The Impact of Molecular Diagnostics on Contemporary Cancer Management

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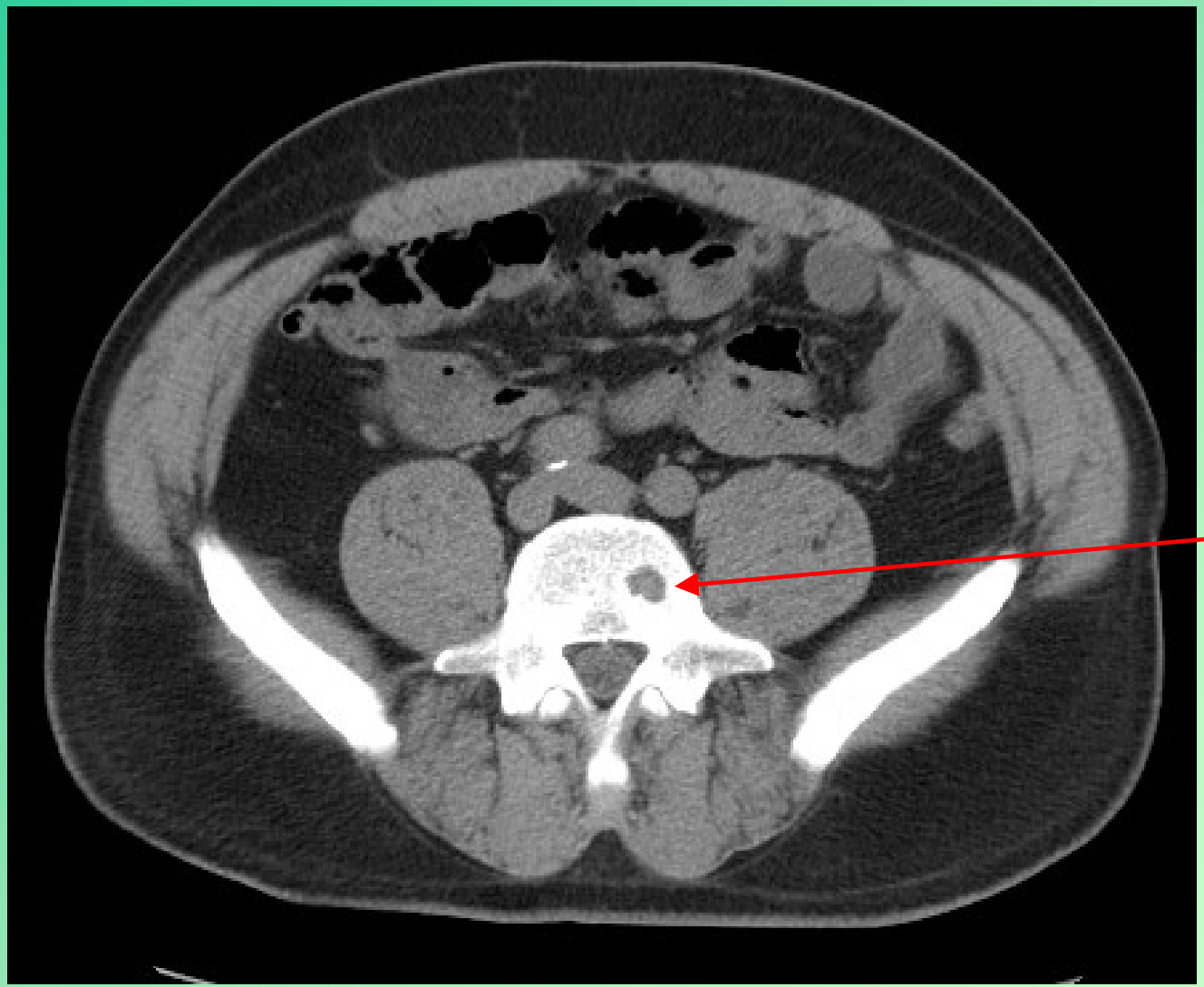
Case Presentation

- 54 y.o. man presented in February, 2009, with widespread lytic lesions of bone – referred as possible multiple myeloma
- Only relevant history is strong family history of prostate cancer
- CBC and all chemistries normal
- While waiting for initial lab workup to return he developed symptoms of impending spinal-cord compression with mild leg weakness and urinary retention
- Admitted to the hospital for emergency decompression and tissue diagnosis

CT scan of spine



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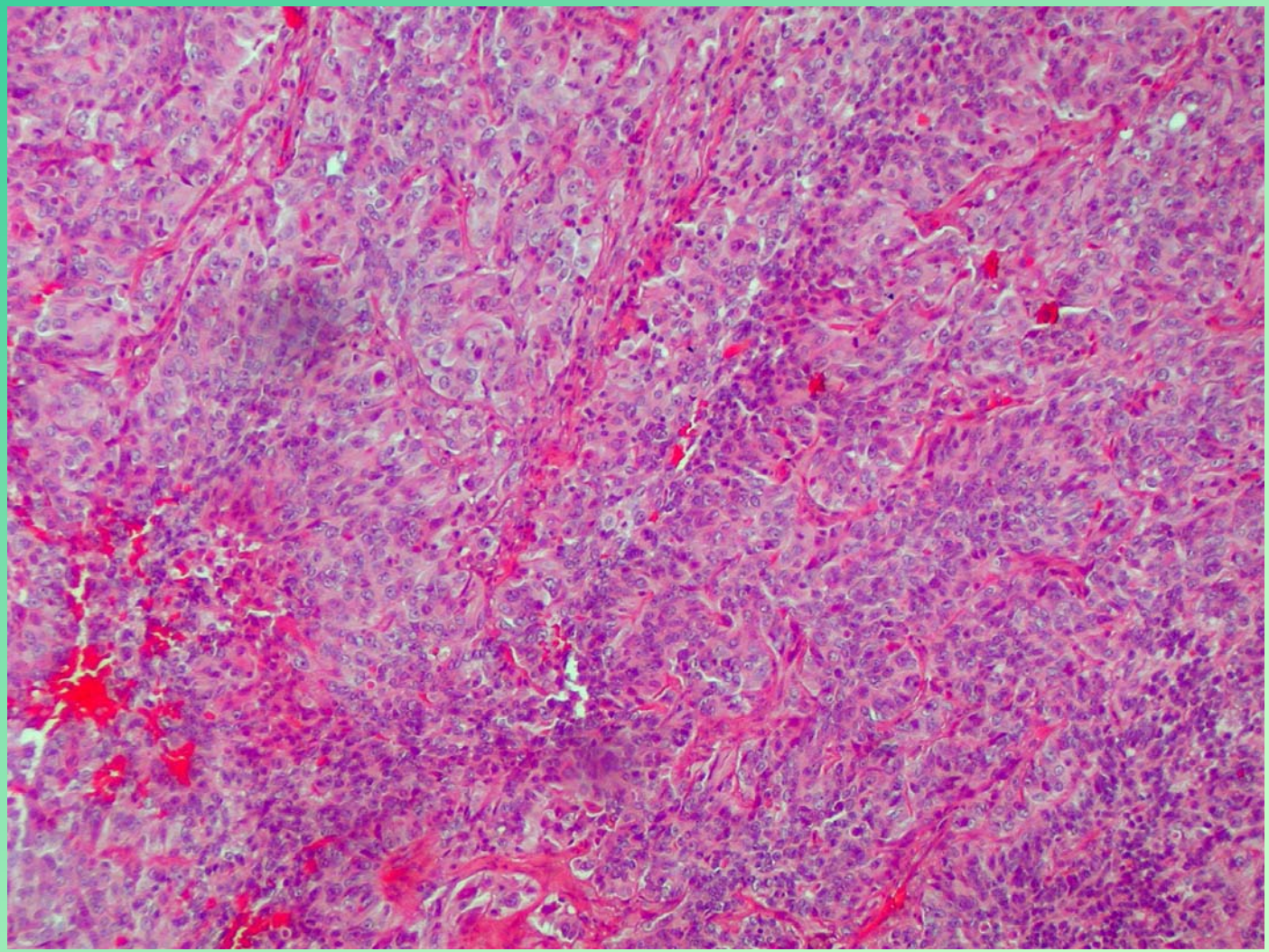
Pathology – Low Power



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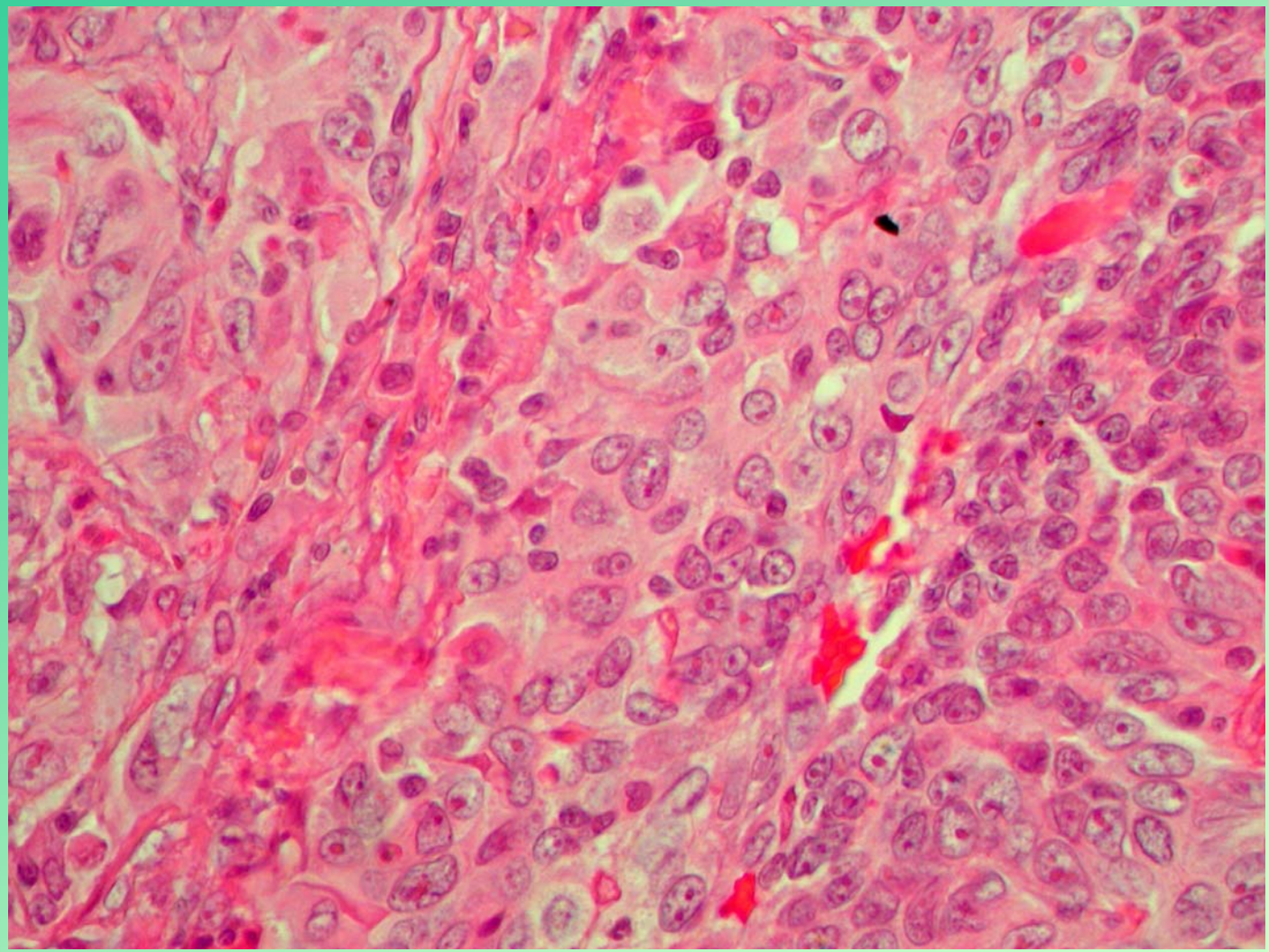
Pathology – High Power



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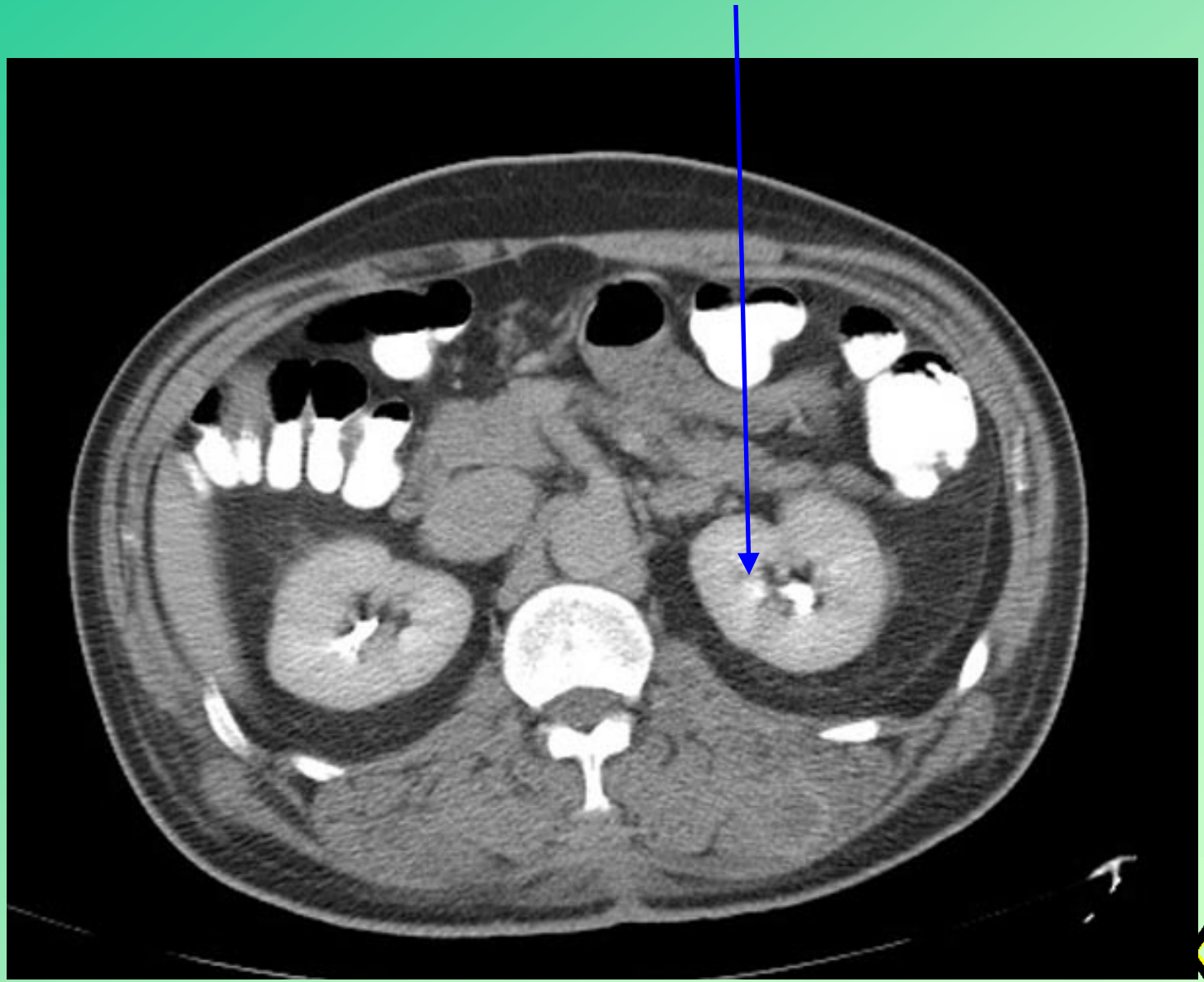
Additional Work-Up

- CT showed many small lung nodules
- Tissue stained for β-subunit HCG and α-fetoprotein as well as PSA – all negative
- Serum markers including CEA, CA27-29, CA 19-9 and above tests all negative
- Myeloma workup looking for paraprotein negative
- Rapidly developed new bone lesions
- Specimen of paraffin-embedded tissue block sent to reference lab for “Cancer Type ID” assay (m-RNA analysis) in hopes of finding a primary
- Details of assay to follow….

Molecular Work-Up of Our Patients' Tumor

- Concluded with 99% confidence that he had renal cell carcinoma
- New CT scan showed new small suspicious lesion in left kidney and increase in size of previously noted lung lesions…

New Renal Lesion: Is this the Primary?



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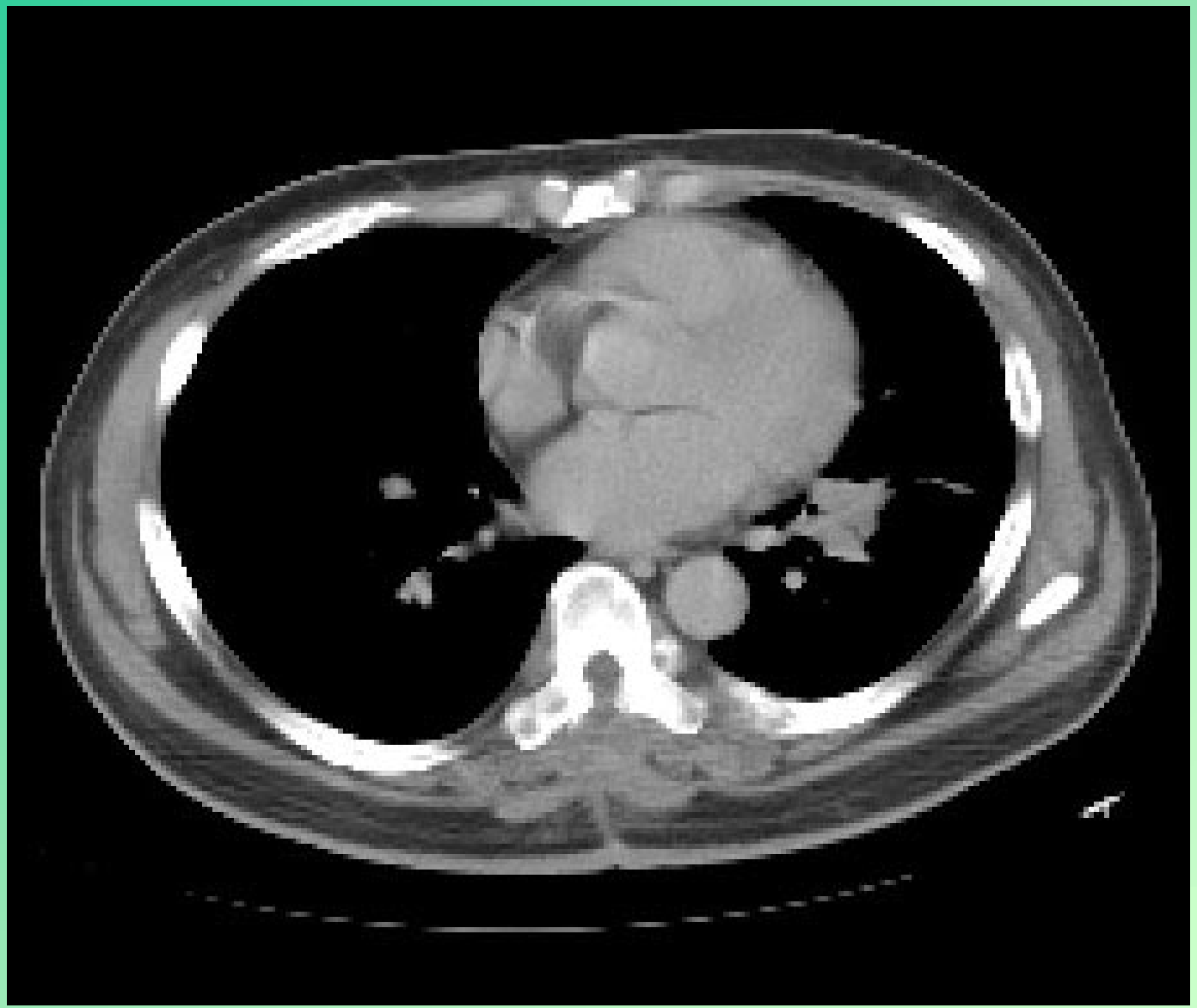
Lung Lesions and Hilar Mass



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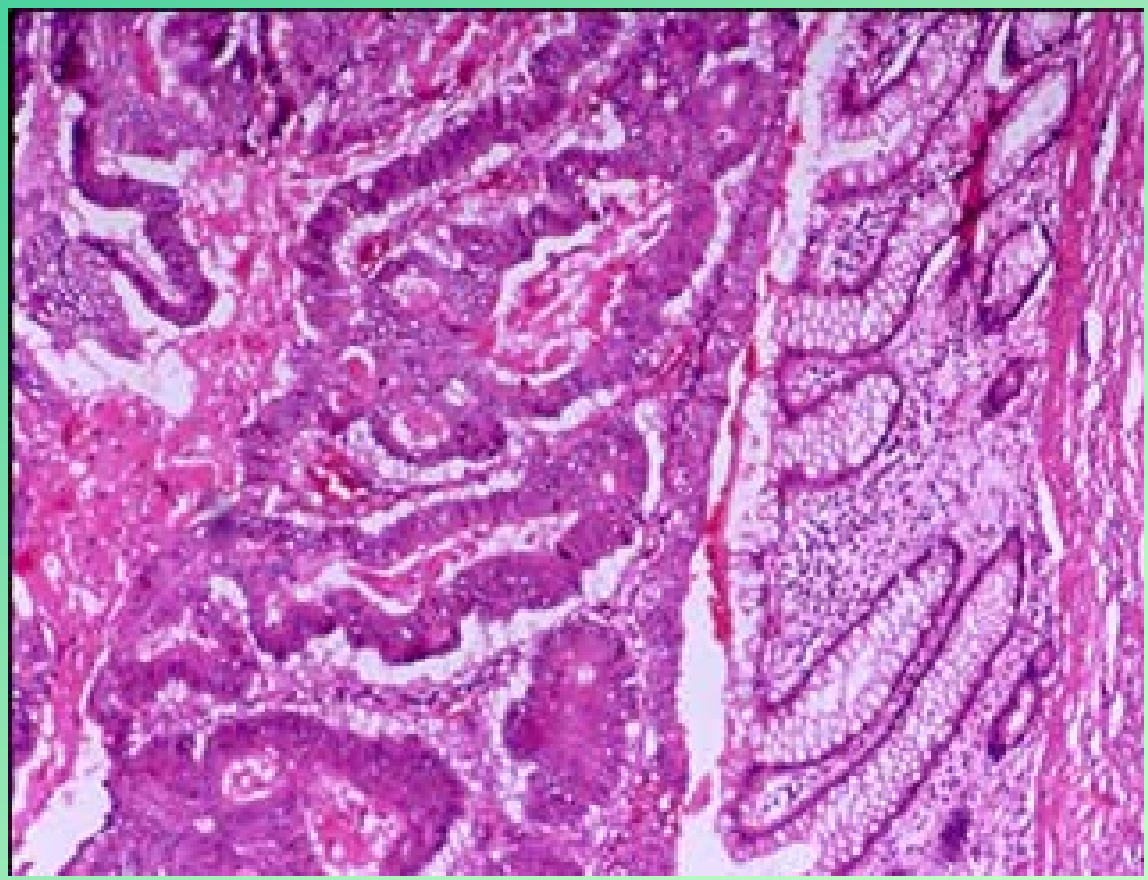
Case, continued

- On the basis of the m-RNA analysis he was started on Sunitinib
- By the time this was started he was almost bed-ridden; he pursued a downhill course and died before we could ascertain whether this drug really did him any good
- No “empiric” chemotherapy was ever given

A Brief History of Molecular Diagnostics

- For a long time H&E staining was the mainstay of histologic diagnosis of cancer
- Pattern recognition differentiated:
  - Carcinoma
    - Adeno, Squamous, Small Cell, etc.
  - Sarcoma
  - Myeloid Malignancies
  - Lymphoid Malignancies
  - Neuro-endocrine Malignancies

Classic Colonic Adenocarcinoma



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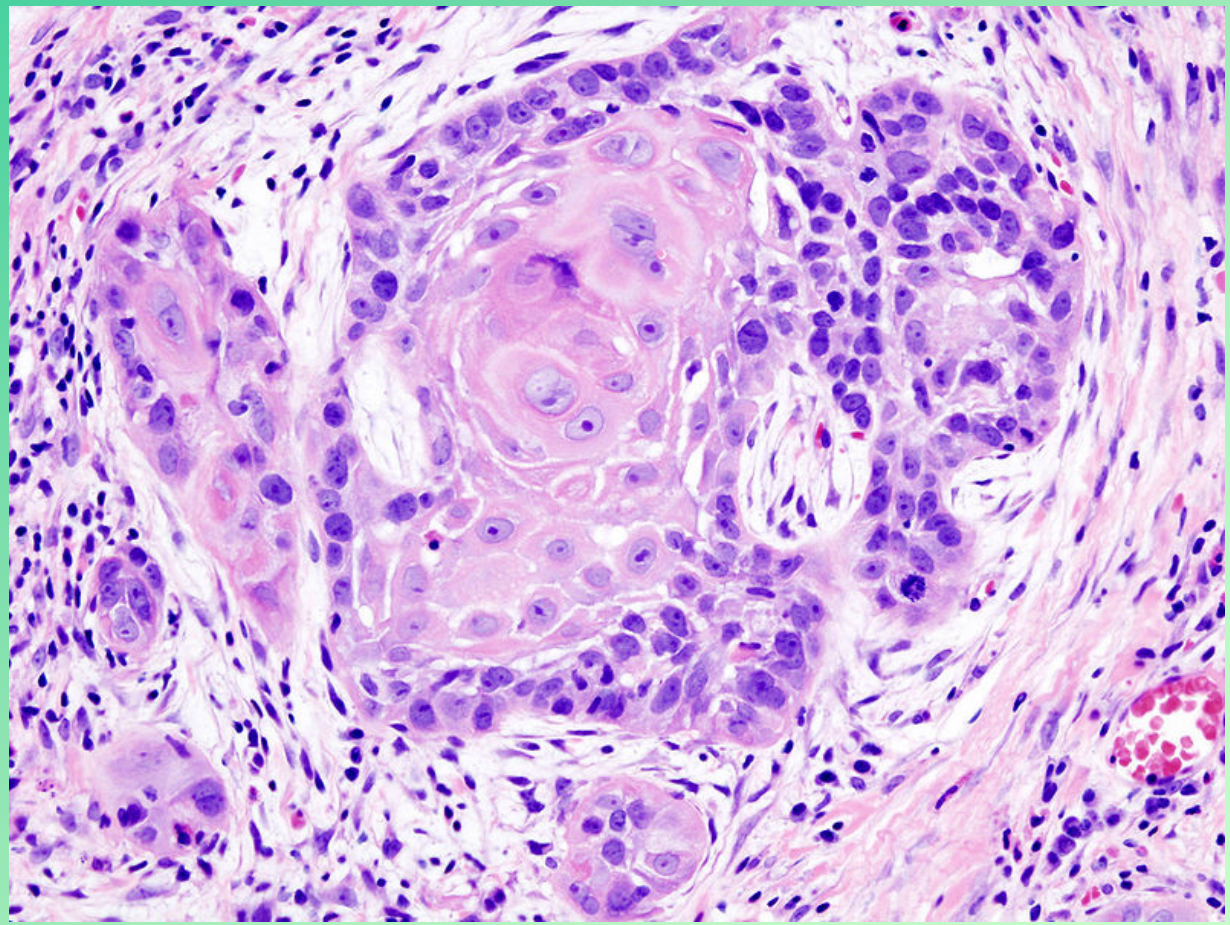
Oral Cavity Squamous Cell Carcinoma



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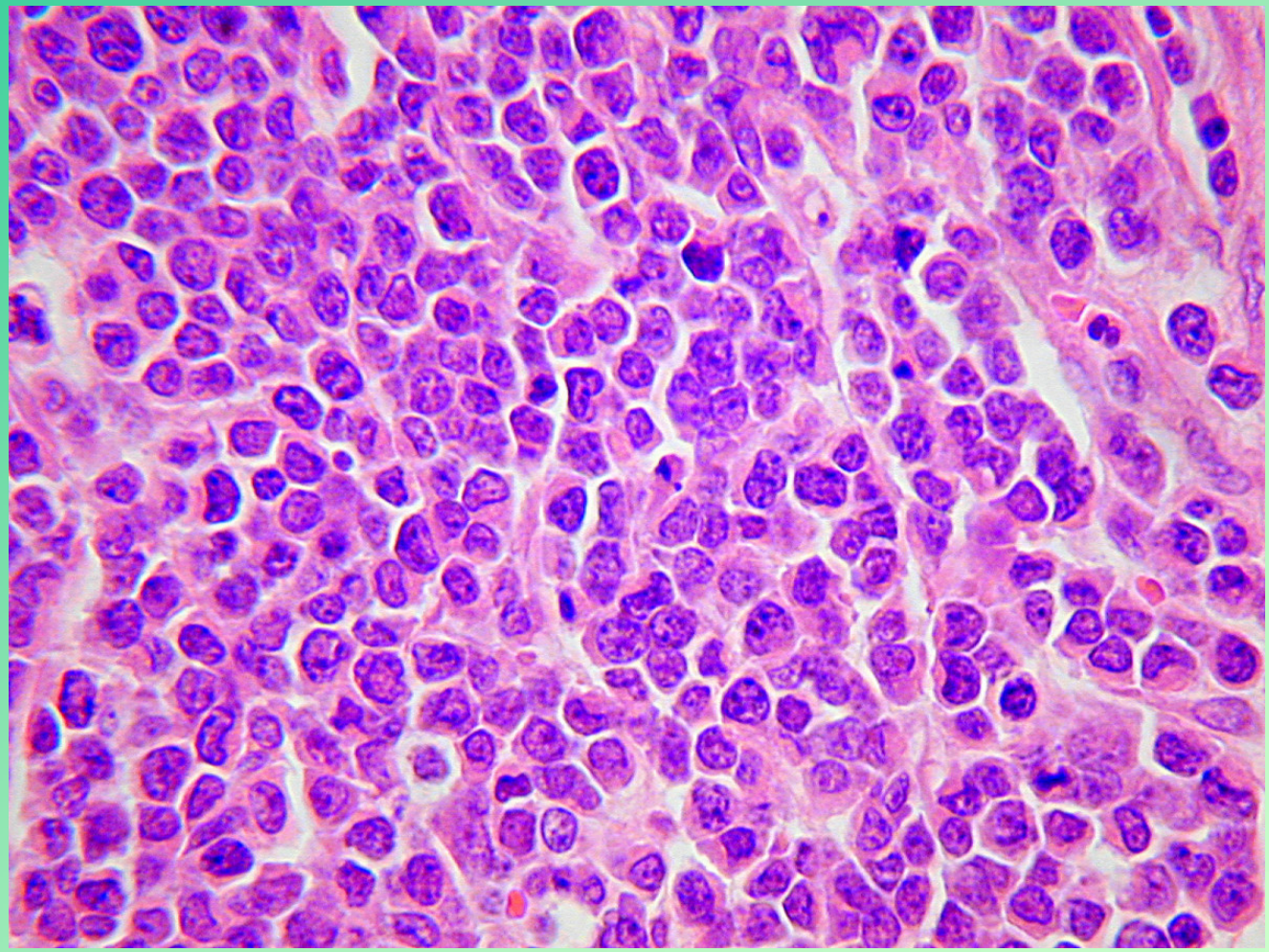
Small Cell Lung Cancer



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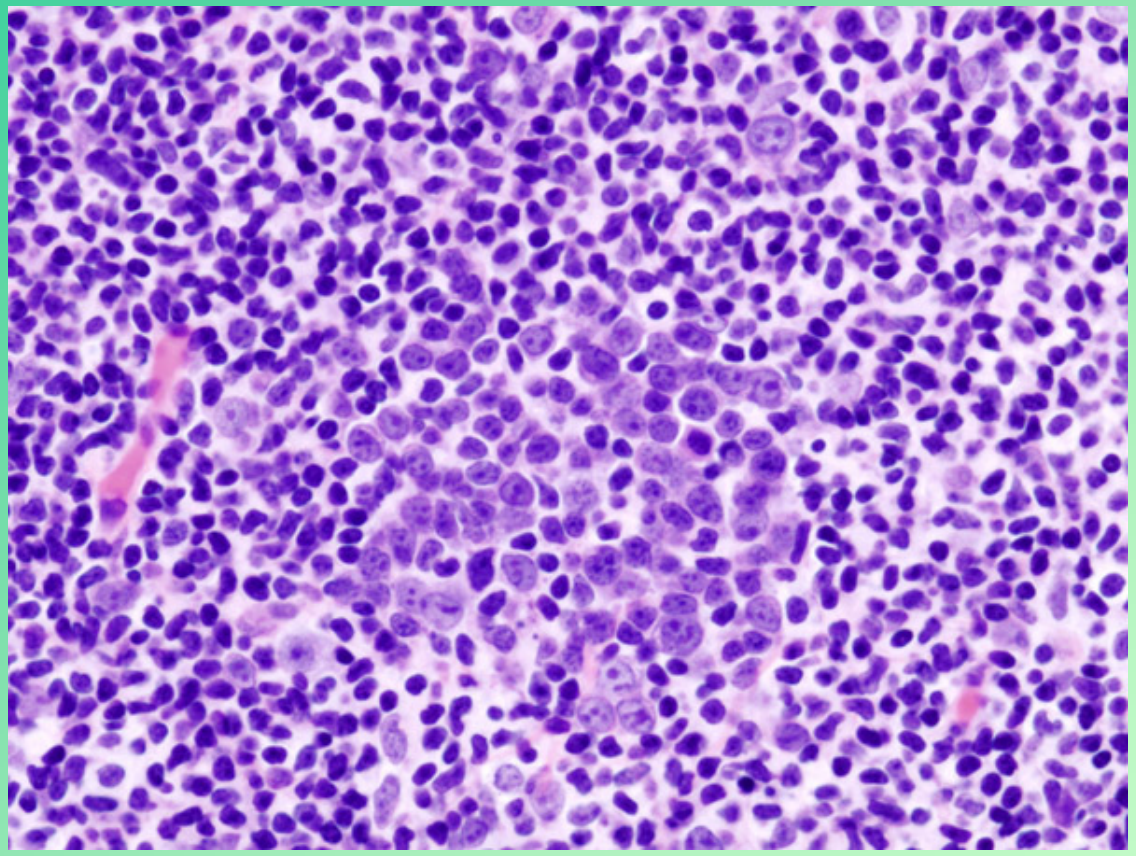
Small Cell Carcinoma or Lymphoma?



By immunoperoxidase staining this is B-cell lymphoma.

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The Impact of Molecular Diagnostics on Contemporary Cancer Management

- The following presentation gives highlights from a burgeoning field, with signficant advances occurring on a daily basis
- It cannot be encyclopedic in its approach
- Rather I hope to hit the high points in a very active and exciting area of basic and clinical research
- First…some relevant history

First Breakthroughs in Solid Tumors – the 1970's

- The Hormone Receptor
- Immunoperoxidase staining looking for proteins expressed on the surface or in the cytoplasm of cells – as in the last example (small cell carcinoma vs. lymphoma)

The Hormone Receptor Story

- Marc Lippman at NCI and others recognized that breast and other cancers expressed hormone receptors in their cytoplasm
- Those receptors bind steroid hormones; that complex then migrates into the nucleus of the cell and affects genes within the cell that control cell growth

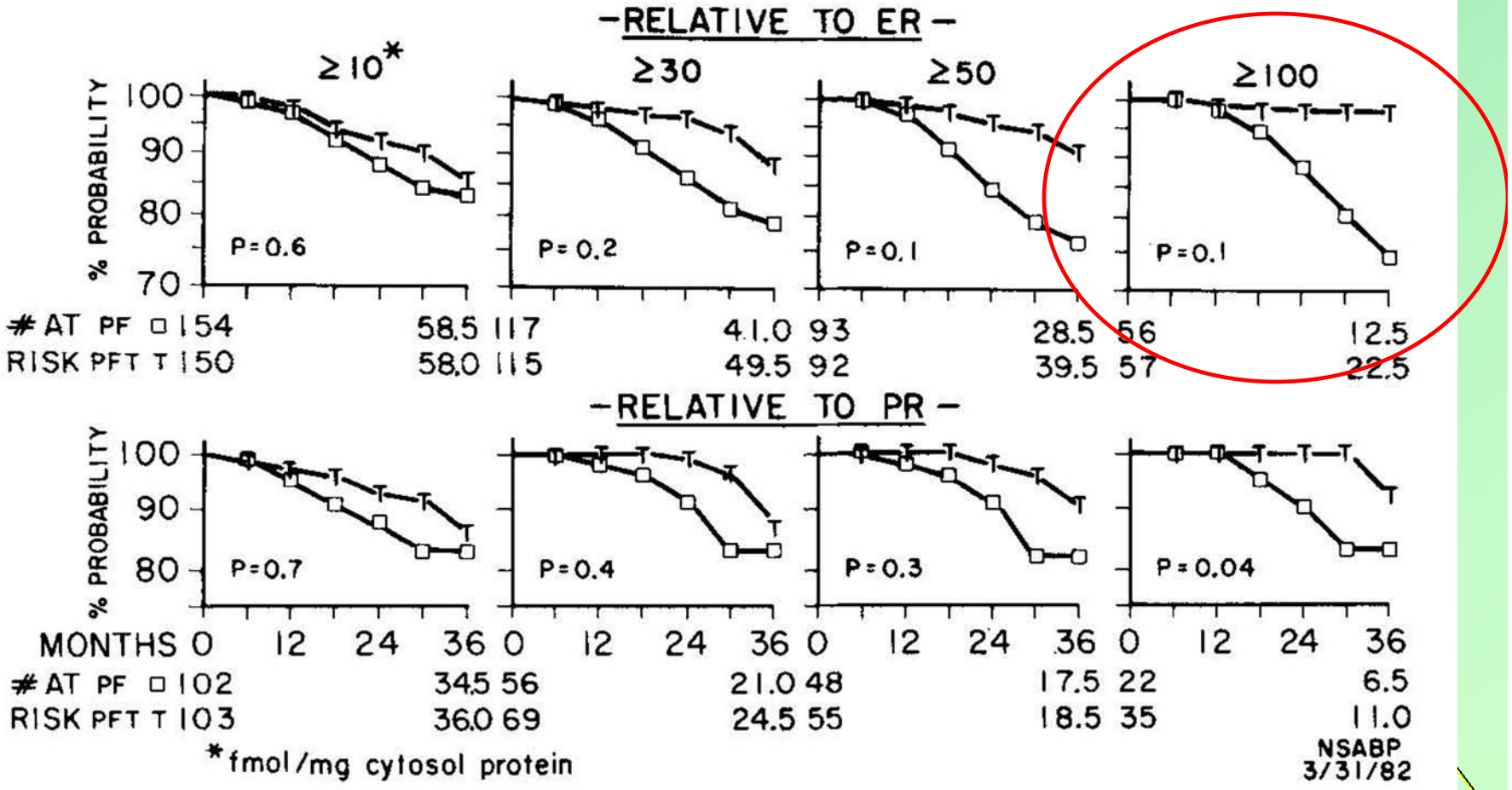
The Significance of the Presence of the Hormone Receptor

- Lends credence to the site of origin of the tumor as breast if the tumor assayed is in a metastatic site rather than the breast
- Guides therapy towards the use of hormonal manipulation rather than cytotoxic chemotherapy

The Significance of the Hormone Receptor in the History of this Field

- Applied technology already known in the 70's to solving a new problem
- Only recently have newer techniques (Northern Blot and Polymerase Chain Reaction) been used investigationally to improve the accuracy of this test
- Represented a benchmark in going beyond conventional histology in determining structure *and* function of a cancer

Further significance of the Estrogen Receptor: Disease-Free Survival with Adjuvant Tamoxifen versus concentration of quantitative ER and PR in women over 50 (NSABP B09)



Fisher et al. *J. Clin. Oncol.* 1(4)227-41, 1983

The Importance of Surface Immunoglobulins to this Story

- Lymphomas until the 1970's were classified purely morphologically – nodular vs. diffuse, large vs. small cells
- The discovery of monoclonal surface immunoglobulins on lymphoid tissue changed forever the approach to, and treatment of, non-Hodgkin lymphoma
- The therapy of B-cell neoplasms (lymphomas, multiple myeloma) was changed substantially with the development of an antibody to the CD20 surface molecule expressed on B cells preferentially – i.e., Rituximab
- Molecular diagnostics drove the development of novel therapy

Monoclonal Antibodies in Lymphoid Diseases, continued

- Next important antibody was OKT3 used therapeutically in the treatment of allograft rejection
- Directed against the T-cell and its role in graft rejection
- As a marker can help distinguish T- from B-cell lymphoma in difficult cases
- Being investigated in the treatment of T-cell malignancies

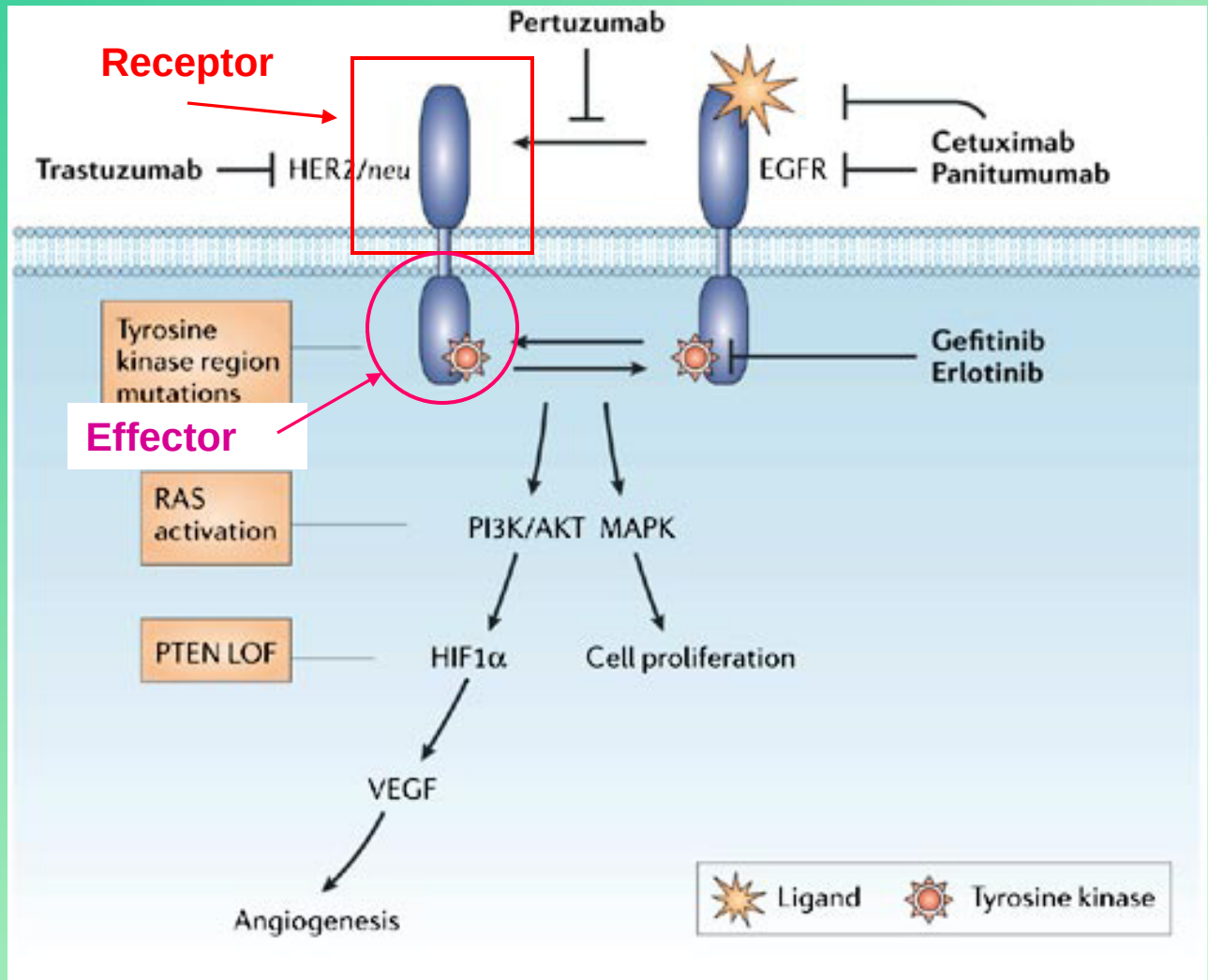
Other Monoclonal Antibodies in the Treatment of Lymphoid Malignancies

- Alemtuzumab (Campath) directed against CD-52 in refractory CLL
- Ofatumumab picks up patients refractory to chemotherapy and Alemtuzumab – just approved by FDA and marketed as Arzerra
- All of these therapies are based on utilizing unique molecular aspects of lymphoid cells as targets for novel therapies

Newer targets for the Treatment of Cancer: the Her-2/neu Oncogene

- 185 KD transmembrane glycoprotein receptor with intracellular tyrosine kinase activity
- Overexpressed in 18-20% of breast cancers
- Presence predicts for more virulent disease

The Her-2/neu Oncogene



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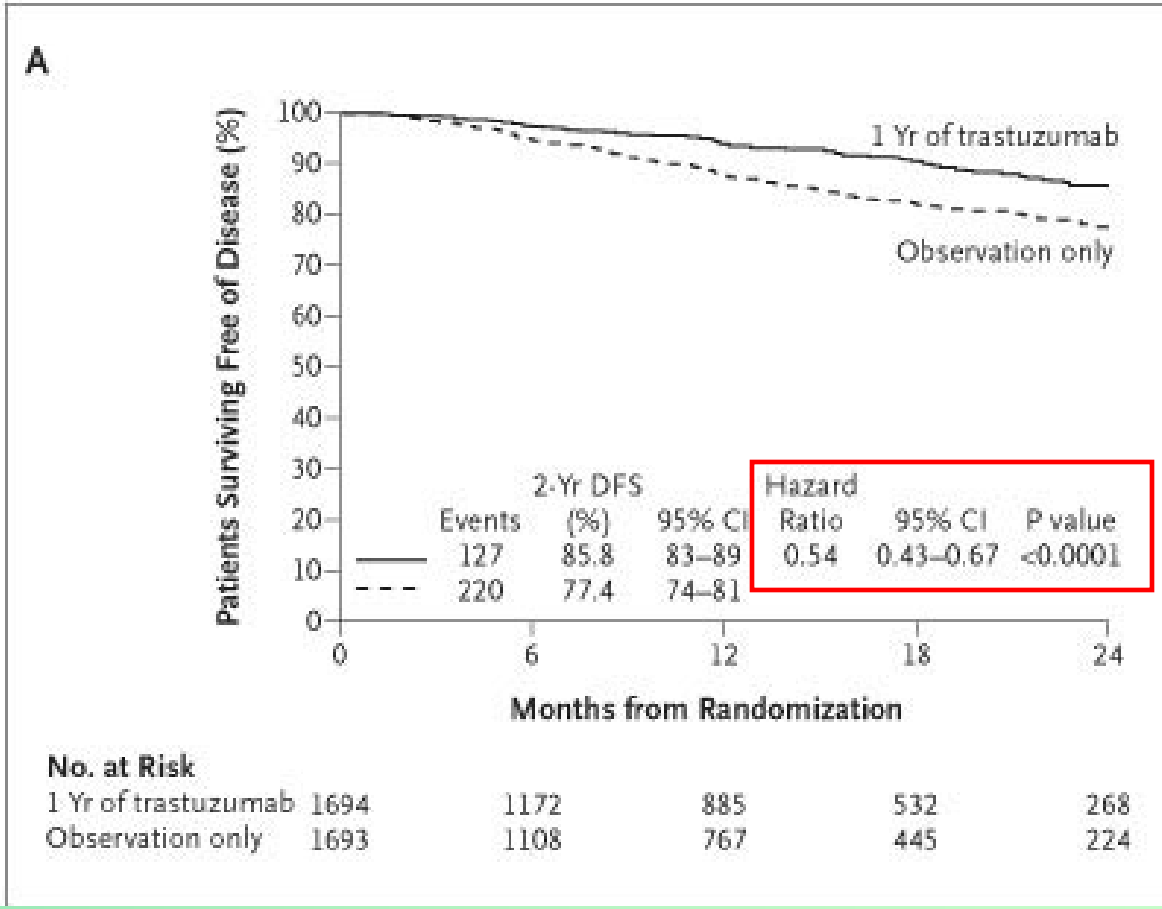
Her-2, continued

- Patients who are “Her-2+” are so by virtue of making numerous copies of this transmembrane protein as part of the defect in regulation associated with the mutation
- Such patients historically (prior to the development of Trastuzumab) had a much higher overall mortality

Her-2, continued

- The addition of Trastuzumab to conventional chemo therapy in the adjuvant and metastatic setting has resulted in marked improvement in the outcome of such patients
- The classic adjuvant trial…the HERA trial

Disease-Free survival in patients getting adjuvant chemotherapy with or without subsequent Trastuzumab



Piccart-Gebhart M et al. *N Engl J Med* 2005;353:1659-1672

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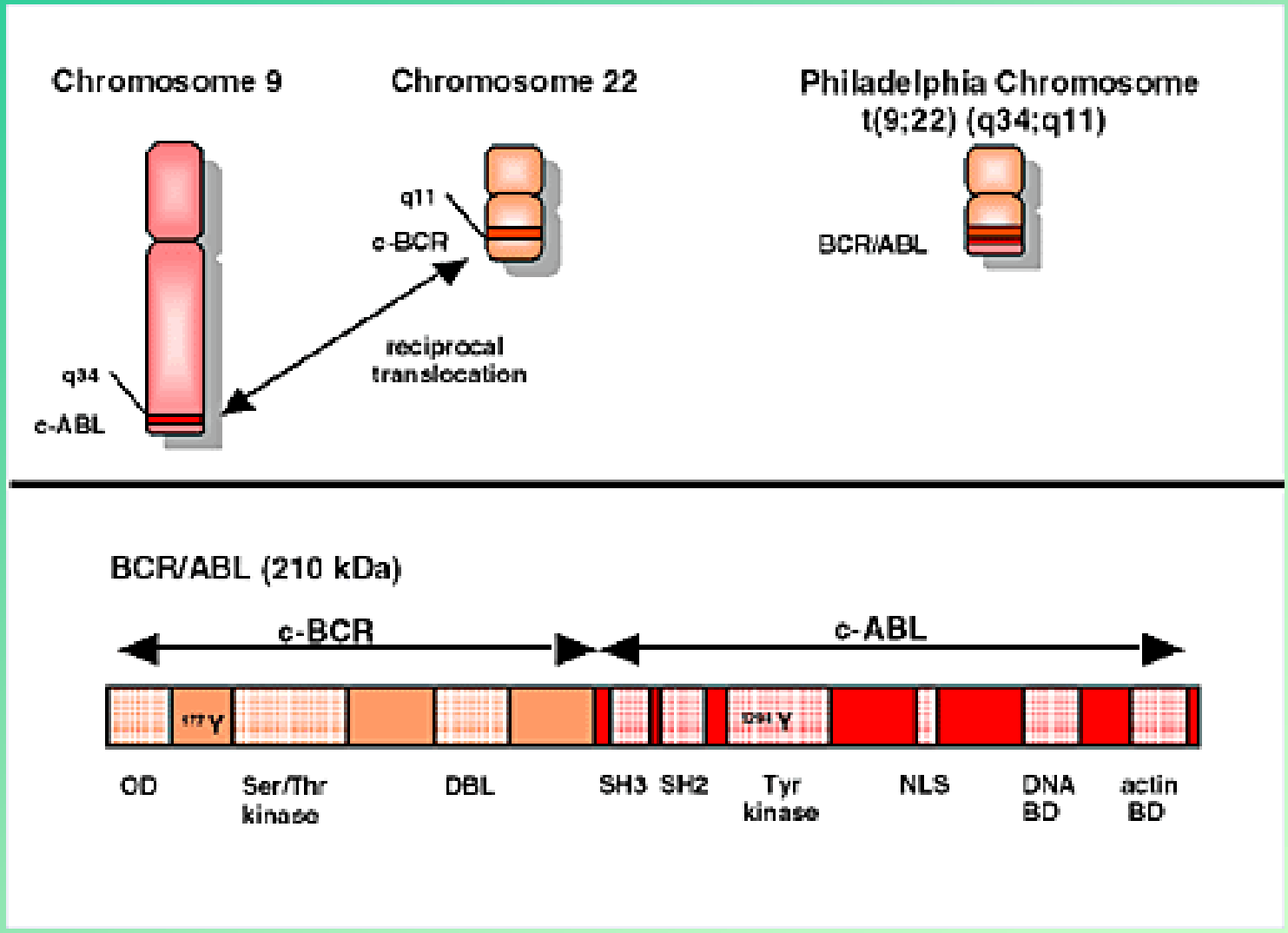
Adjuvant Herceptin, continued

- These seminal observations have been extended to other groups of patients
- This treatment became the overnight standard of care for patients with Her-2 positive breast cancer, either primary or metastatic
- The original observation about a unique gene on the surface of breast cancer cells started the cascade of developments which has led to revolutionary new treatment of breast cancer

The Philadelphia Chromosome in the Era of Molecular Biology

- For decades the exchange of genetic material between chromosomes 9 and 22 in CML has been well known…

Schematic of BCR-ABL



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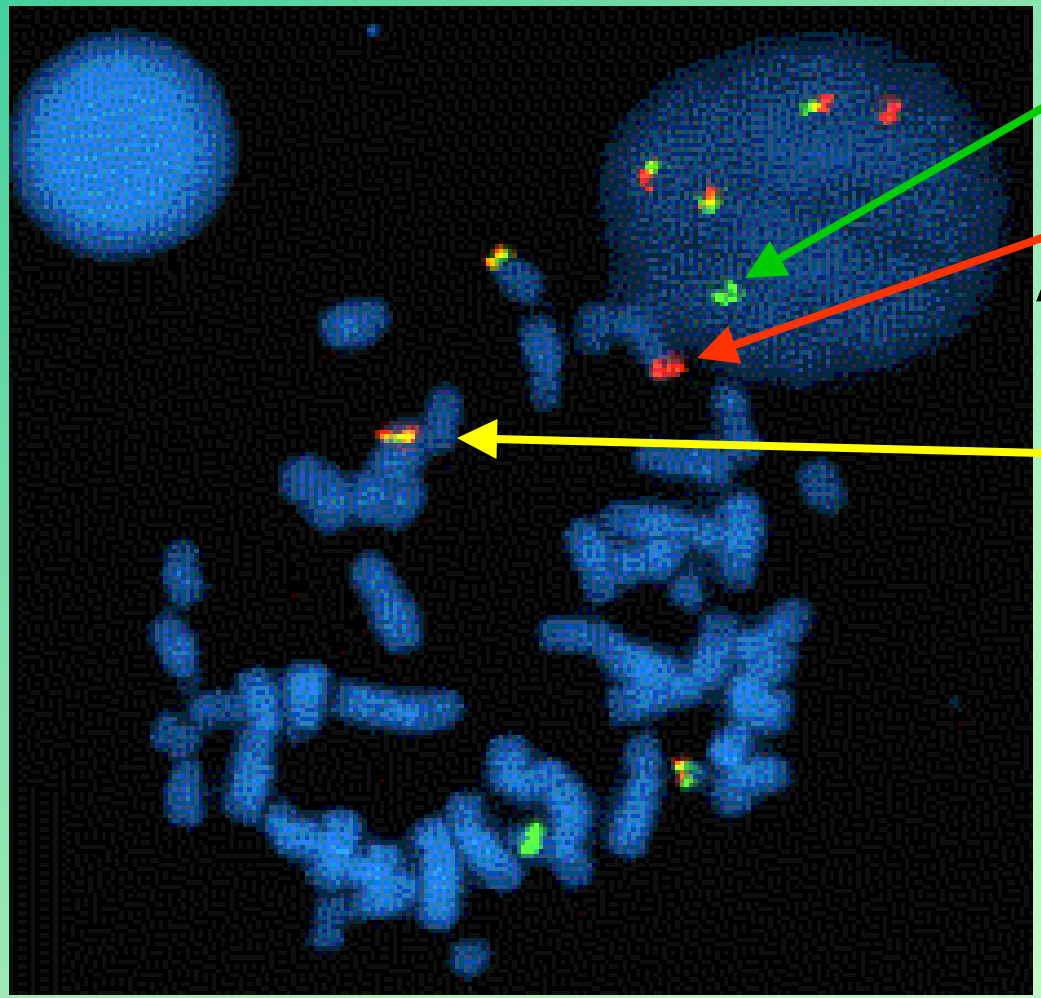
The Philadelphia Chromosome in the Era of Molecular Biology

- For decades the exchange of genetic material between chromosomes 9 and 22 in CML has been well known
- The ability to find a single cell with the translocation in a sea of normal cells is a relatively new development: Fluorescence in-situ hybridization (FISH)

FISH

- Uses short pieces of DNA which are complementary to a genetic sequence of interest (a probe)
- Probe binds specifically to target DNA sequence
- Probe is linked to a fluorescent compound for visualization
- 200 cells typically scored
- Always targeted to a specific mutation;
- Not a hunt for any mutation

FISH: When you know what you are looking for…
In this case the novel BCR-ABL sequence



BCR green

ABL orange

Fusion signal yellow

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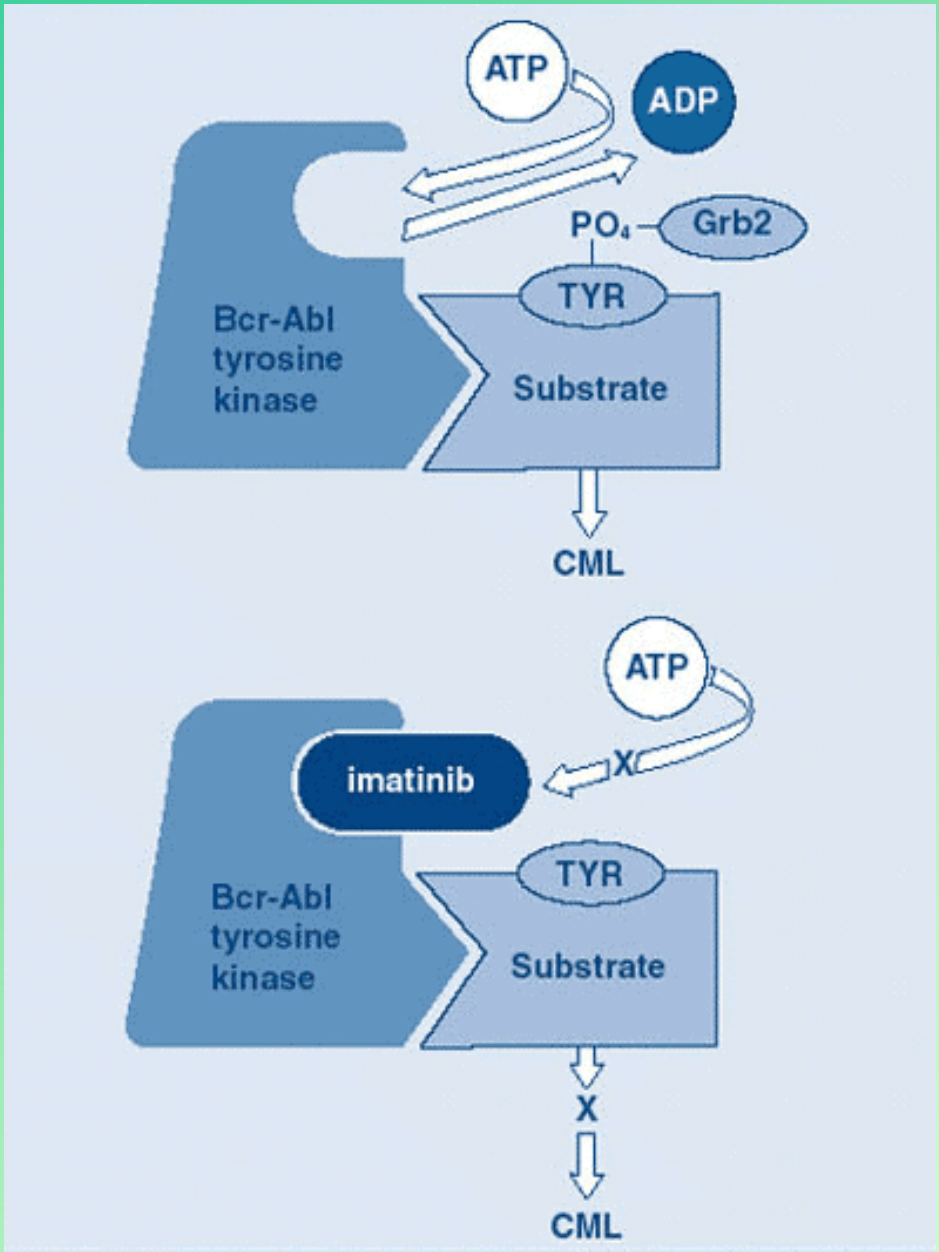
How Imatinib Works



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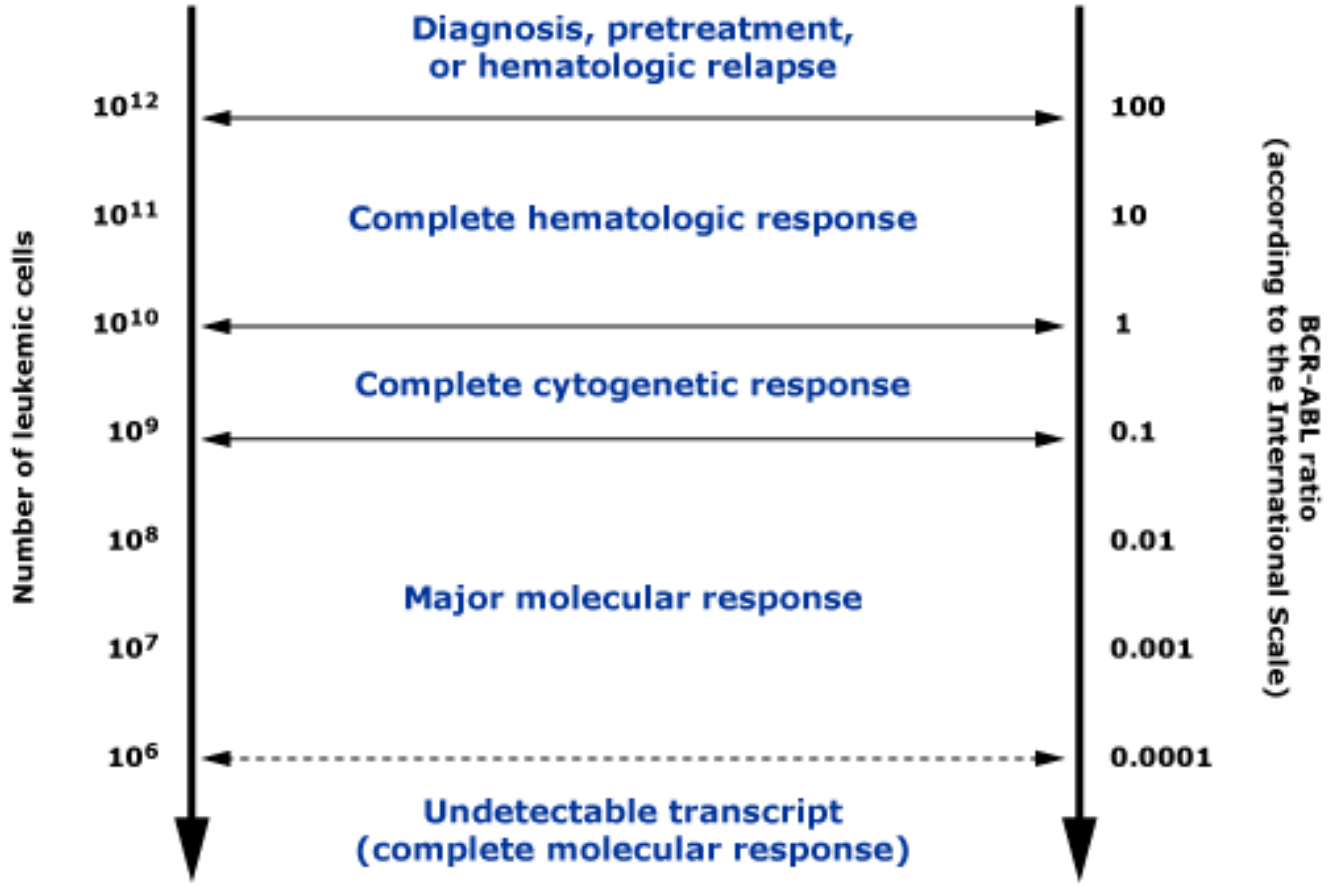
Outcomes in Patients with CML treated with Imatinib

- A high percentage of patients with convert to FISH-negative in marrow and peripheral blood
- A smaller number will have complete disappearance of disease by Polymerase Chain Reaction techniques

Approximate relationship between response, the putative number of leukemic cells, and the level of BCR-ABL transcripts



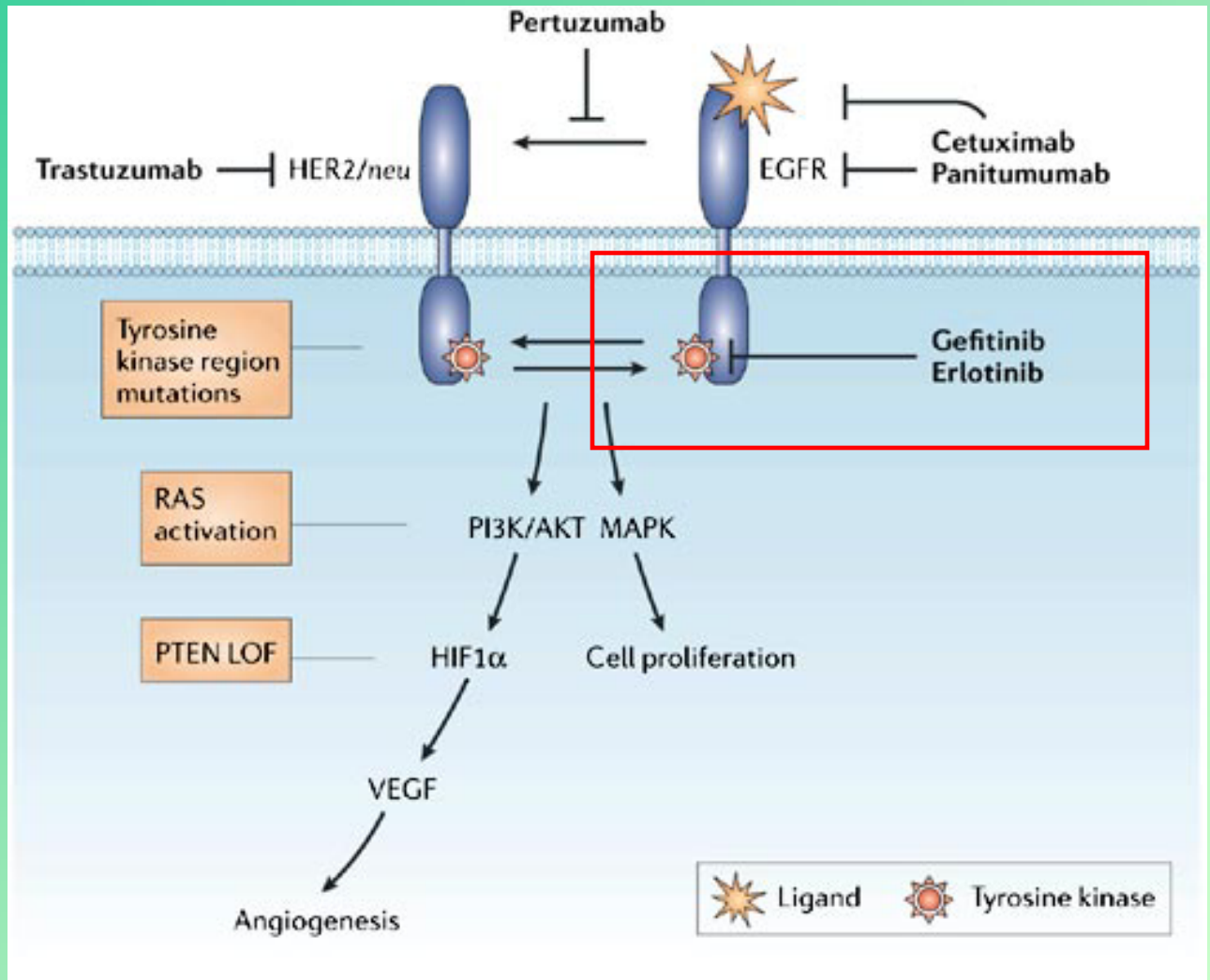
Long-Term Results

- The greater the log reduction in tumor burden the more likely that the patient will stay in morphologic remission
- After ten years of experience with Imatinib medial survival of original group of patients has not yet been reached
- Previously median survival of patients with newly diagnosed CML receiving best therapy was 3-4 years

The Role of the Epidermal Growth Factor Receptor in Lung Cancer

- Required for tumor growth
- Mutated about 15-20% of the time
- After several years of research on Erlotinib (Tarceva), investigators determined that this mutation was critical to success with this drug

The Many Targets of Tyrosine Kinase Inhibition



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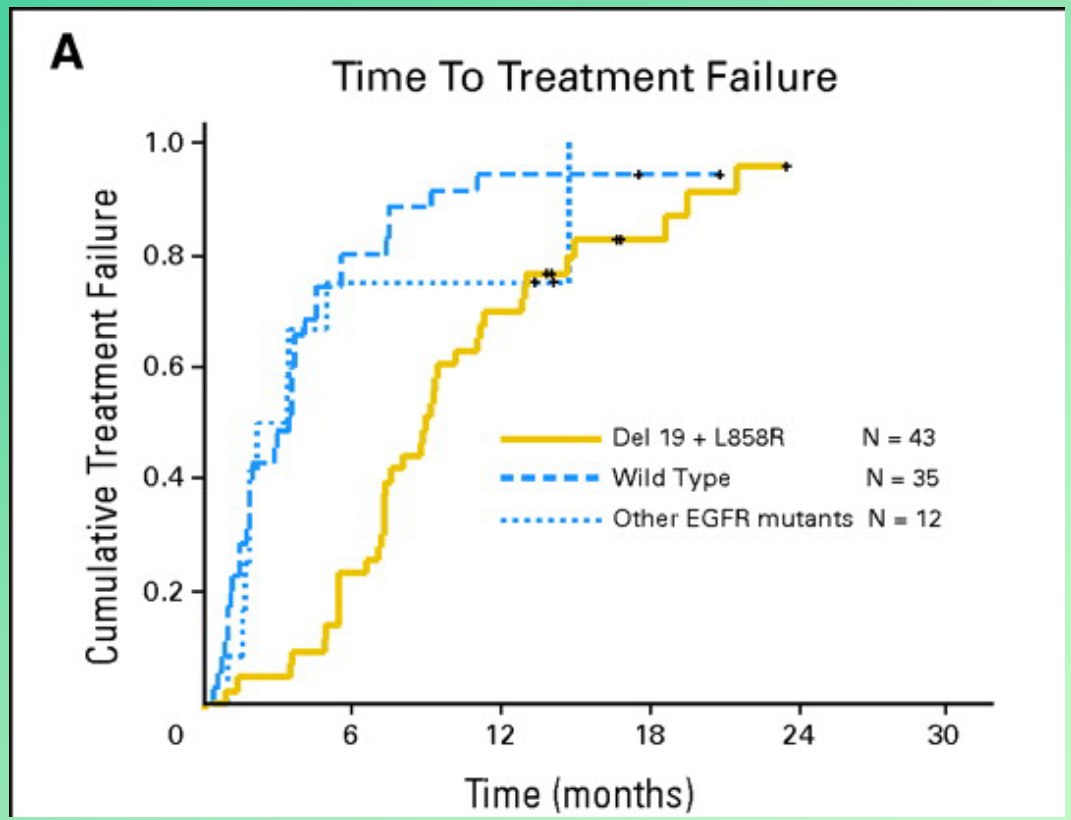
Time to treatment failure of patients treated with Erlotinib grouped by EGFR mutational status



Yang, C.-H. et al. *J Clin Oncol*; 26:2745-2753 2008

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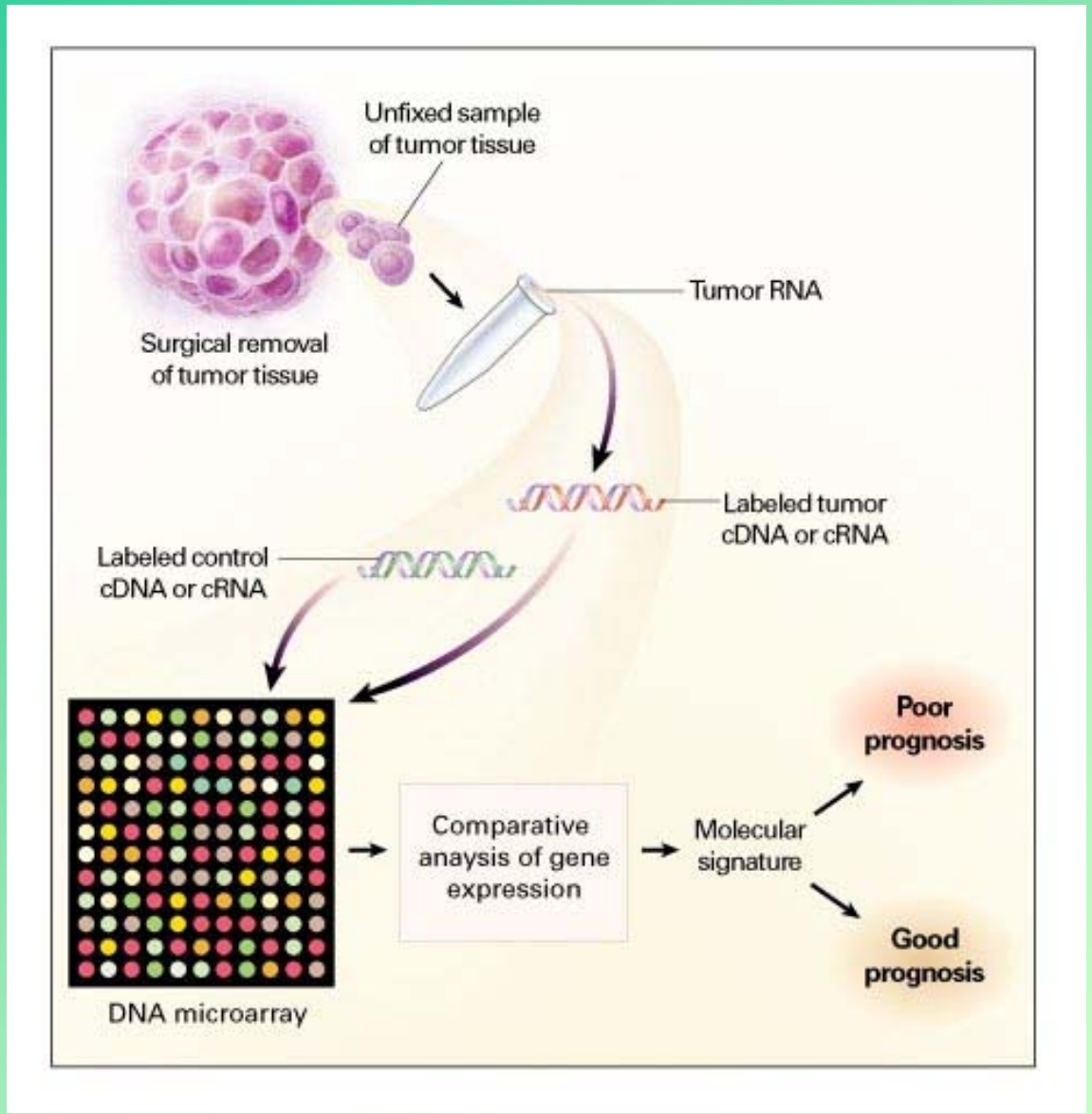
The next step in this adventure: Gene-Expression Profiling



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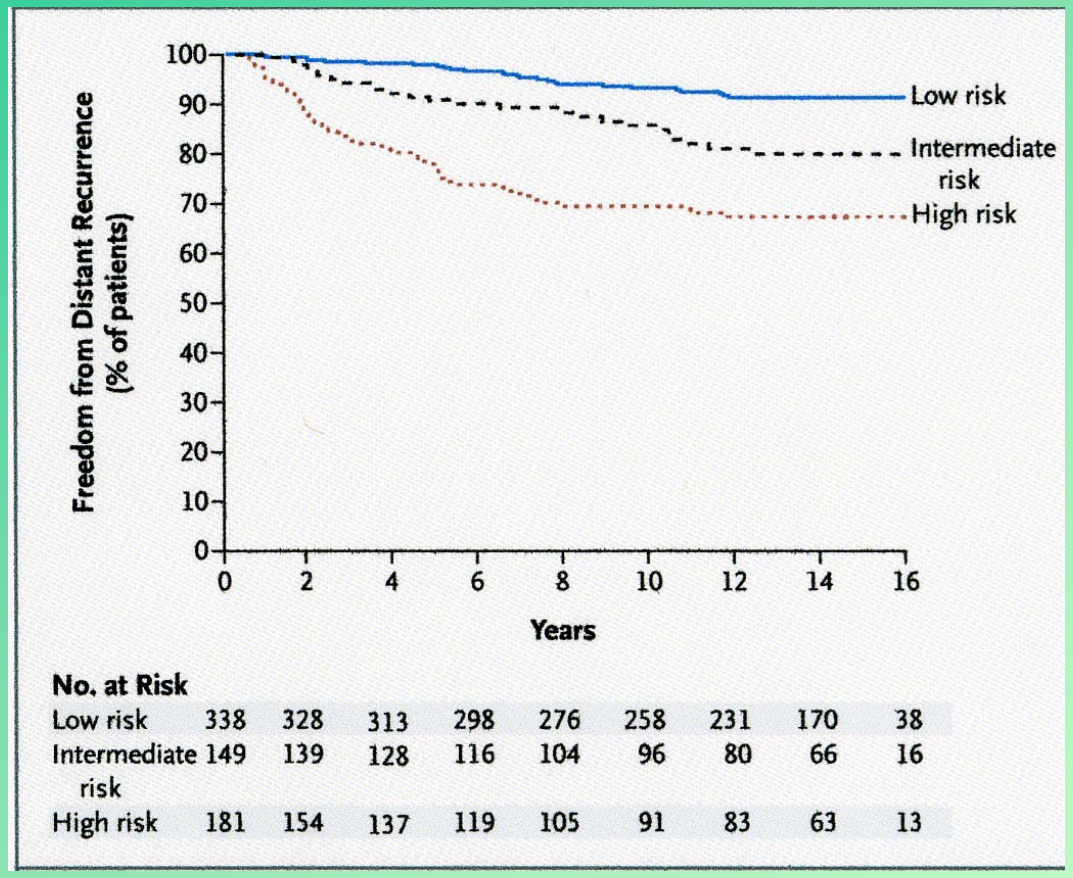
Using Gene-Expression Profiling to Create Prognosis in Primary Breast Cancer: the Oncotype DX test



No. at Risk									
Low risk	338	328	313	298	276	258	231	170	38
Intermediate risk	149	139	128	116	104	96	80	66	16
High risk	181	154	137	119	105	91	83	63	13

By regression analysis 21 genes were picked which, if mutated, alter prognosis. Those genes are analyzed in this test and a risk-of-recurrence score is derived based on the types of mutations seen.

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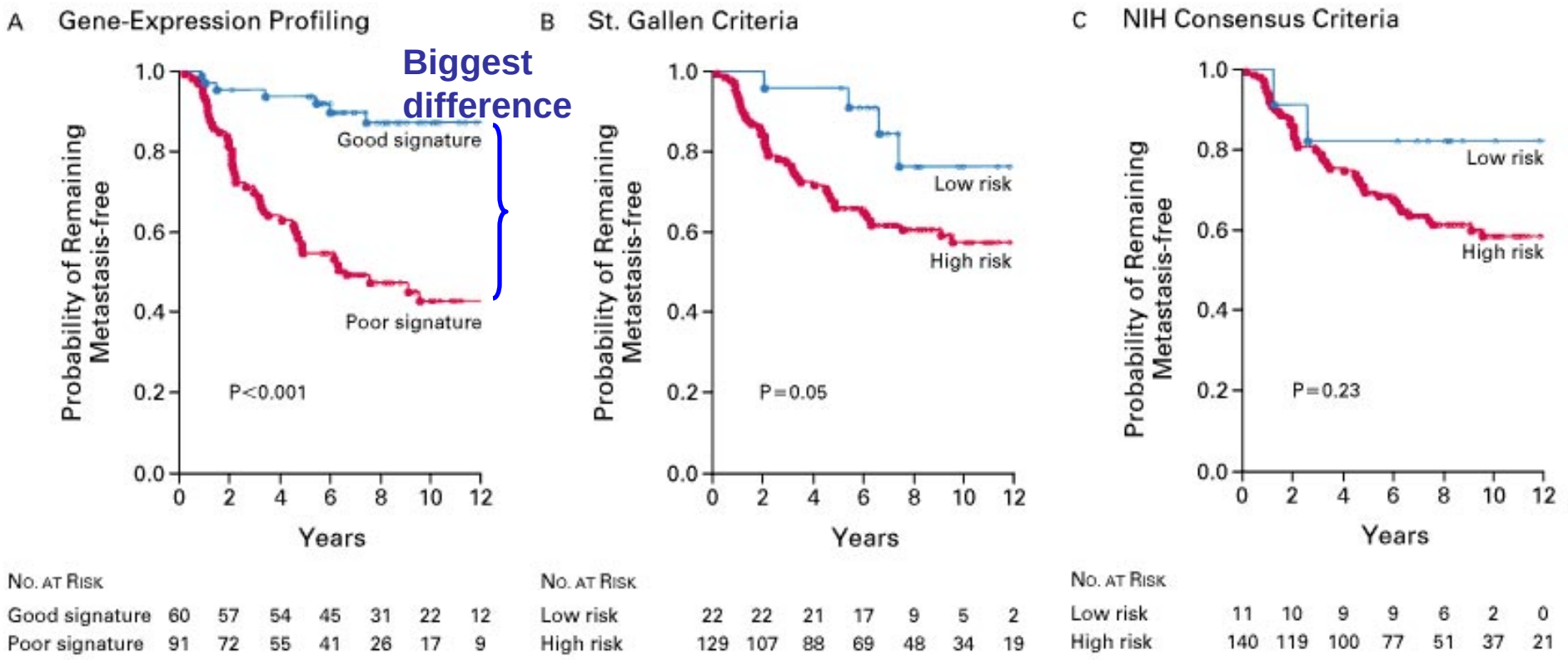
Probability That Patients Would Remain Free of Distant Metastases among 151 Patients with Lymph-Node-Negative Breast Cancer with the Use of Gene-Expression Profiling, the St. Gallen Criteria, and the National Institutes of Health (NIH) Consensus Criteria)



van de Vijver, M. et al. *N Engl J Med* 2002;347:1999-2009

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Using Recently Acquired Technology to Address the Problem of the Unknown Primary

- 4-5% of cancers present as unknown primaries with metastasis being the first evidence of cancer
- Until now there have been only a limited number of ways to analyze their tumors *ante-mortem*
- A variety of genes can now be sequenced and compared to a library of genetic mutations compiled for a wide variety of tumors
- Genes looked at include genes for transcription factors, trans-membrane proteins and tumor-specific genes (e.g., TTF-1 for lung cancer)

Commercial Test Addresses Problem: The CancerTYPE ID® Gene Characteristics

- 92 genes not normally measured by routine laboratory testing:
  - Transcription factors (e.g.,HOX-A9, HOX-B8)
  - Plasma membrane proteins (e.g., HTR3A, CHRM3)
  - Uncommonly measured tumor-specific markers (e.g., ESR1 for breast, PRAME for melanoma)
  - Compared genetic sequence in these genes of the unknown tumor against a library of 2000+ tumors whose site of origin was known

Ma XJ, et al. *Arch Pathol Lab Med*. 2006;130:465-473

Impact of Diagnostic Procedures on Healthcare Systems

Misclassifications	In a retrospective study reviewing the frequency and impact of errors in ~24000 cases, 45% of gynecologic errors and 39% of non-gynecologic errors were associated with harm[1]
Cost	Traditional diagnostic methods often fail to diagnose hard-to-identify cancers, even after extensive work-ups that average nearly $18,000[2,3]

There is an unmet need for standardized assays to support diagnostic evaluation and reduce diagnostic uncertainty

[1]Raab SS, et al. *Cancer* 2005;104:2205-2213; [2]Levine MN, et al. *CMAJ*. 1985;133:977-987;
[3]Schapira DV, Jarrett AR. *Arch Int Med*. 1995;155:2050-2054

Applying this technology to a series of twenty unknown primary tumors

	Age/Sex	Biopsy Site	Light Microscopic Histology	Molecular Assay Diagnosis	Actual Primary Site
Correct Primary Site Identified (N=15)	59 F	Axillary node	PDC	Breast	Breast
	65 F	Axillary node	PDA	Breast	Breast
	51 F	Bone	PDC	Breast	Breast
	64 F	Supraclavicular node	PDA	Breast	Breast
	85 F	Chest wall mass	PDA	Ovary	Primary peritoneal
	69 F	Inguinal node	Adenocarcinoma	Ovary	Primary peritoneal
	87 F	Omentum	PDA	Ovary	Primary peritoneal
	68 F	Paratracheal mass	PDC	Ovary	Ovary
	49 F	Mesenteric node	PDA	Intestine	Colon
	61 M	Liver	PDA	Intestine	Colon
	42 F	Brain	PDA	NSCLC	NSCLC
	67 M	Subcutaneous mass	Squamous carcinoma	NSCLC	NSCLC
	59 M	Brain	PDA	NSCLC	NSCLC
	74 M	Bones	Adenocarcinoma	Gastric	Gastric
	76 M	Axillary node	PDC	Melanoma	Melanoma
Primary Site Indeterminate by Assay (N=2)	60 M	Small intestine	PDC	Unclassifiable	NSCLC
	38 M	Mediastinal node	PDA	Unclassifiable	NSCLC
Incorrect Primary Site Identified (N=3)	61 M	Supraclavicular node	PDC	Testis	Pancreas
	62 M	Retroperitoneal node	PDA	Colorectal	Gastric
	75 F	Chest wall mass	PDC	Soft tissue sarcoma	NSCLC

Greco A et al. *J Clinical Oncology* 27:15s, 2009 (Abstract 11070)

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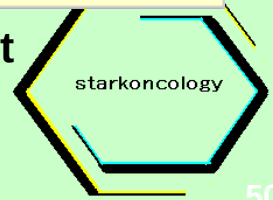
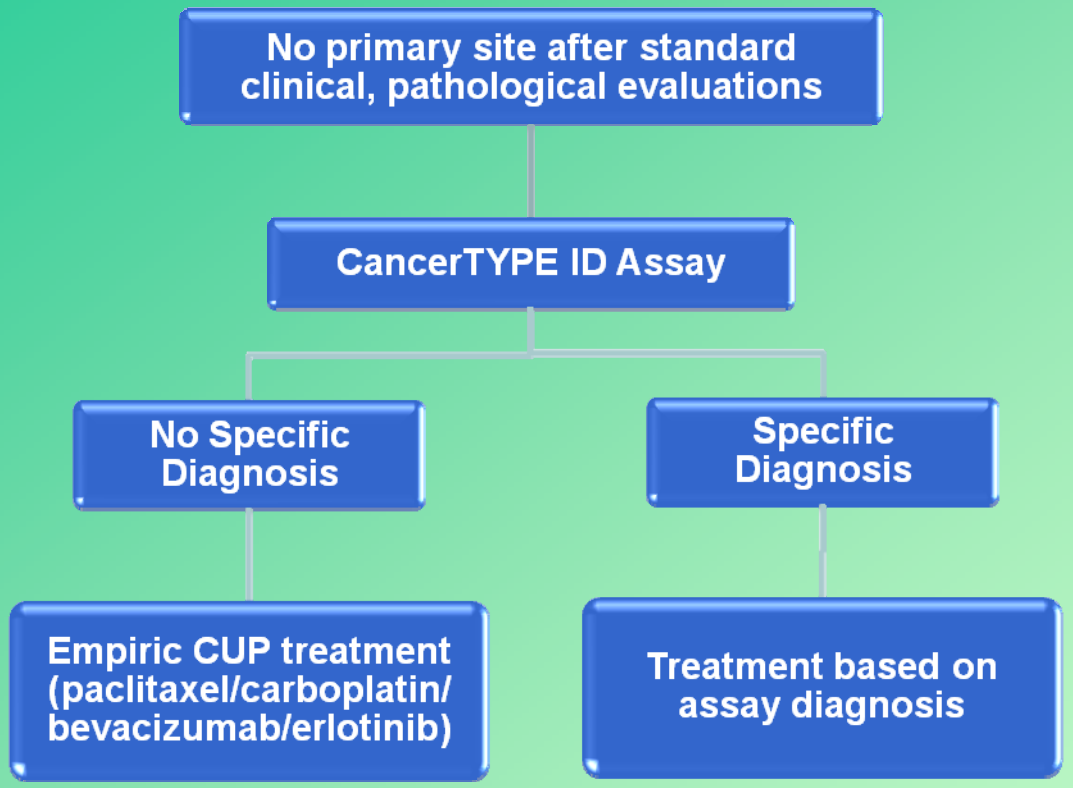
Potential Changes in Treatment for Cases with Accurate Predictions

Patient	Primary site suspected	Treatment for	Molecular Assay Diagnosis	Likely Change in Treatment & Outcome
1	Breast	Breast	Breast	No
2	Breast, Lung	Lung	Breast	No
3	Lung	Lung	Breast	Yes
4	Lung, Pancreas, Gastric	Lung	Breast	Yes
5	Lung, Breast	Lung	Ovary	Yes
6	Lung, Breast, Ovary	Lung	Ovary	Yes
7	Lung, Ovary, Breast	Lung	Ovary	Yes
8	Lung, Pancreas	Lung	Ovary	Yes
9	Colorectal	Colorectal	Intestine	No
10	Colorectal	Colorectal	Intestine	No
11	NSCLC	Lung	NSCLC	No
12	Lung, Head/Neck	Lung	NSCLC	No
13	NSCLC	Lung	NSCLC	No
14	Lung, Renal, Pancreas	Lung	Gastric	Yes
15	Unknown	None	Melanoma	Yes

Results from the 92-gene molecular assay had the potential to change treatment in 53% of CUP cases



A Phase II Study of Chemotherapy Treatment Based on Molecular Profiling Diagnosis for Patients with CUP

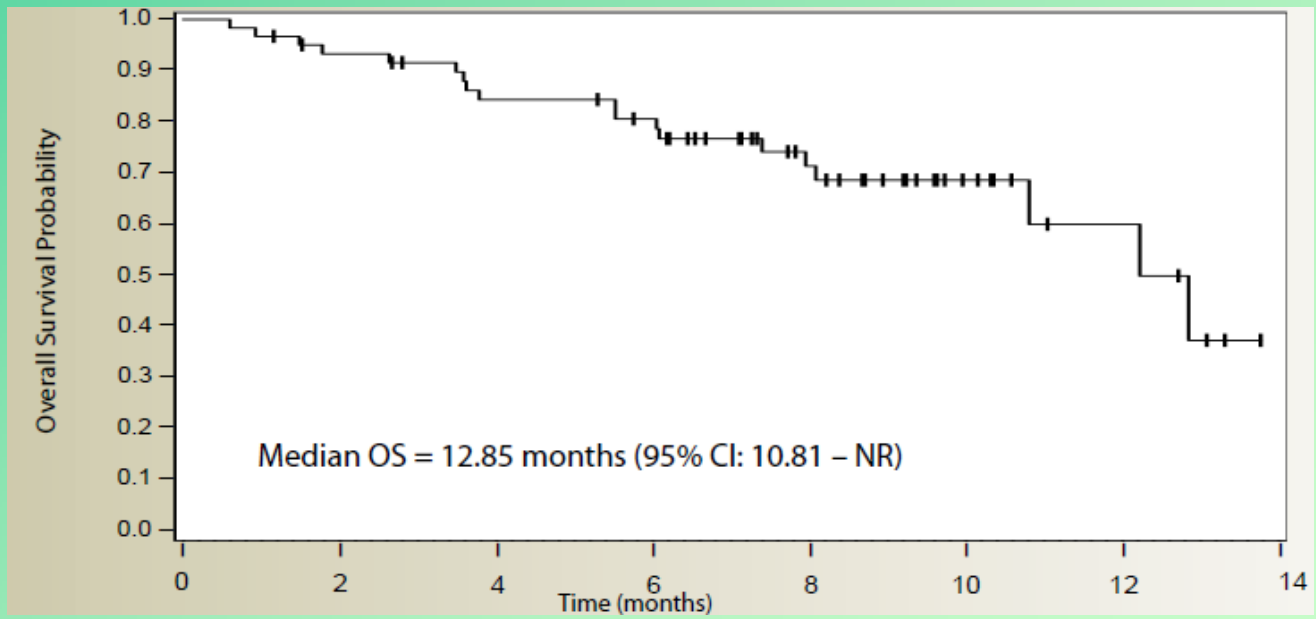


First-Line Treatments Administered for Specific Assay Diagnoses

Diagnosis	Treatment
Non-small cell lung cancer	Platinum-based doublet +/- bevacizumab
Breast ca	Paclitaxel/bevacizumab
Ovarian cancer	Paclitaxel/carboplatin +/- bevacizumab
Pancreas cancer	Gemcitabine/erlotinib
Colorectal cancer	FOLFOX (or FOLFIRI) + bevacizumab
Renal cell carcinoma	Sunitinib or bevacizumab
Other specific diagnoses	Standard first-line treatment per treating MD

Interim Results

- Current regimens for CUP patients have a median survival of 7- 11 months
- CTID provided a prediction in 98 of 110 patients (89%)
- 61 patients received assay directed therapy



Hainsworth J et al. *J Clin Oncol* 2010; 28 (15 suppl): Abstract 10540.

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The Current State of the Unknown Primary and Genetic Variance

- A number of competing technologies are being developed to look at a variety of ways of comparing sequences in RNA versus an established library of tumors
- The winning technology has not yet emerged
- All of the technologies represent an advance over what was previously available

Summary

- In the last forty years cancer diagnostics has advanced beyond morphologic analysis
- The understanding of the relationship between abnormal structure and function has progressed rapidly
- Therapies designed to exploit the differences between normal and abnormal structure have advanced in number and sophistication
- All the current advances in Medical Oncology are coming in the area of “targeted” therapy with few new all-purpose chemotherapy drugs coming on line in the last few years
- As our understanding of structure and function of normal versus abnormal becomes more advanced, cancer therapy will progress accordingly