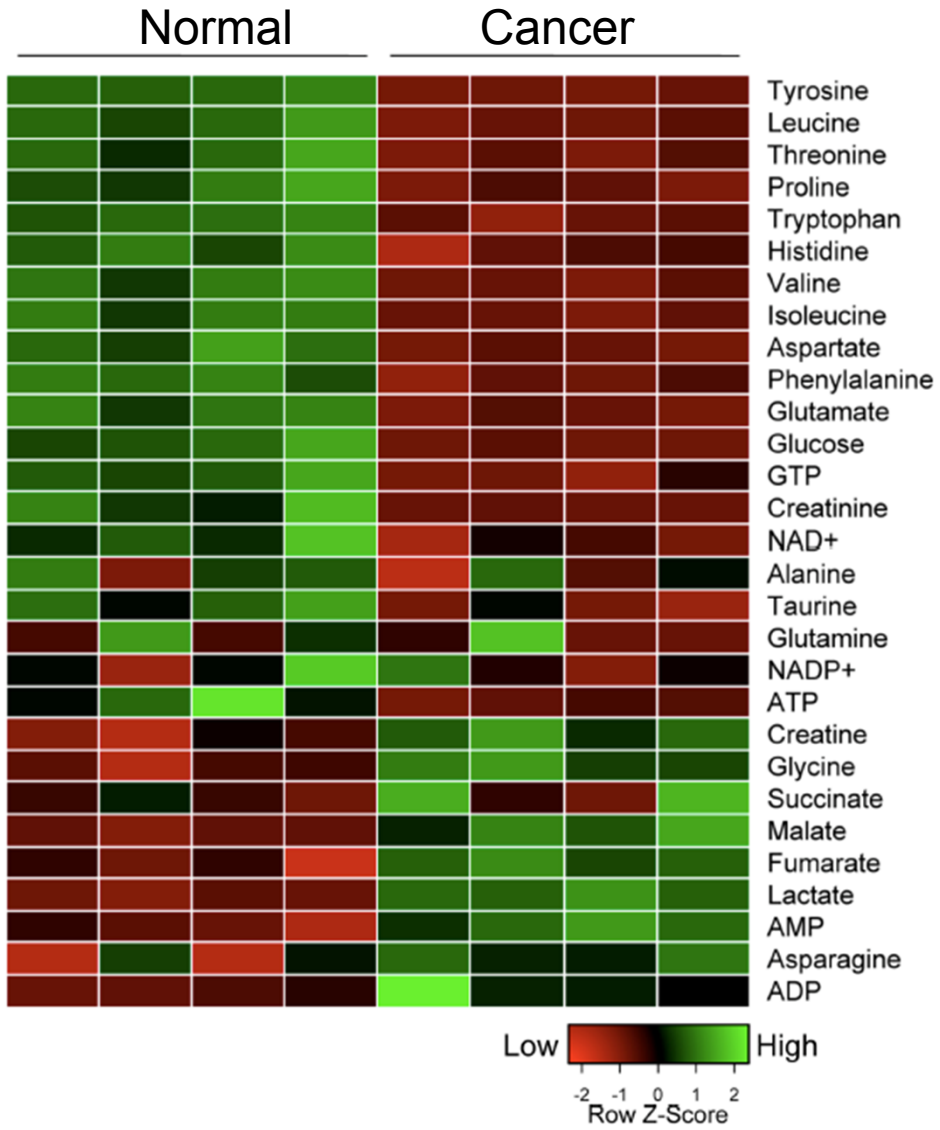
Human Metabolomes (2015)



Metabolomics & Cancer







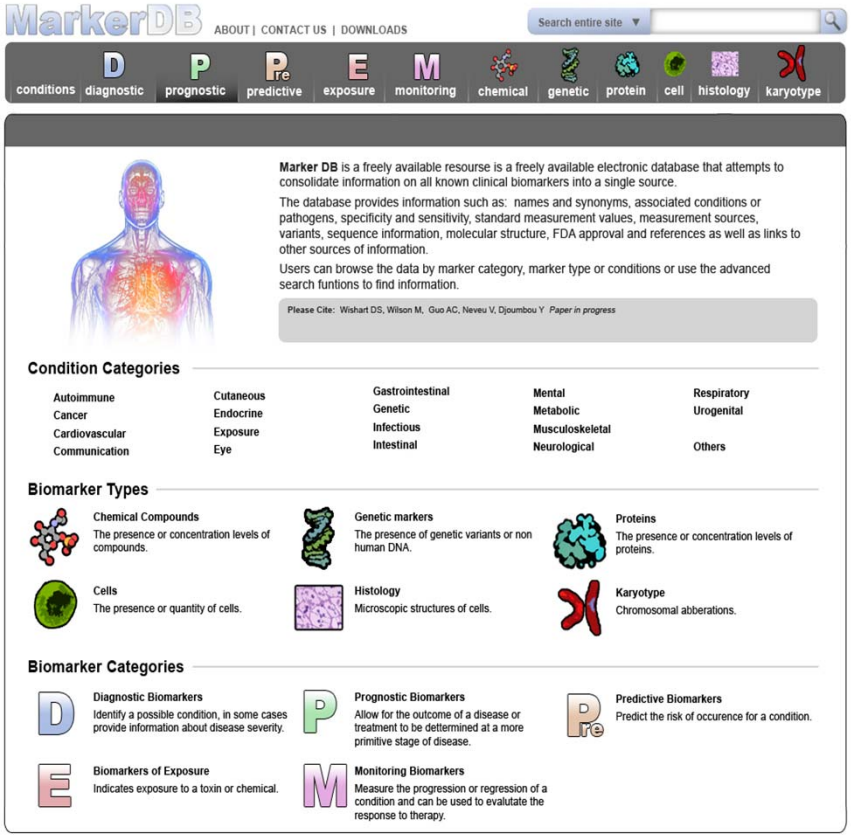
Metabolomics is Discovering Oncometabolites

Oncometabolite	Effect or Mechanism
Lactate	Promotes tumor metastasis
2-Hydroxyglutarate	Alters histone/DNA methylation
Fumarate	HIF activation/alters DNA methylation/binds GSH
Succinate	HIF activation/alters DNA methylation
Glucose	Fuels Warburg effect
Sarcosine	Promotes tumor metastasis
Kynurenine	Activates aryl hydrocarbon receptor, tumorigenesis
Glutamine	Fuels glutaminolysis, promotes tumor growth
Glycine/Serine	Promotes tumor growth, reverse Warburg effect

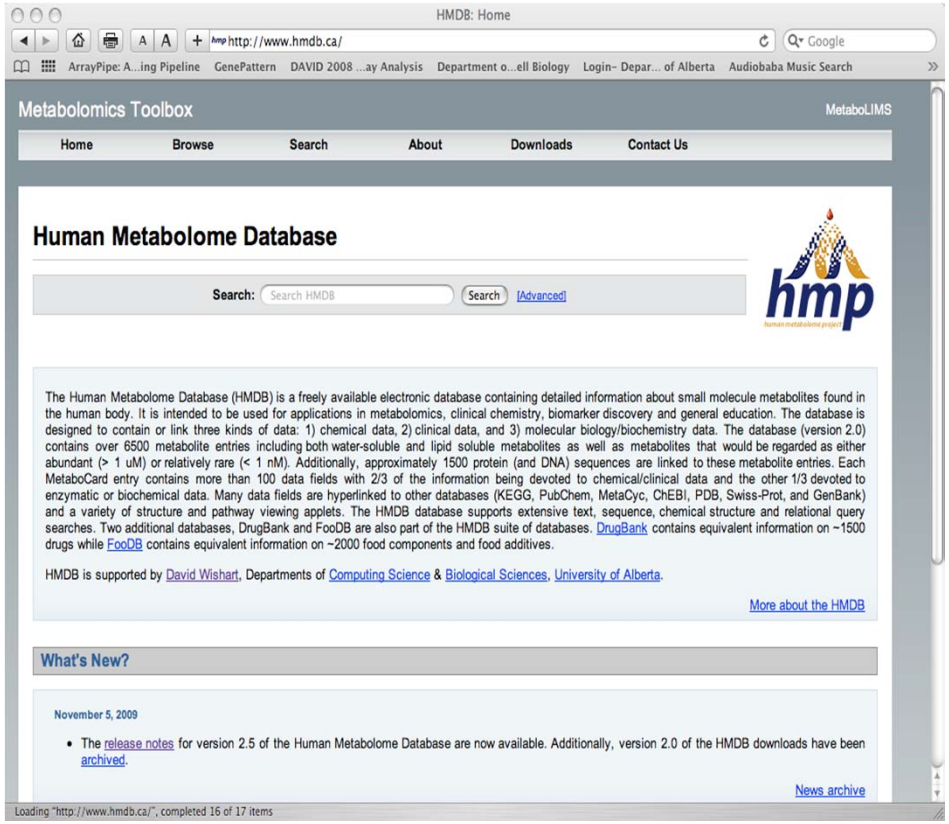
Metabolomics is Discovering Cancer Biomarkers

- Vanillylmandelic acid (neuroblastoma + pheochromocytomoa)
- 3-Hydroxymandelic acid (neuroblastoma)
- 3,4-Dihydroxymandelic acid (neuroblastoma)
- Homovanillic acid (neuroblastoma)
- Sarcosine (metastatic prostate cancer)
- 2-hydroxyglutarate (glioma + acute myeloid leukemia)
- Ribothymidine (breast cancer)
- 1-methylguanosine (breast cancer)
- 1-methyladenosine (cholangioma + cervical cancer)
- Cadaverine (pancreatic cancer)
- 5-hydroxyindoleacetic acid (carcinoid tumors)
- 3-methoxytyramine (carcinoid tumors)
- Testosterone glucuronide (adrenocortical tumors)
- 3a,16a-dihydroxyandrostenone (adrenal carcinoma)
- 5-methoxyindoleacetate (lung + stomach + colon cancer)
- 21-deoxycortisol (testicular cancer)
- 3,5-diiodothyronine (brain tumors)
- Androstendione (thyroid cancer)
- Thromboxane A2 (Hepatocellular carcinoma)
- Deoxypyridinoline (Multiple myeloma)

Cancer & Metabolite Biomarkers



www.markerdb.ca



www.hmdb.ca

Building Better Biomarkers

Metabolomics. 2013 Apr;9(2):280-299. Epub 2012 Dec 4.

Translational biomarker discovery in clinical metabolomics: an introductory tutorial.

Xia J[1], Broadhurst DI, Wilson M, Wishart DS.

⊕ Author information

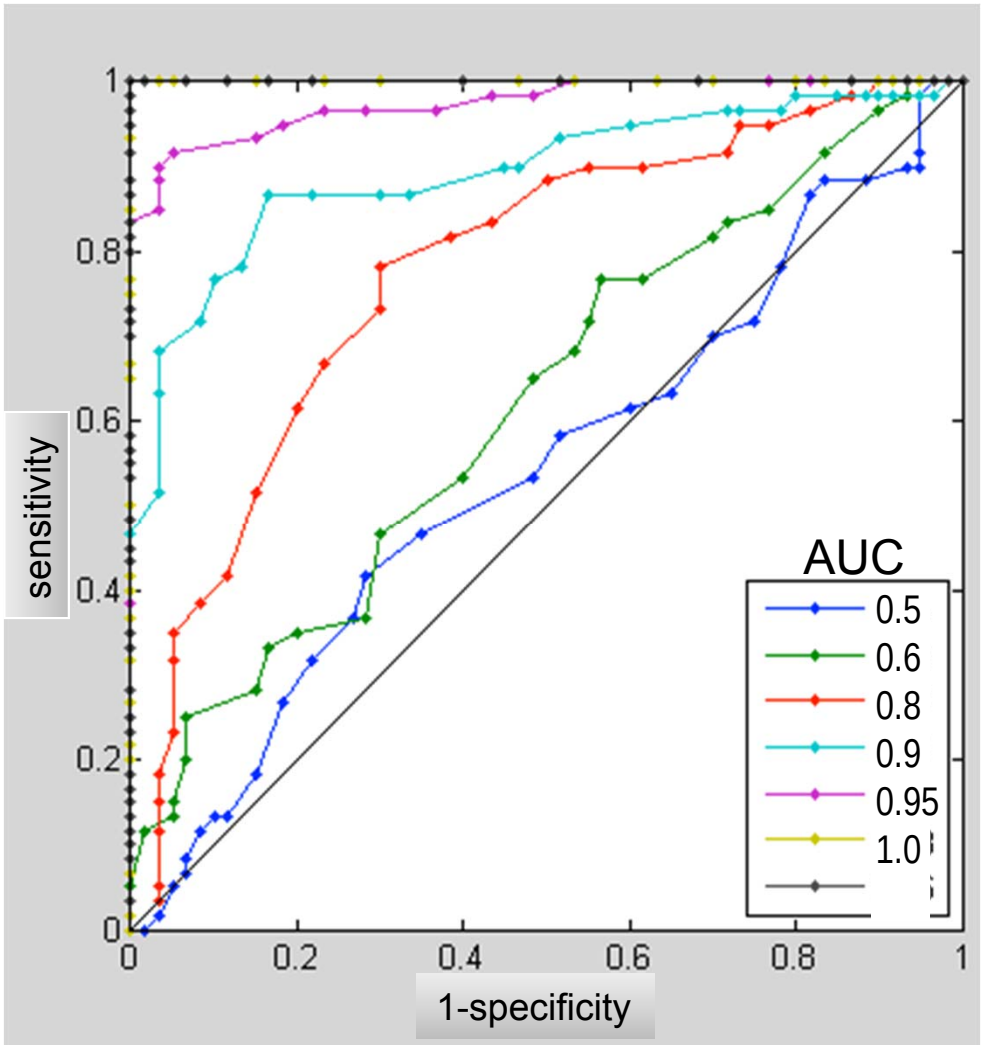
Abstract

Metabolomics is increasingly being applied towards the identification of biomarkers for disease diagnosis, prognosis and risk prediction. Unfortunately among the many published metabolomic studies focusing on biomarker discovery, there is very little consistency and relatively little rigor in how researchers select, assess or report their candidate biomarkers. In particular, few studies report any measure of sensitivity, specificity, or provide receiver operator characteristic (ROC) curves with associated confidence intervals. Even fewer studies explicitly describe or release the biomarker model used to generate their ROC curves. This is surprising given that for biomarker studies in most other biomedical fields, ROC curve analysis is generally considered the standard method for performance assessment. Because the ultimate goal of biomarker discovery is the translation of those biomarkers to clinical practice, it is clear that the metabolomics community needs to start "speaking the same language" in terms of biomarker analysis and reporting-especially if it wants to see metabolite markers being routinely used in the clinic. In this tutorial, we will first introduce the concept of ROC curves and describe their use in single biomarker analysis for clinical chemistry. This includes the construction of ROC curves, understanding the meaning of area under ROC curves (AUC) and partial AUC, as well as the calculation of confidence intervals. The second part of the tutorial focuses on biomarker analyses within the context of metabolomics. This section describes different statistical and machine learning strategies that can be used to create *multi-metabolite* biomarker models and explains how these models can be assessed using ROC curves. In the third part of the tutorial we discuss common issues and potential pitfalls associated with different analysis methods and provide readers with a list of nine recommendations for biomarker analysis and reporting. To help readers test, visualize and explore the concepts presented in this tutorial, we also introduce a web-based tool called ROCCET (ROC Curve Explorer & Tester, http://www.roccet.ca). ROCCET was originally developed as a teaching aid but it can also serve as a training and testing resource to assist metabolomics researchers build biomarker models and conduct a range of common ROC curve analyses for biomarker studies.

KEYWORDS: AUC; Biomarker analysis; Biomarker validation and reporting; Bootstrapping; Confidence intervals; Cross validation; Optimal threshold; ROC curve; Sample size

PMID: 23543913 [PubMed] PMCID: PMC3608878 **Free PMC Article**

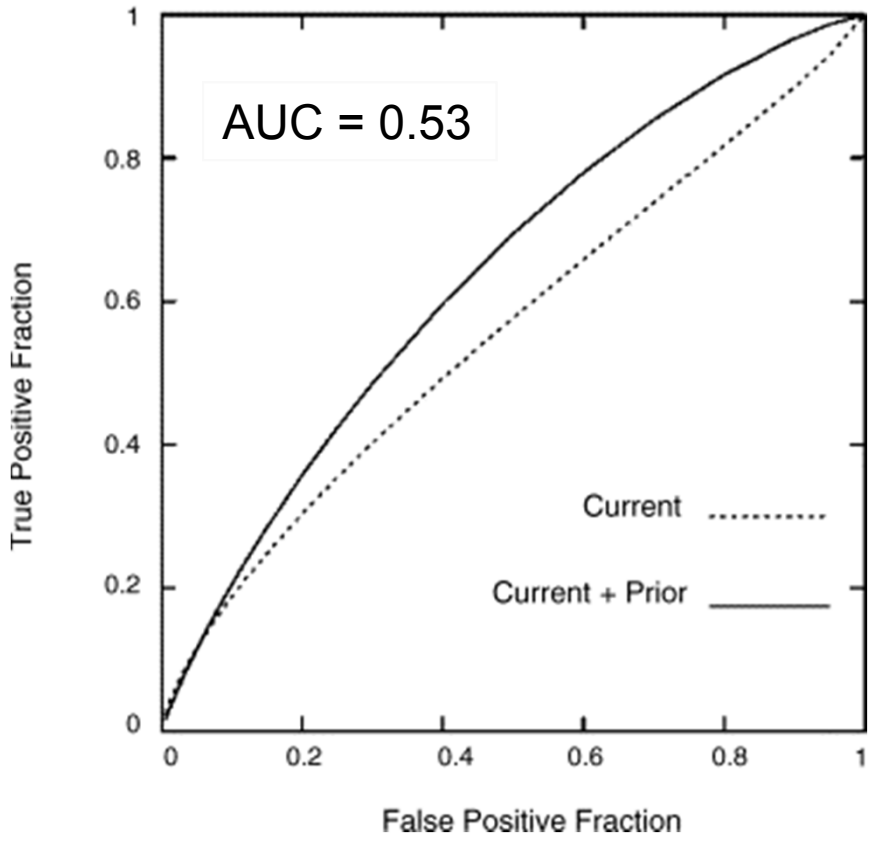
Assessing Biomarkers with ROC Curves



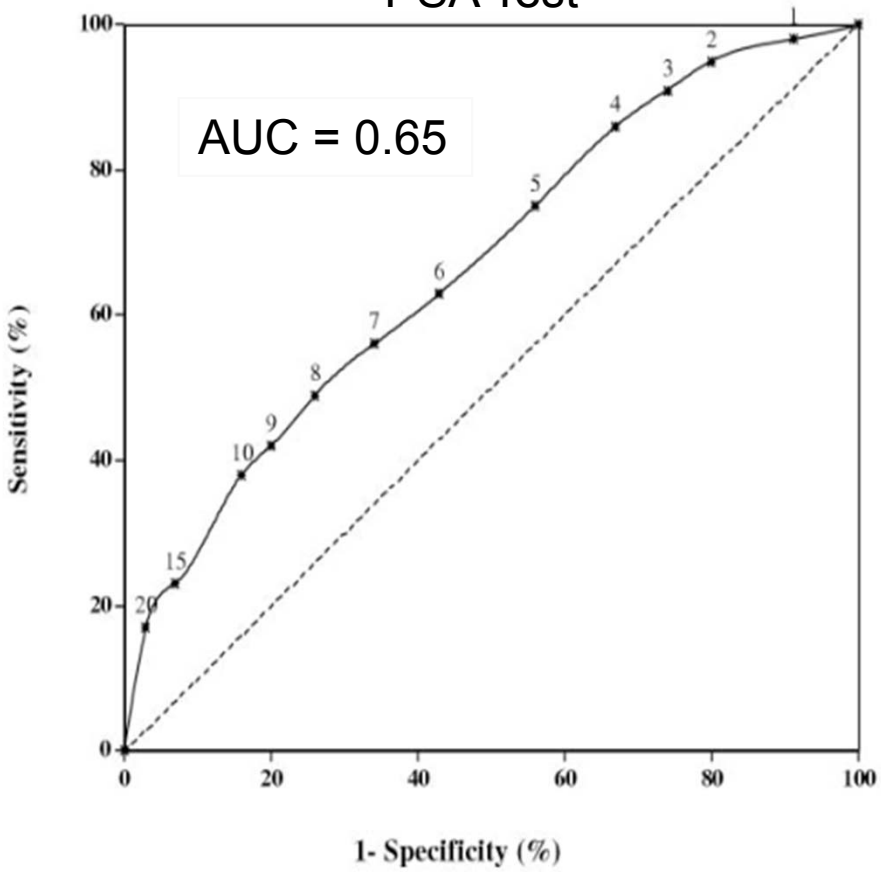
- Plots sensitivity (%TP) vs. specificity (%TN)
- A poor ROC curve would be a straight line with a slope of 1
- The area under an ROC (AUROC) curve is a good measure of the quality of the biomarker
- AUCs of >0.75 are good, AUCs of 0.5 are terrible, AUCs of 1.00 are perfect

AUCs of Common Tests

Mammogram (Benign vs. Malignant)

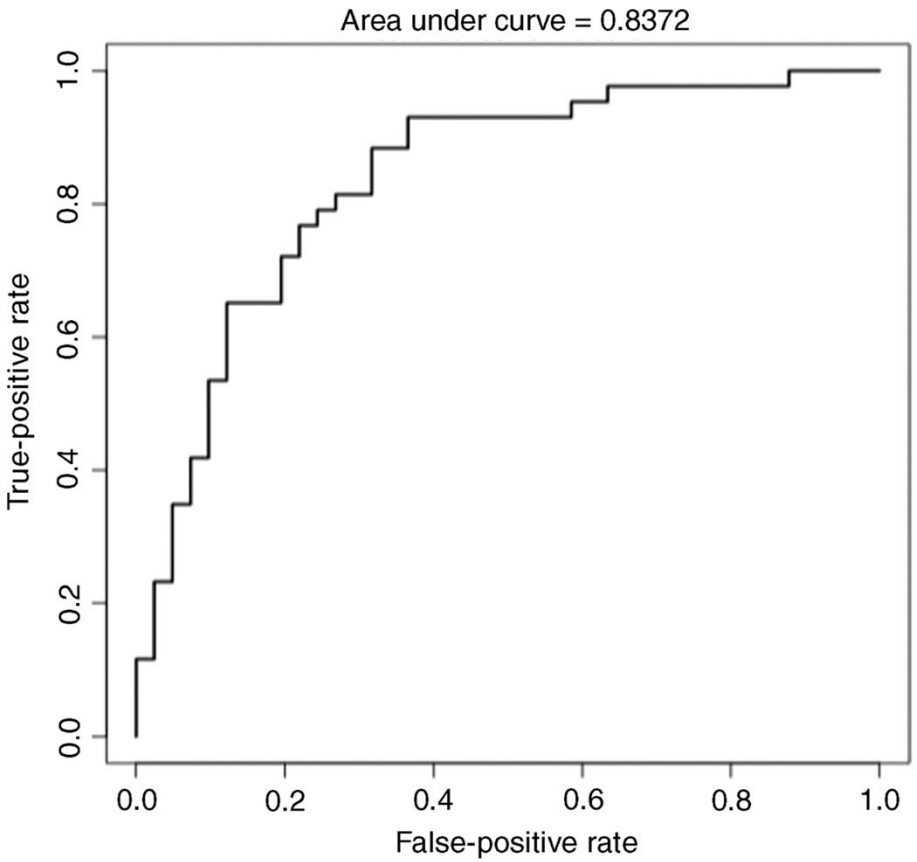


PSA Test



How Does Metabolomics Do?

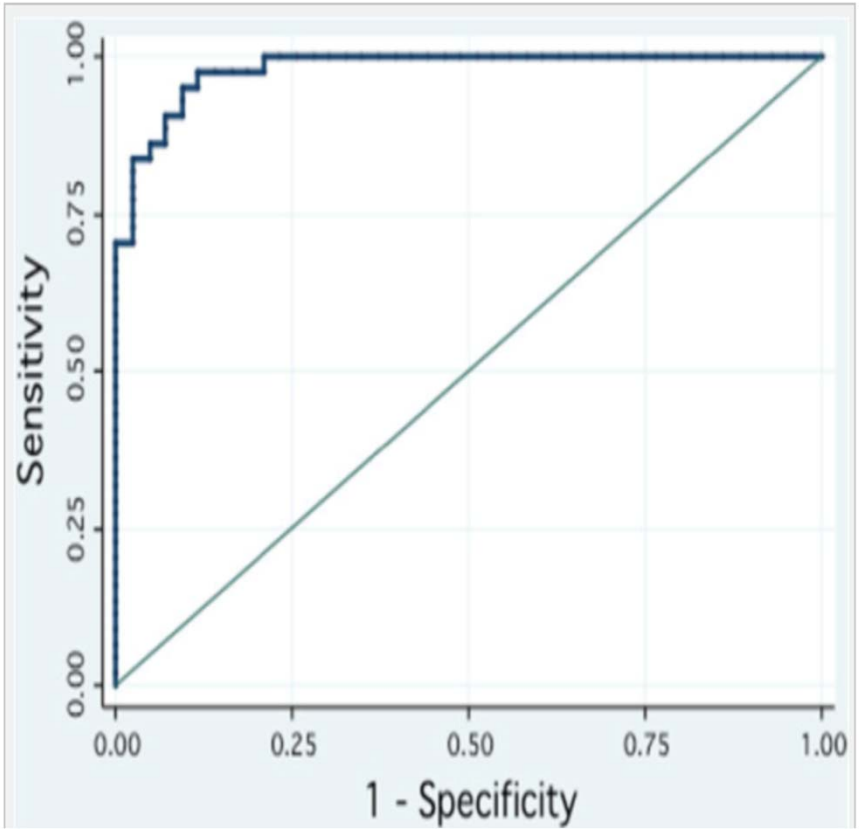
Diagnosing Pancreatic Cancer



- Adult Serum Samples
- 43 cases, 41 controls
- NMR metabolomics
- AUC = 0.84 using 8 metabolites
- Glutamate, acetone, 3-hydroxybutyrate, glucose, glutamine, creatine, phenylalanine, formate

Bathe OF, Shaykhutdinov R, Kopciuk K. et al – Cancer Epid Biomark Prev. (2011) Jan;20:140-147.

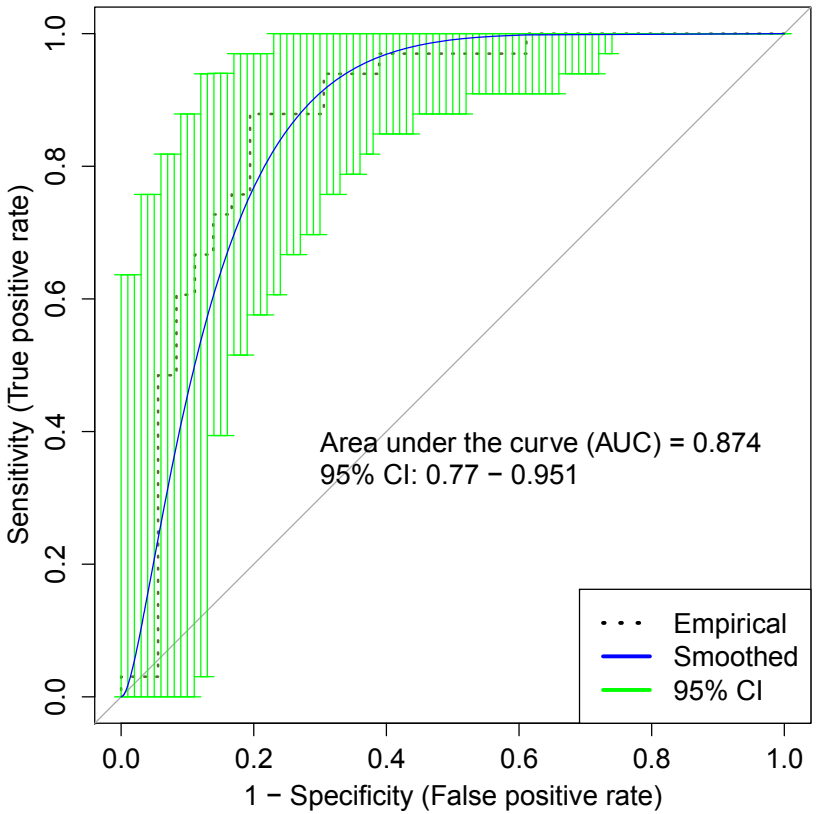
Diagnosing Esophageal Cancer



- Adult Urine Samples
- 44 cases, 75 controls
- NMR metabolomics
- AUC = 0.98 using 7 metabolites
- Urea, acetate, acetone, formate, succinate, pantothenate, 2-hydroxyisobutyrate

Davis VW, Schiller DE, Eurich D, Sawyer MB - World J Surg Oncol (2012) Dec 15;10:271.

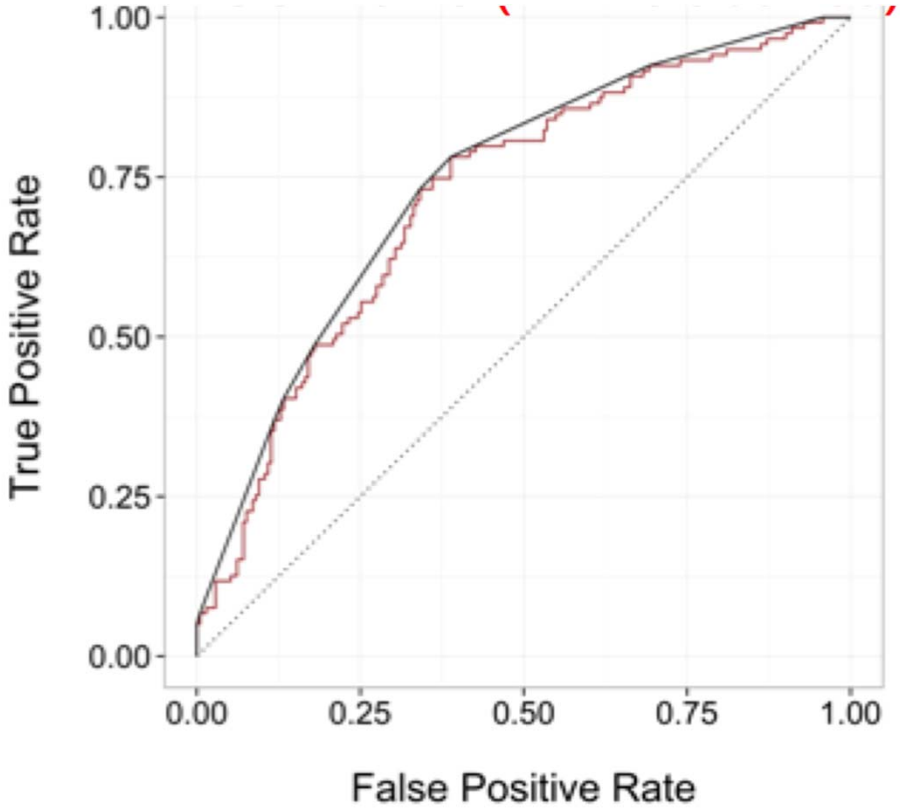
Diagnosing Endometrial Cancer



Bahado-Singh R, Mandal R, Wishart DS (unpublished)

- Adult Serum Samples
- 40 cases, 41 controls
- MS metabolomics
- AUC = 0.88 using 3 metabolites
- C18:2, PC ae C40:1, C6 (C4:1-DC)
- Very strong correlation with BMI
- Pap smear AUC=0.55

Predicting Colon Cancer (Polyps)

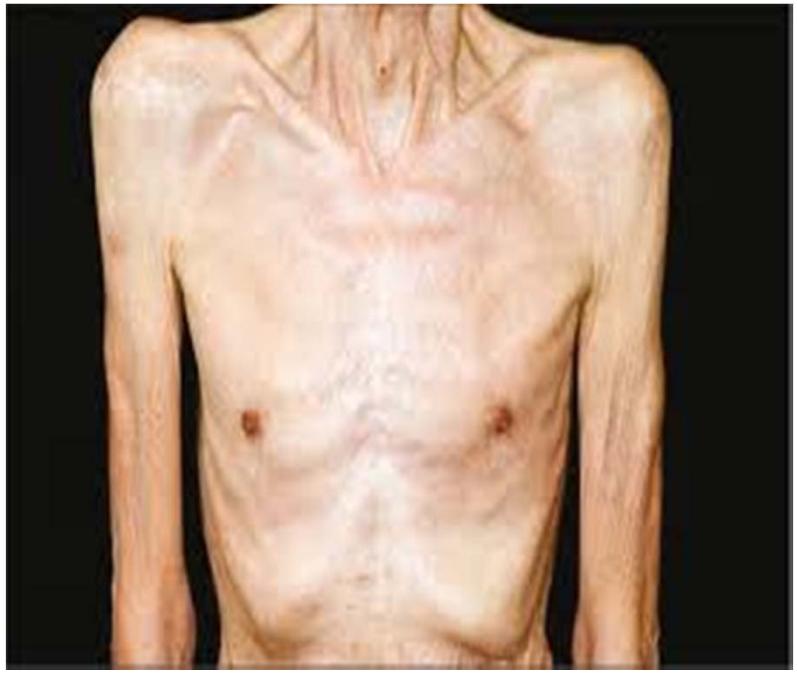


Wang H, Tso V, Wong C, Sadowski D, Fedorak RN. Clin Transl Gastroenterol. (2014) Mar 20;5:e54

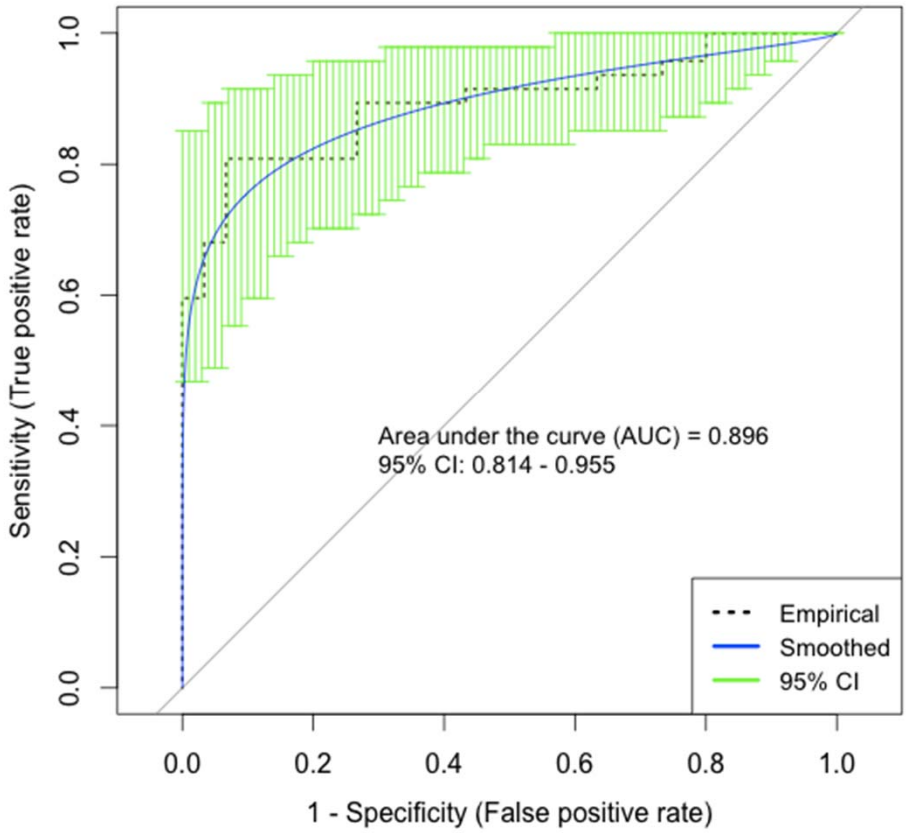
- Adult Urine Samples
- 162 cases, 422 controls
- NMR metabolomics
- AUC = 0.75 using 17 metabolites
- Butyrate, serine, methanol, beta-alanine, methylhistidine, 3-hydroxybutyrate, acetone, benzoate

Cancer Cachexia

- Adverse metabolic effect from cancer (negative energy balance due to tumor burden, loss of skeletal muscle mass)
- Responsible for significant morbidity and significantly earlier mortality
- Early detection, prediction & prevention could save lives



Predicting Cancer Cachexia via Metabolomics



Eisner R, Stretch C, Eastman T, et al. Metabolomics (2011) March; 7:25-34.

- Adult Urine Samples
- All with cancer
- 44 cachetic, 29 non-cachectic
- NMR metabolomics
- AUC = 0.90 using 8 metabolites
- Creatine, creatinine, branched chain AAs, glucose

Using Metabolomics to Phenotype Cancer

- Most cancers generate large quantities of glycolysis biomarkers (lactate, formate, glucose, succinate)
- Some cancers produce large quantities of glutaminolysis biomarkers (glutamate, glutamine)
- Certain cancers exhibit dysregulated one-carbon metabolism biomarkers (choline, sarcosine, glycine, serine, hydroxyglutarate)
- Most cancers produce excesses of metabolites belonging to certain cell classes (indoleacetate, homovanillate)
- MRS (chemical shift) & PET imaging or metabolite profiling allows precise phenotyping of cancers

Using Metabolomics To Phenotype Those At Risk

- Is there a metabolome that pre-disposes one to cancer?
- How to measure the GxE interactions via metabolomics?
- Metabolites that harm: oncometabolites, uremic toxins, transformed xenobiotics
- Metabolites that heal: butyrate, bicarbonate, uric acid, glutathione

But Metabolomics Tests Will Never Be Approved…

Almost Everyone <25 Has Had A Metabolomic Test

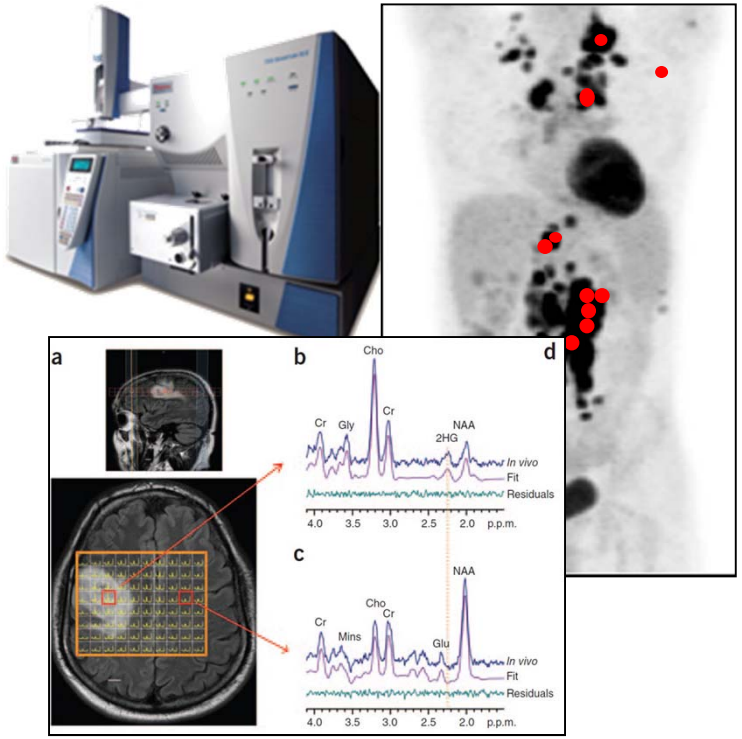
Newborn Screening

Metabolomics is Moving to the Bedside

- Number of "approved" tests arising from Metabolomics/Clinical Chem. – 195
- Number of "approved" tests arising from or using Genomics – 100-110
- Number of "approved" single Protein tests (ELISA) – 60
- Number of "approved" tests arising from or using Transcriptomics – 5
- Number of "approved" tests arising from or using Proteomics - 0

Re-Thinking Precision Medicine

BRCA1/2 Testing



Cancer Phenotyping

Key Points

- **Cancer is a metabolic disease**
  - Cancer cells exhibit a 200x increase in glucose consumption
  - Most known oncogenes and tumor suppressors fundamentally alter glucose metabolism
  - Oncometabolites promote cancer
  - Antimetabolites stop cancer
  - High abundance metabolites play key cancer signaling roles
  - Metabolic disorders such as diabetes and obesity increase cancer risk substantially
  - Cachexia (a metabolic disorder) is a manifestation of cancer
  - Some of the best cancer biomarkers are metabolites

New Opportunities

- **If cancer is a metabolic disease…**
  - New kinds of drug targets
  - New methods for cancer prevention (diets?)
  - New approaches for early diagnosis
  - New methods for risk prediction
  - New techniques to look at cancer
  - New ways of integrating genomics with metabolomics
  - New kinds of drugs…

Cancer Drugs That Reverse The Warburg Effect

Drug	Mechanism
Gleevec	Inhibits Bcr-Abl, downregulates HK & G6PDH
Dicholoracetate (DCA)	Targets and inhibits pyruvate dehydrogenase kinase
Orlistat	Targets and inhibits fatty acid synthase
Metformin	Downregulates mTOR, Activates AMPK
Rapamycin	Inhibits mTOR
Trastuzumab	Inhibits glycolysis via LDH and HSF1 downregulation

Conclusion

Cancer as a genetic disease

- 250 oncogenes
- 700 tumor suppressors
- ~10,000-50,000 Additional mutations, CNVs or chromosomal variants in each cell
- *1 million+ different diseases*

Cancer as a metabolic disease

- Aerobic glycolysis
- Glutaminolysis
- One-carbon metabolism
- *3-5 different diseases*

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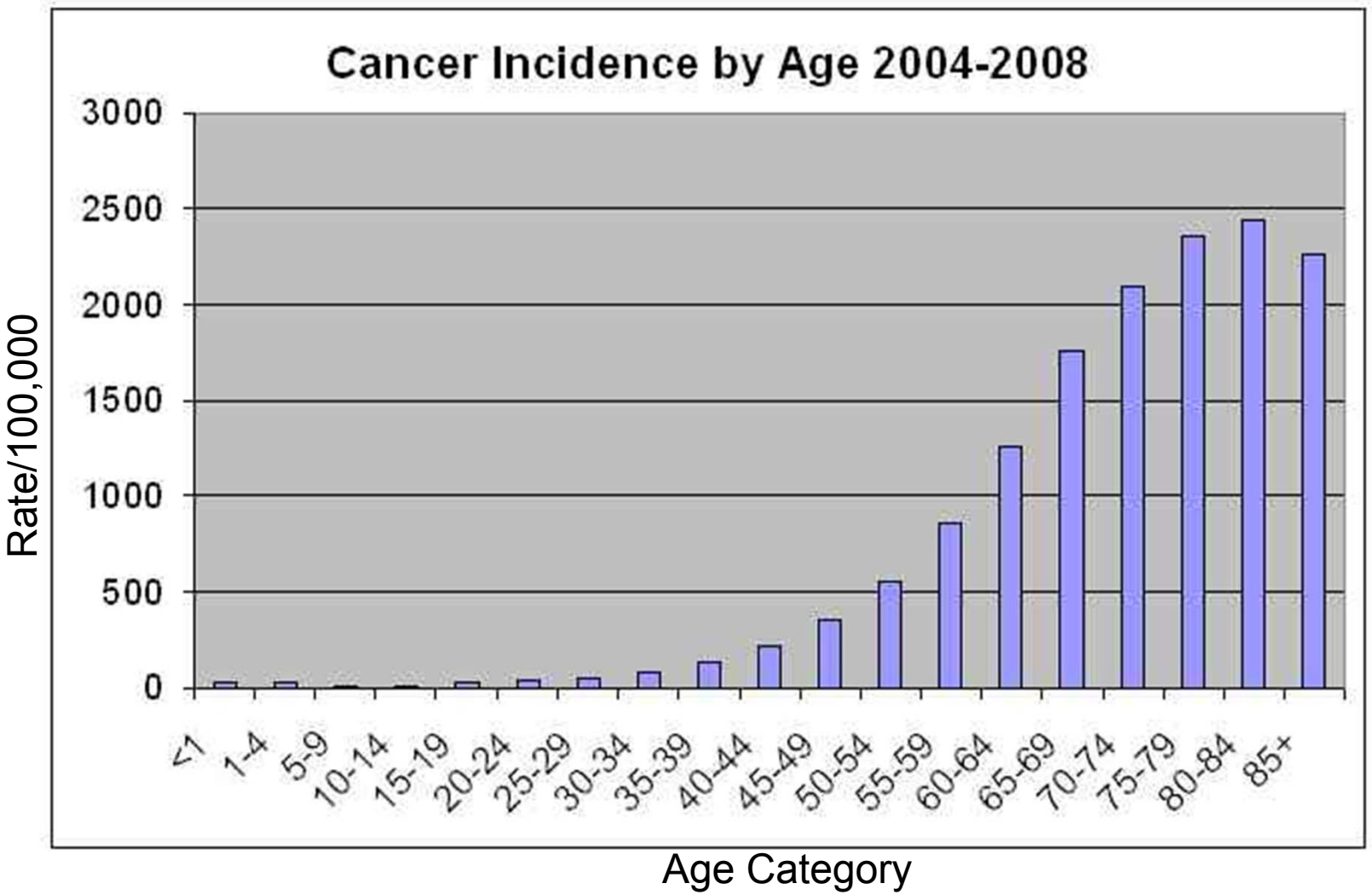
Canadian Institutes of Health Research

Instituts de recherche en santé du Canada

Cancer & Age


Cancer Incidence by Age 2004-2008
Rate/100,000
3000
2500
2000
1500
1000
500
0
<1
1-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-69
70-74
75-79
80-84
85+
Age Category

What Causes Cancer?

- 5% of all cancers are inherited (germline mutations like BRCA1)
- 15-20% of all cancers arise from infectious organisms (human papilloma virus, hepatitis B/C, HIV, H. pylori)
- 75-80% arise from somatic mutations due to: ionizing radiation, pollution, chemicals, food, chronic inflammation, immunosuppression and aging

Changing Times; Changing Views

- **Warburg dies in 1970**
- **First oncogene (Src) discovered in 1970**
- **Nixon declares "war on cancer" in 1971, shift in research funding to genetics**
- **Varmus & Bishop prove oncogene theory in 1976**
- **Hallmarks of cancer appears in 2000 (no mention of metabolic dysregulation)**
- ***From 1970-2009 the metabolic basis to cancer is largely forgotten***

Hanahan D & Wienberg RA, Cell, Jan 100(1): 57-20

Where To Next?