Tyrosine Kinase: from molecule to bedside

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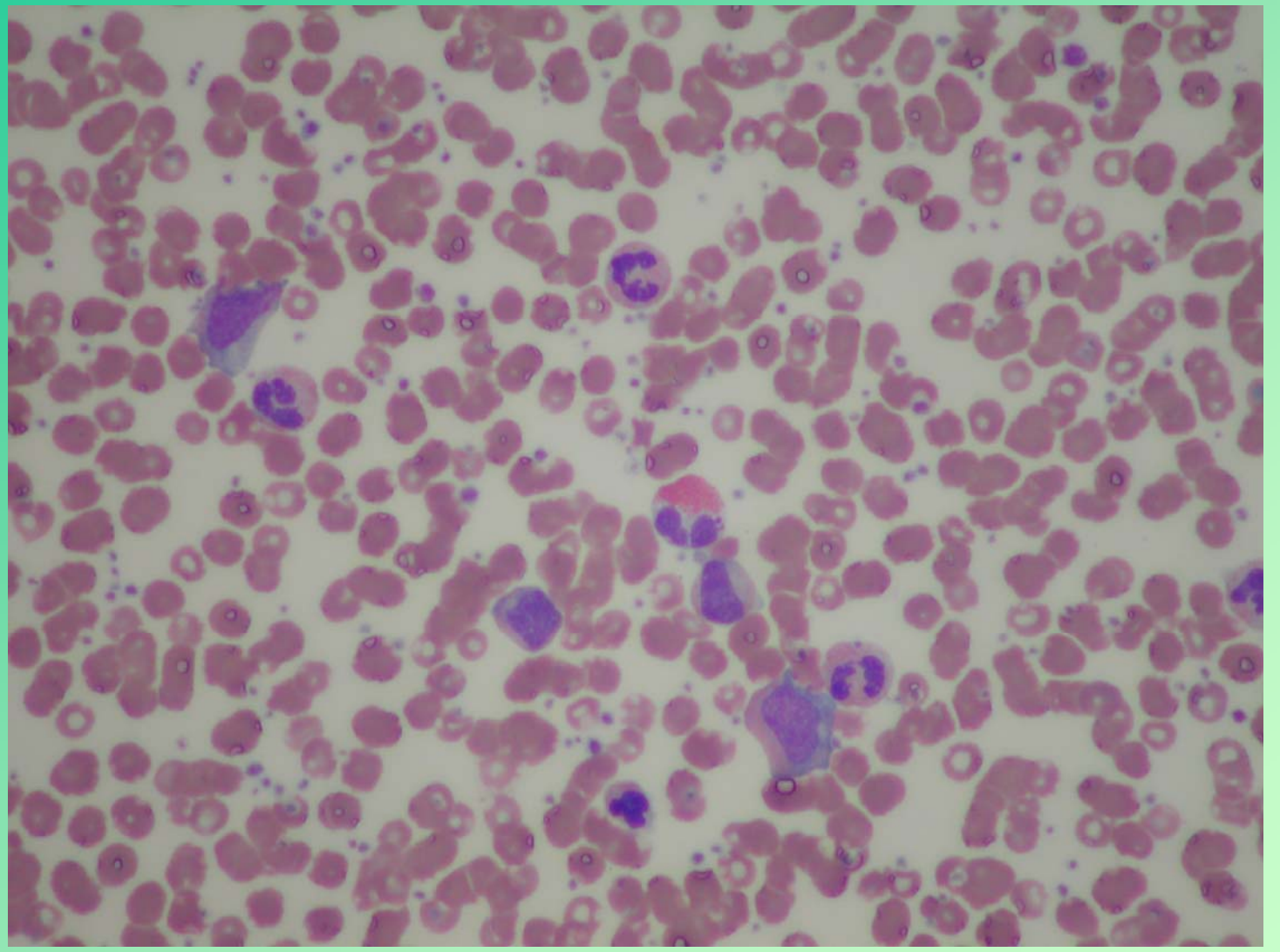
Case Presentation #1

- 78 y.o. lady seen in August 2005 for abnormal blood counts:
  - Hct 44
  - WBC 30,000 with a few promyelocytes and myelocytes
  - Platelet count 820,000
- Recent night sweats
- PE: barely palpable spleen

Peripheral Blood Smear



Case Presentation

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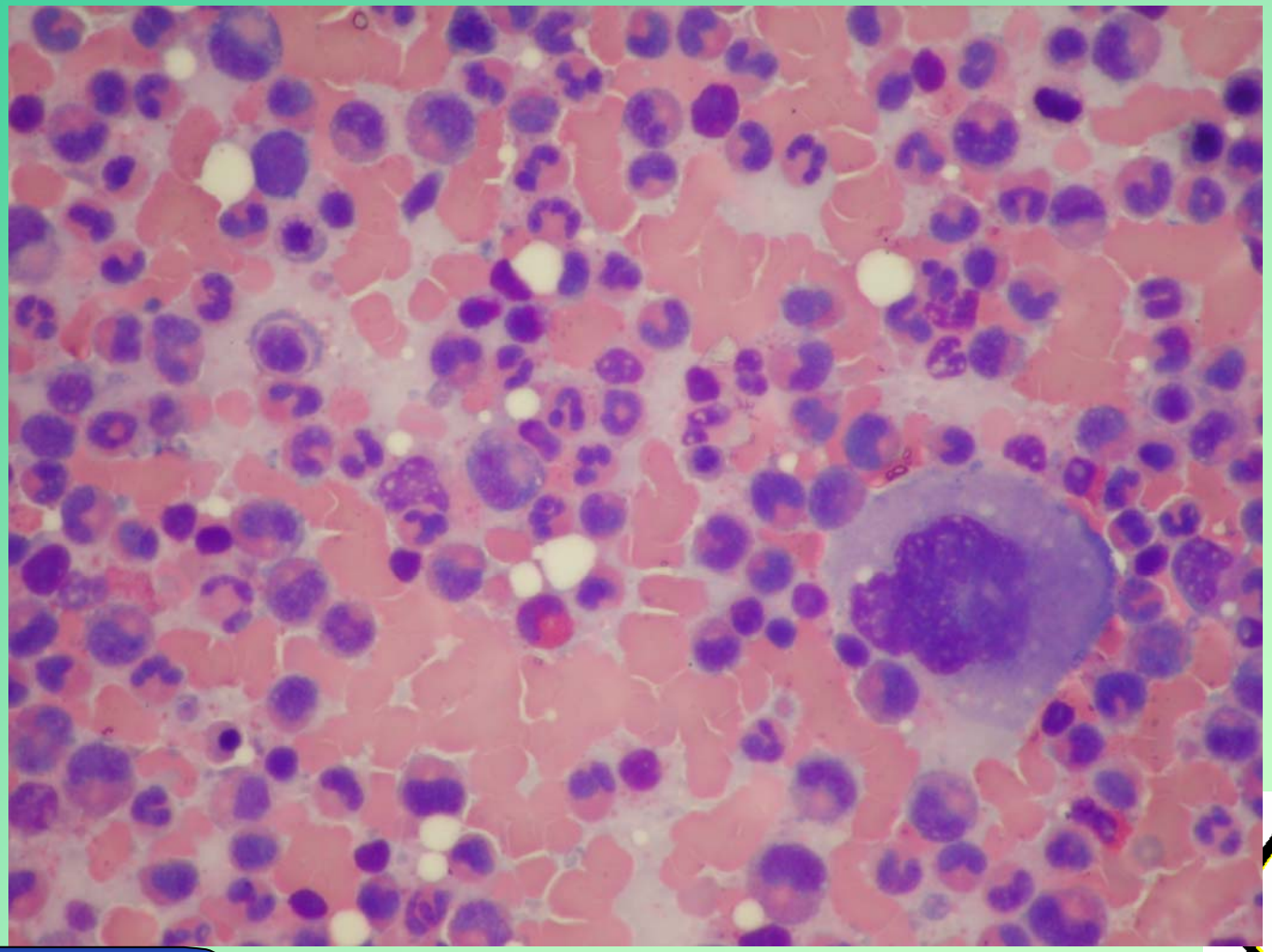
Bone-Marrow Aspiration



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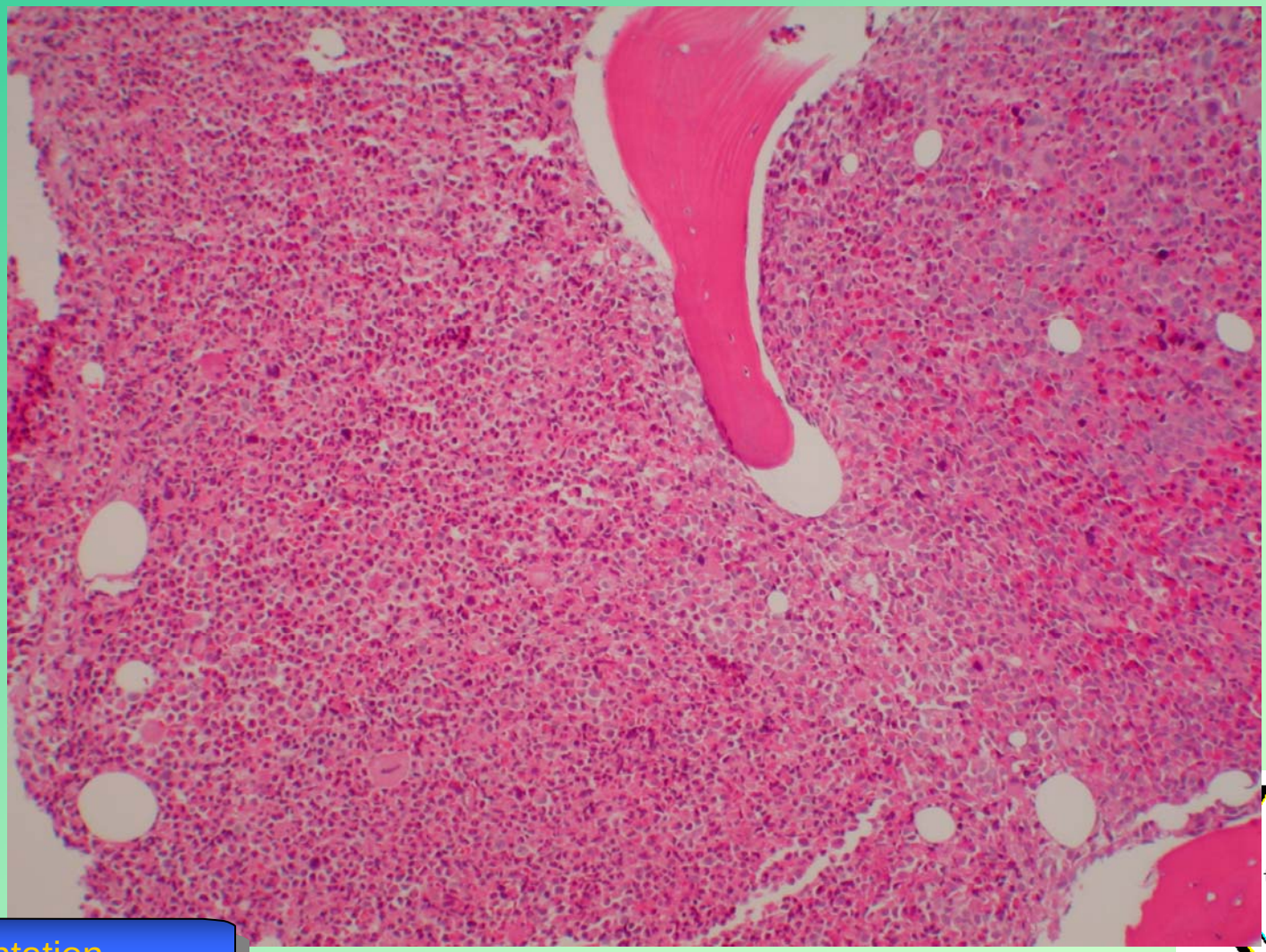
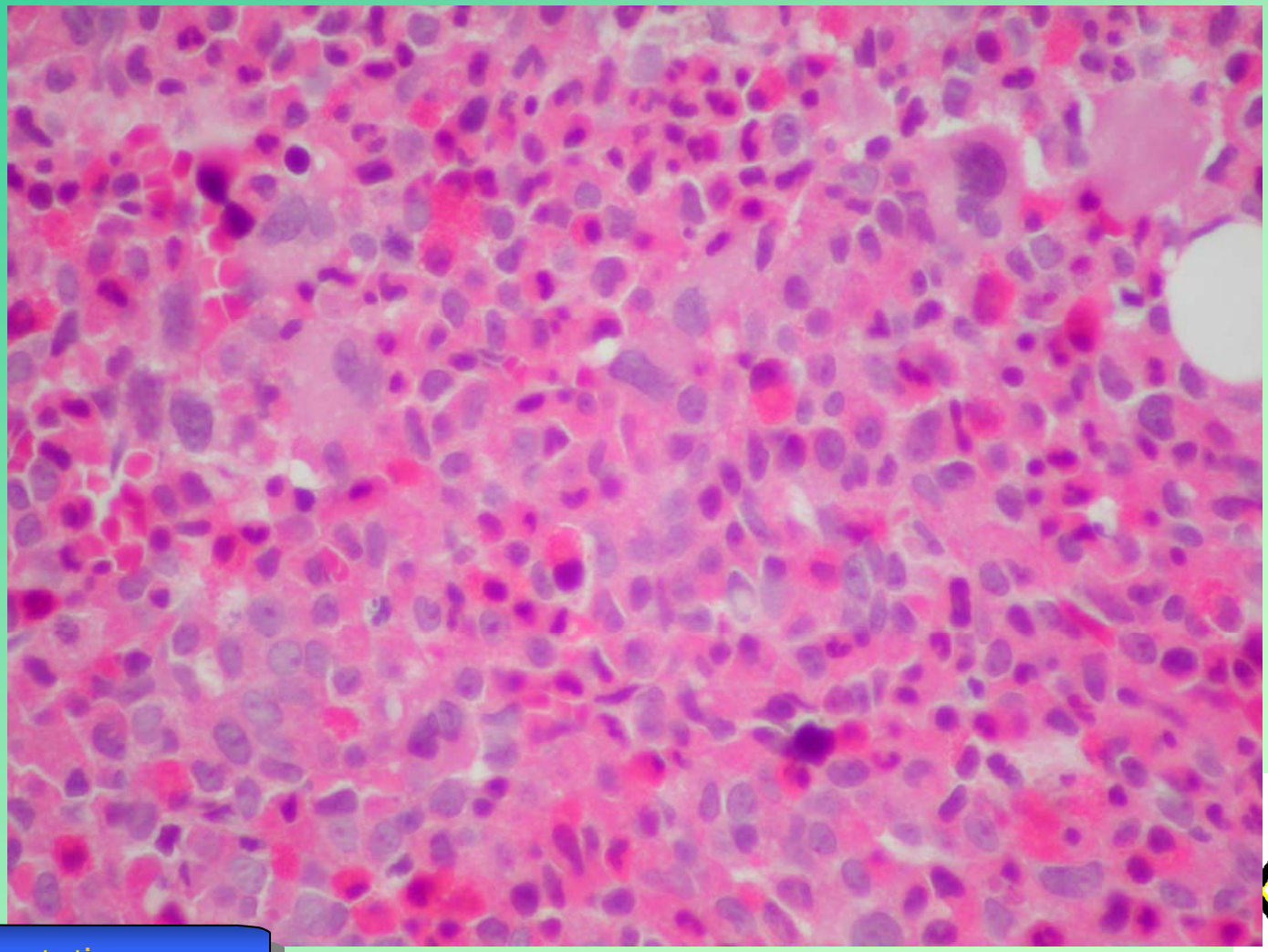
Bone-Marrow Biopsy: low power



Bone-Marrow Biopsy: higher power



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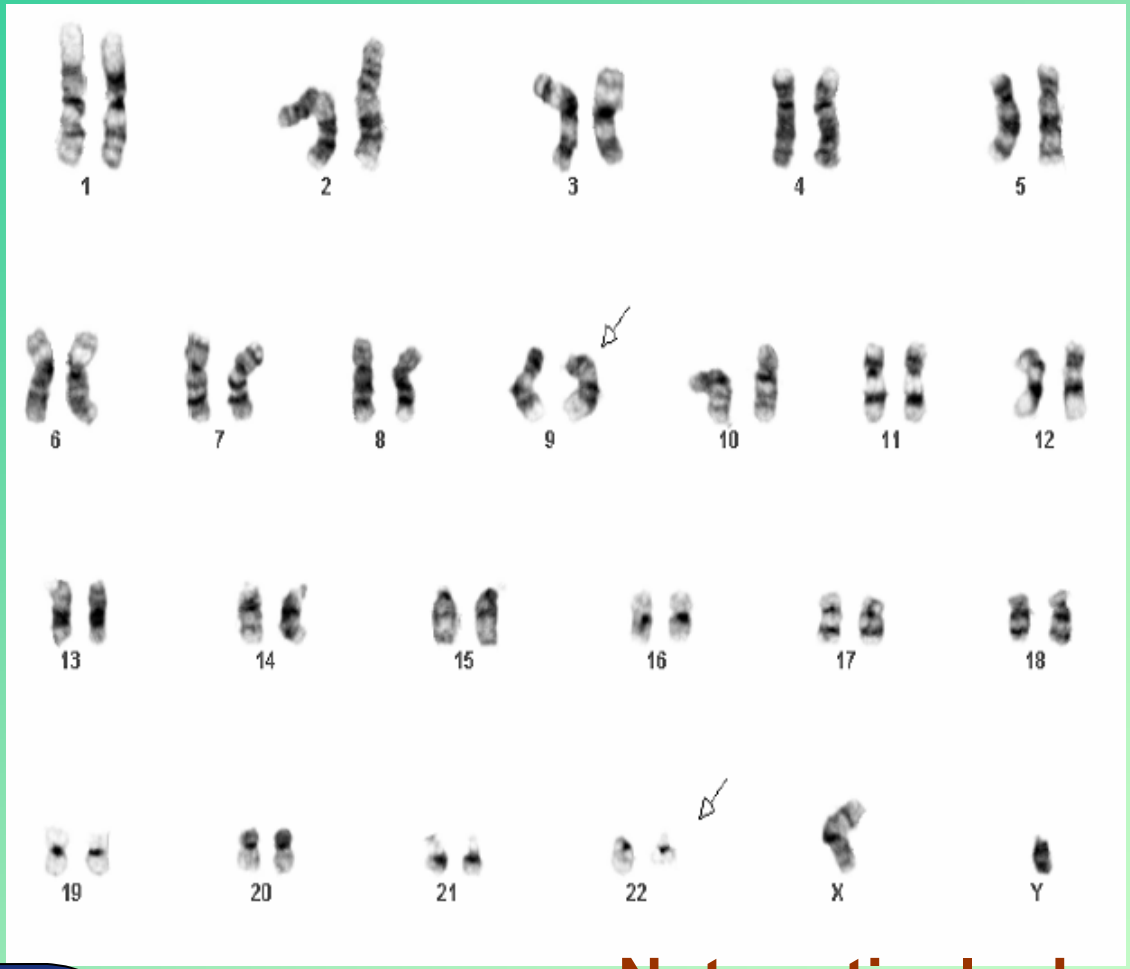
Case Presentation

Cytogenetics of Bone Marrow



Chromosomes 9 and 22 have swapped genetic material: the Philadelphia chromosome translocation

Not routinely done…

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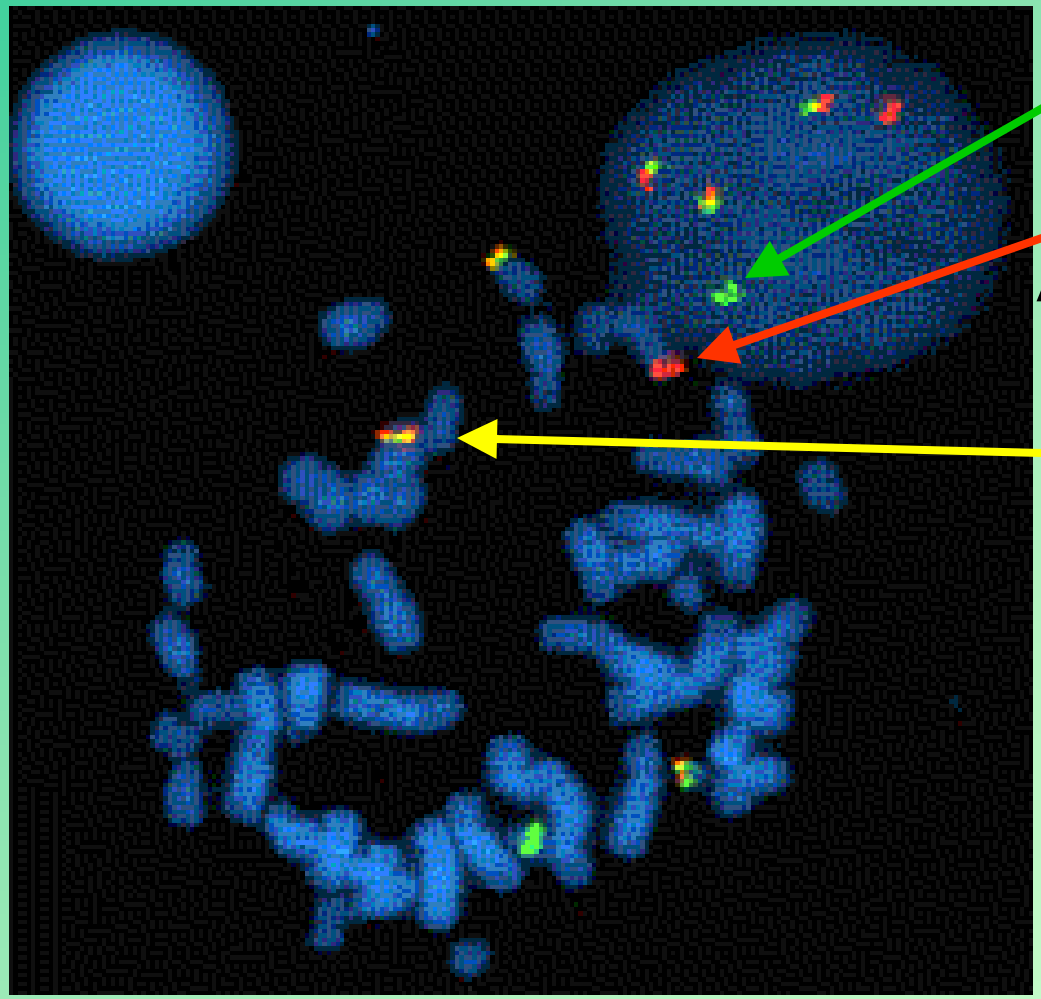
FISH

- 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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Case, continued

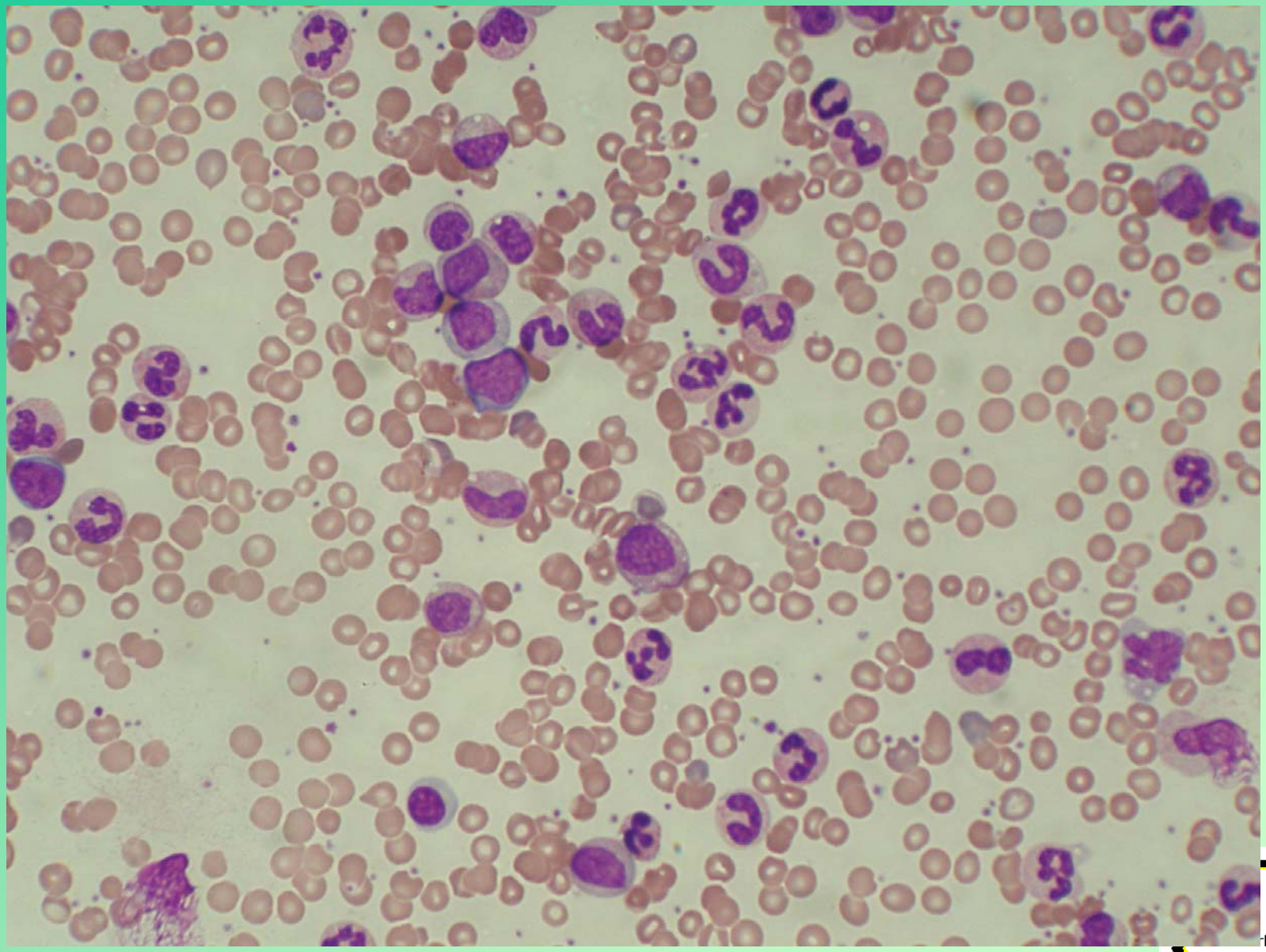
- Started on Imatinib (Gleevec®) upon receipt of chromosome report
- Has since felt better with loss of night sweats
- Blood counts normalized quickly and have remained normal
- Imatinib toxicity (ankle and periorbital edema) mild and well tolerated

Case #2

- 66 y.o. lady with leukocytosis
- Prior history remarkable:
  - Stage II lung cancer in 1990 treated with radiation and chemotherapy without recurrence
  - Non-Hodgkin's lymphoma presenting as an abdominal mass in 1994 treated with "CHOP" chemotherapy with complete and permanent disappearance of disease
  - Second lung cancer in 2001 (different location from the first) treated with surgical removal without recurrence
  - Complex pelvic mass discovered in 2002; operated upon: ovarian cyst

Case #2, continued

- In June, 2004 lost 8 lb and developed a palpable 2 FB spleen
  - WBC 224,000 with smattering of immature forms;
  - Peripheral smear…



Case #2

Case #2, continued

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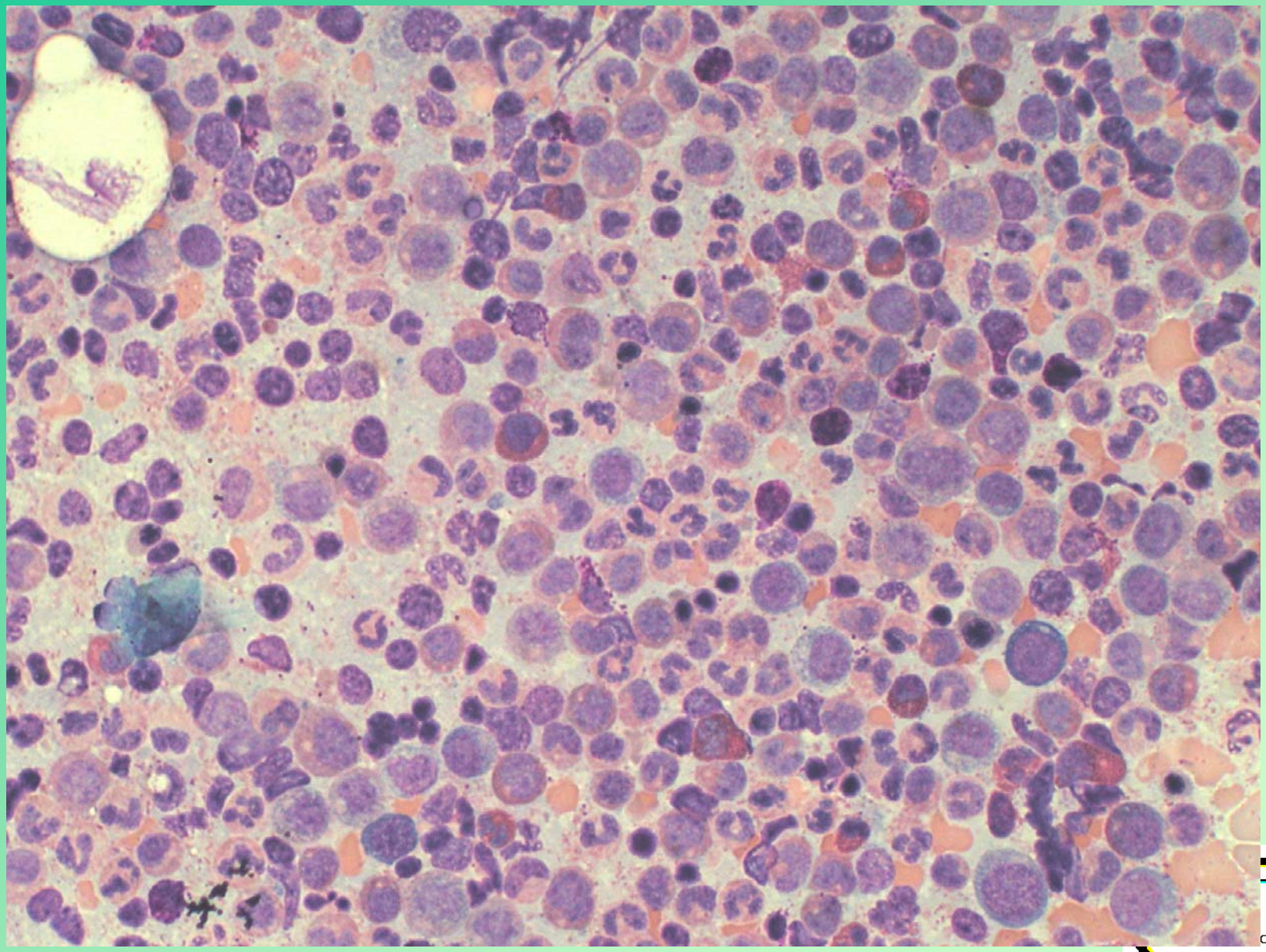

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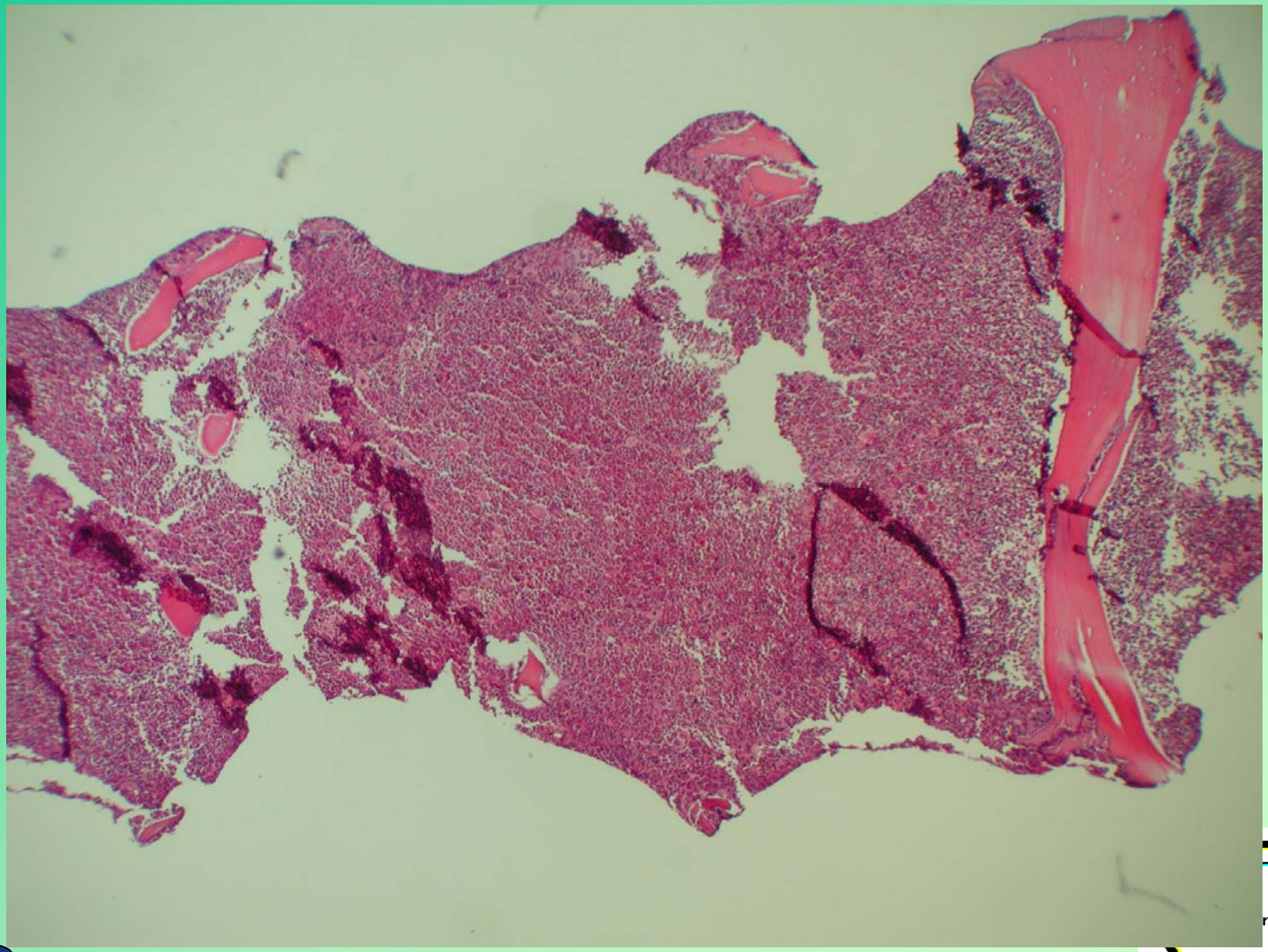
Case #2

Case #2, continued

- In June, 2004 lost 8 lb and developed a palpable 2 FB spleen
  - WBC 224,000 with smattering of immature forms;
  - Peripheral smear…
  - Bone marrow aspiration….
  - Bone marrow biopsy…



Case #2

Case # 2, continued

- Cytogenetics + for 9/22 translocation
- Started on Imatinib 400 mg/day with complete disappearance of disease. Had trouble getting drug because of cost but received most of planned therapy

Case # 2, continued

- In July, 2005 developed tenesmus and rectal bleeding and was found to have carcinoma of the rectum
  - 1/6 positive lymph nodes; post-operative CEA never fell lower than 10
- Decision made not to try to give simultaneous Imatinib and chemotherapy for what was thought to likely be early Stage IV colo-rectal cancer
- CEA rose rapidly and by June, 2006 was found to have overt liver metastases

Case # 2, continued

- Readmitted to Maryview Medical Center in early July, 2006 with fever, rapidly rising WBC (90,000) and platelet count >2,000,000 despite allegedly continuing to take Imatinib
- Cytogenetics obtained on peripheral blood…

Case #2 -- Cytogenetics

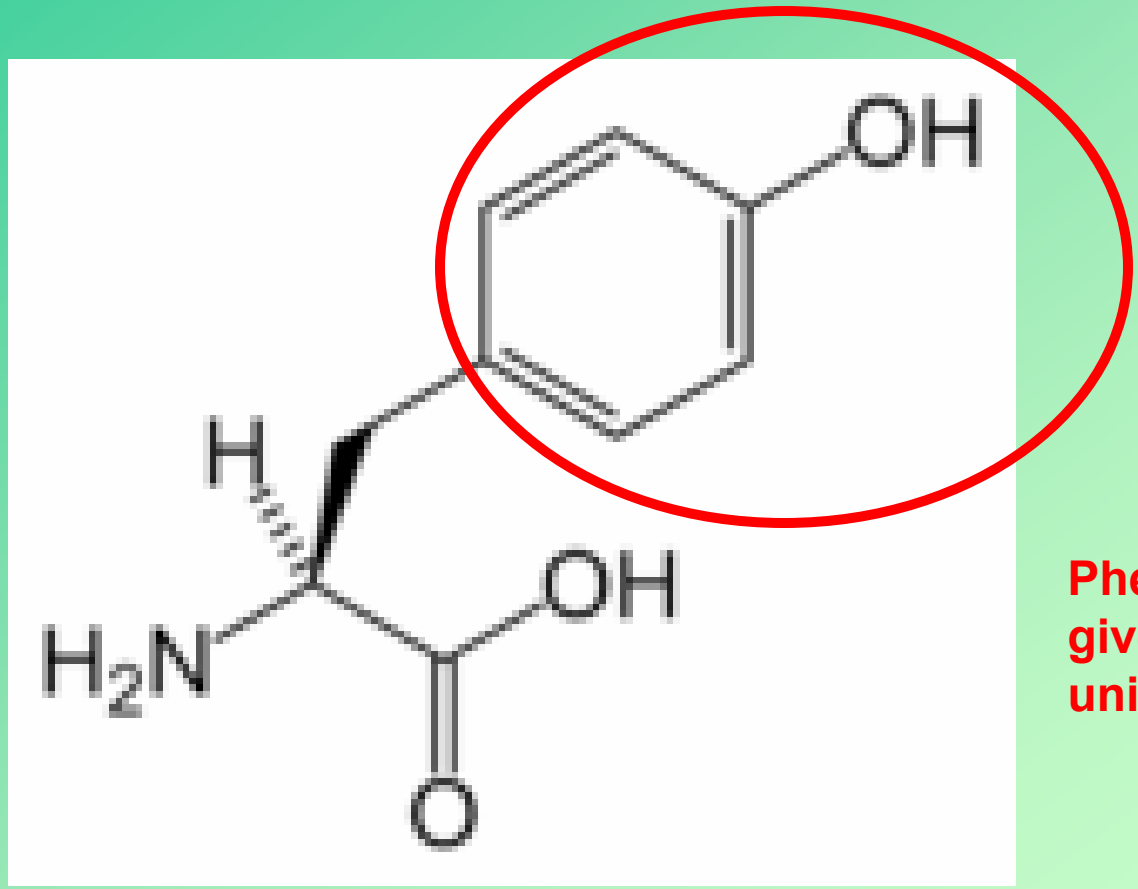
- 18/20 cells in mitosis contained 9/22 translocation
- 4 of those 18 cells contained a second translocation: 7/11 indicating clonal evolution

Case # 2, continued

- Dose of Imatinib increased to 800 mg/day
- Poor response to increased dose; modest change in counts; much toxicity
- Dasatinib started

What does any of this have to do with Tyrosine Kinase??

The Amino Acid Tyrosine



Phenol group gives tyrosine unique structure

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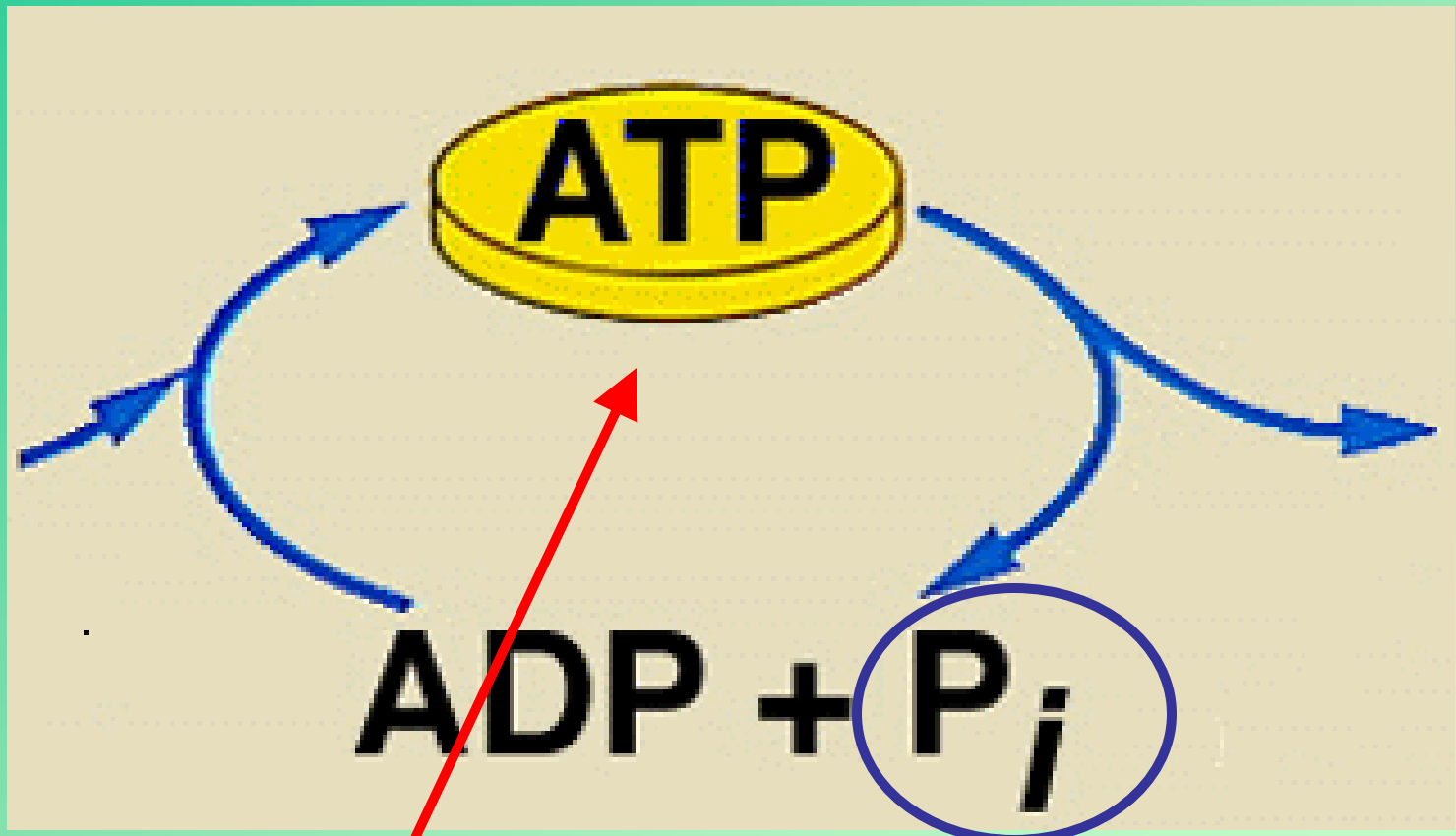
The ATP-ADP cycle



Source of high-energy phosphate

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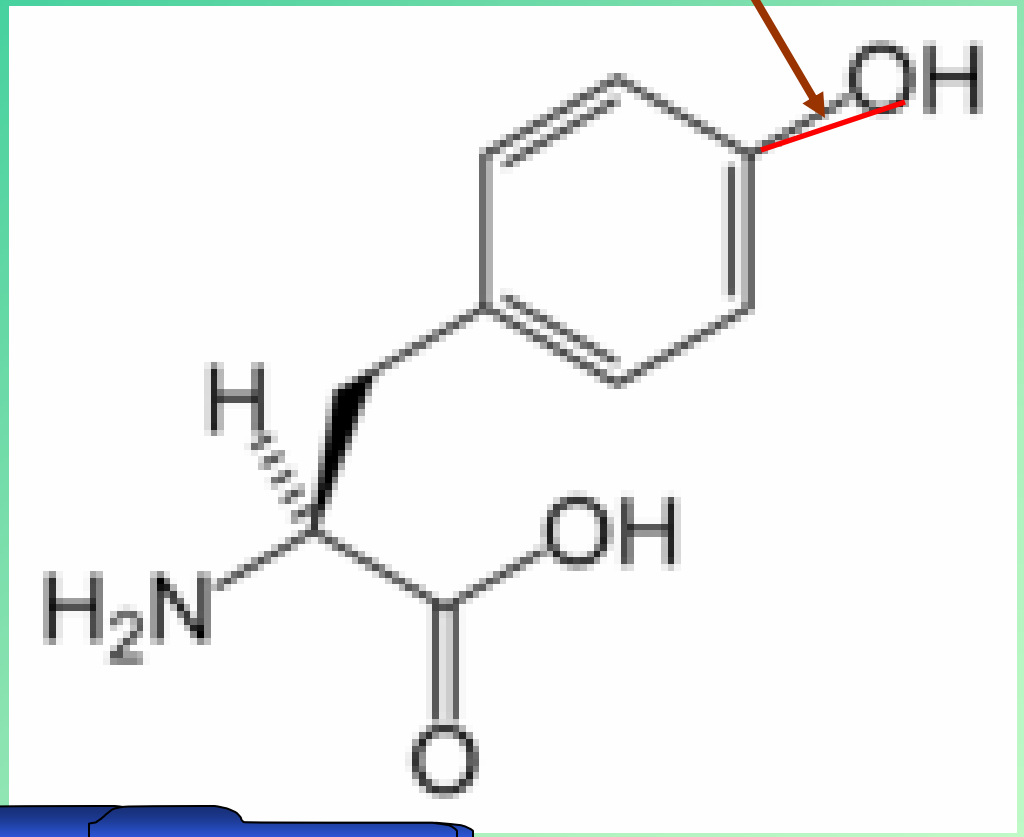
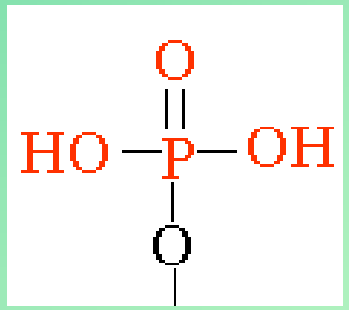
The Phosphorylation of Tyrosine

Action of Tyrosine Kinase





High-energy Phosphate

Phosphorylated Tyrosine

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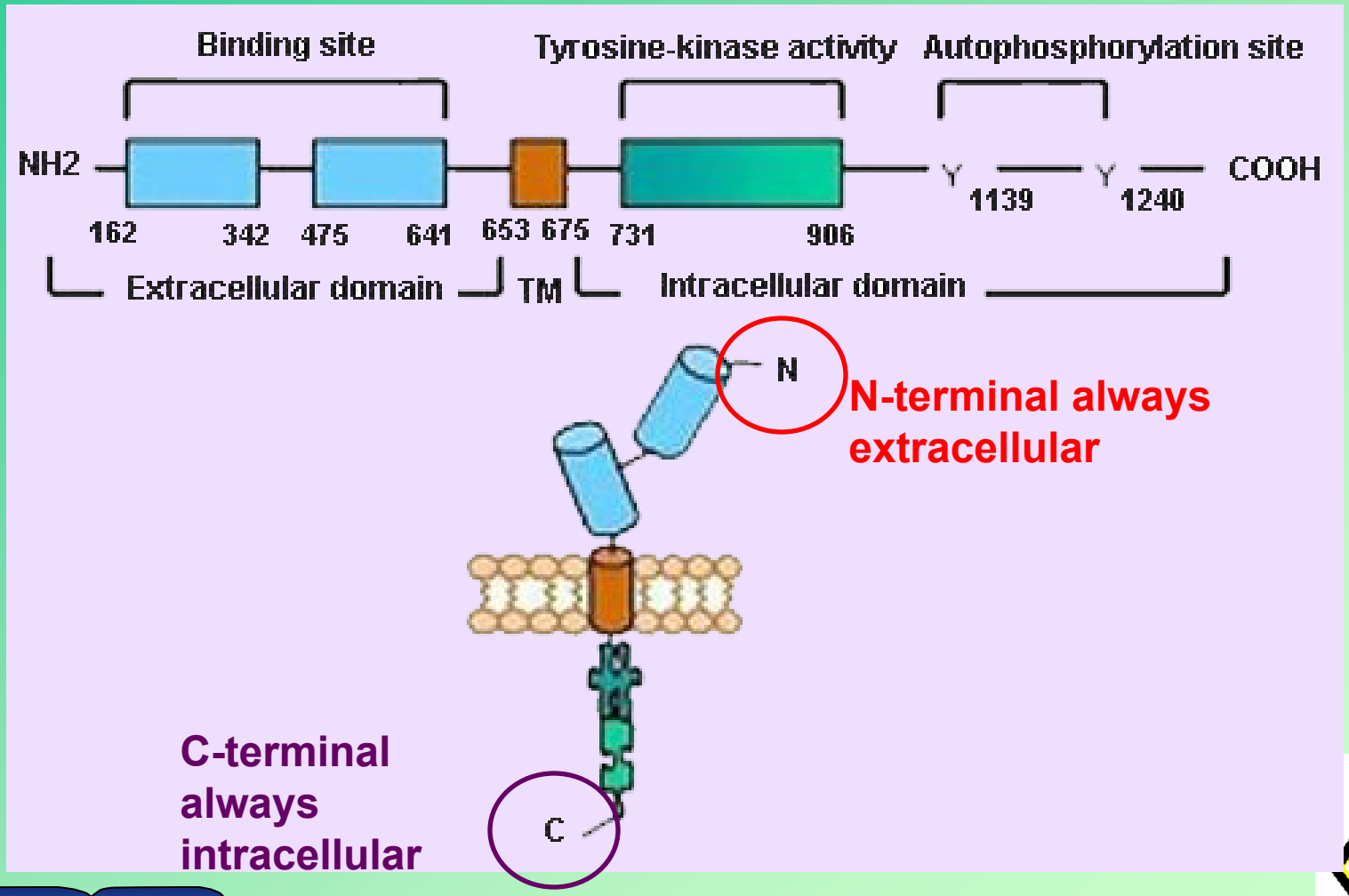
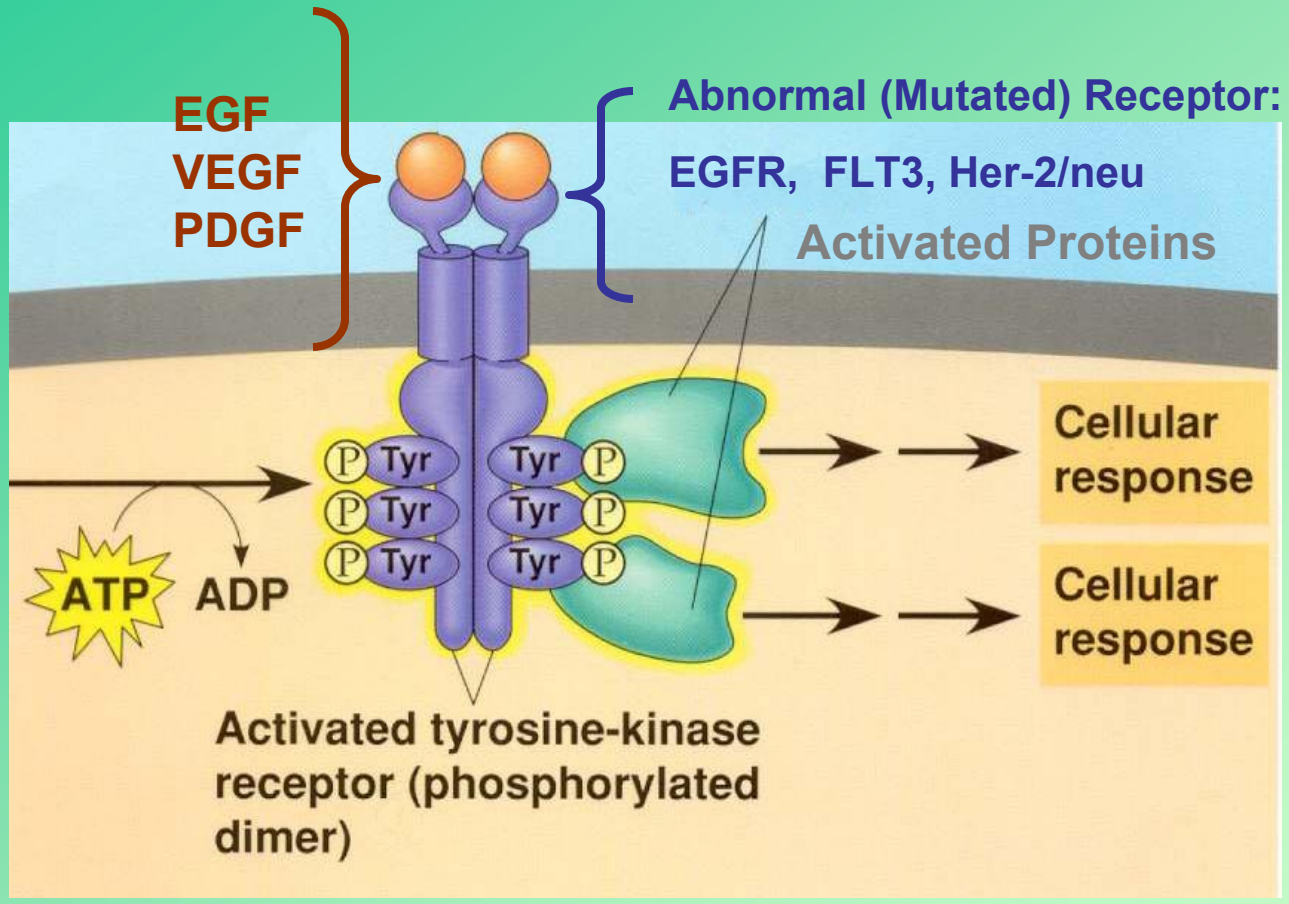
What's the relevance of tyrosine phosphorylation??

- Important signal in higher organisms: signal transduction
  - Membrane phosphorylation leads to transmembrane signaling
  - Intracellular phosphorylation leads to signal transduction within the cell, especially within the nucleus
- The family of tyrosine kinases control all of this; phosphorylation mediates signals

Classic Transmembrane TK



Activation of TK in disease

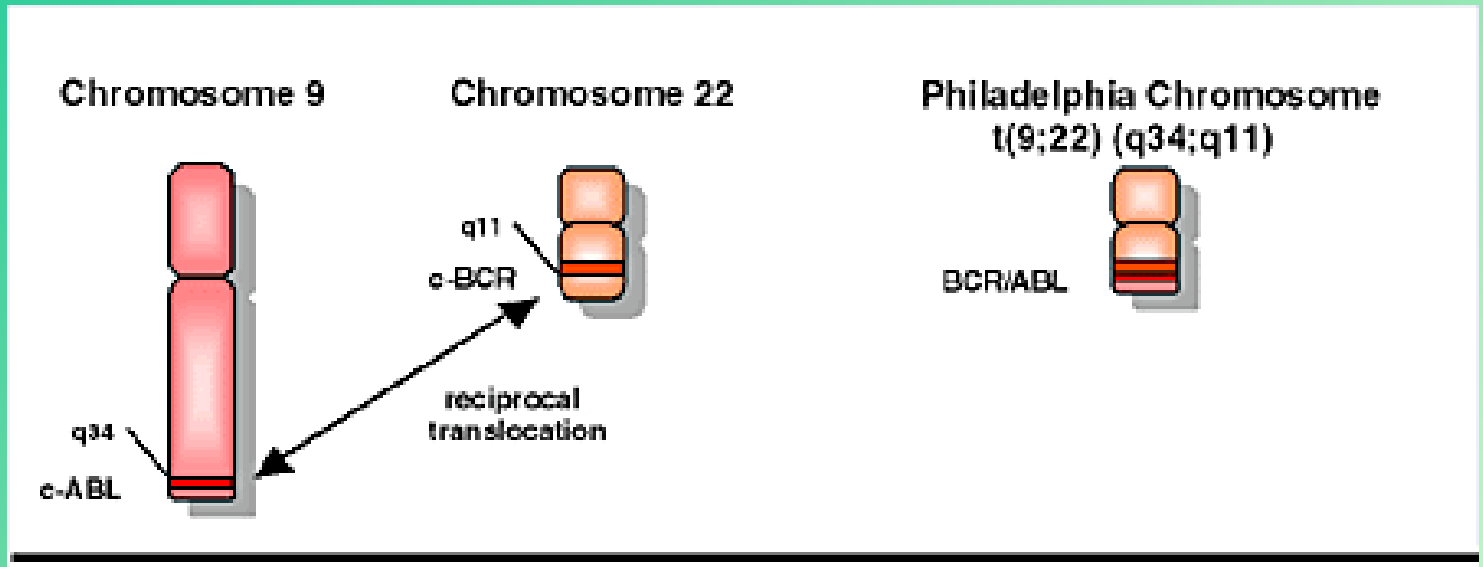


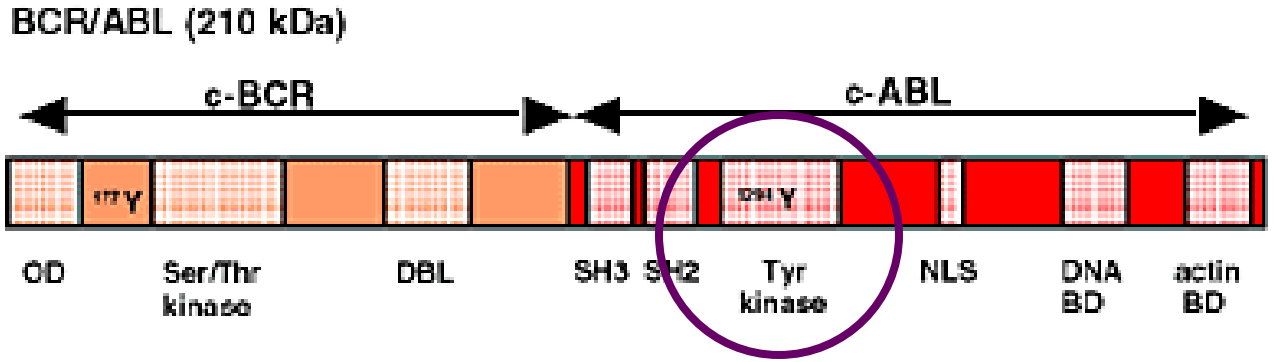
Final common path is abnormal phosphory-lation of tyrosine

Back to our patients…

- 9-22 translocation encodes for novel TK as a result of the novel DNA sequence produced by the swap of genetic material…

Schematic of BCR-ABL





Tyrosine Kinase one of a number of gene products of BCR-ABL

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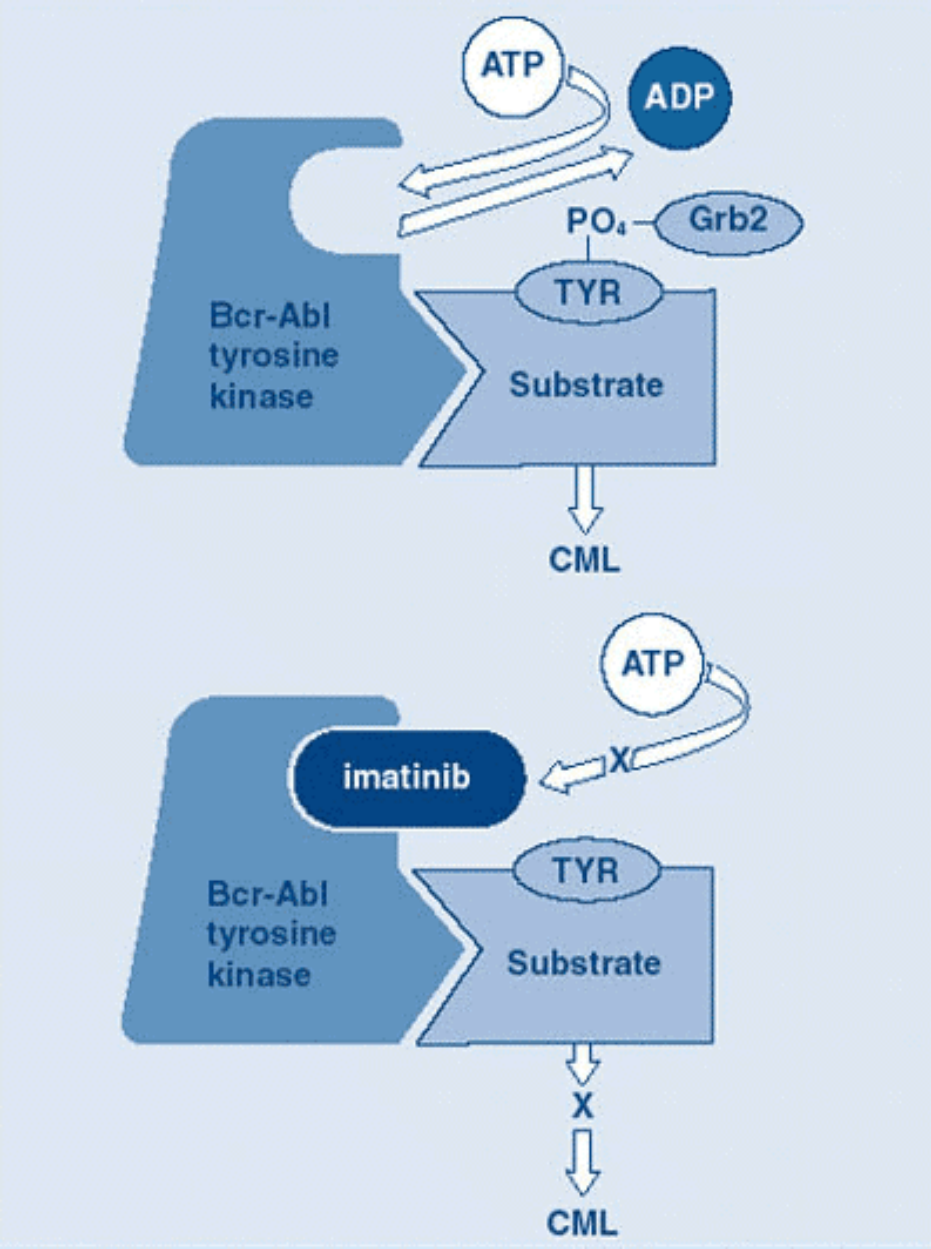
Back to our patients…

- 9-22 translocation encodes for novel TK
- Usual feedback control of phosphorylation is interrupted in this novel, slightly abnormal TK
- Results in uncontrolled phosphorylation of tyrosine and a proliferative advantage of cell lines which have the mutation

How Imatinib Works



Biochemistry

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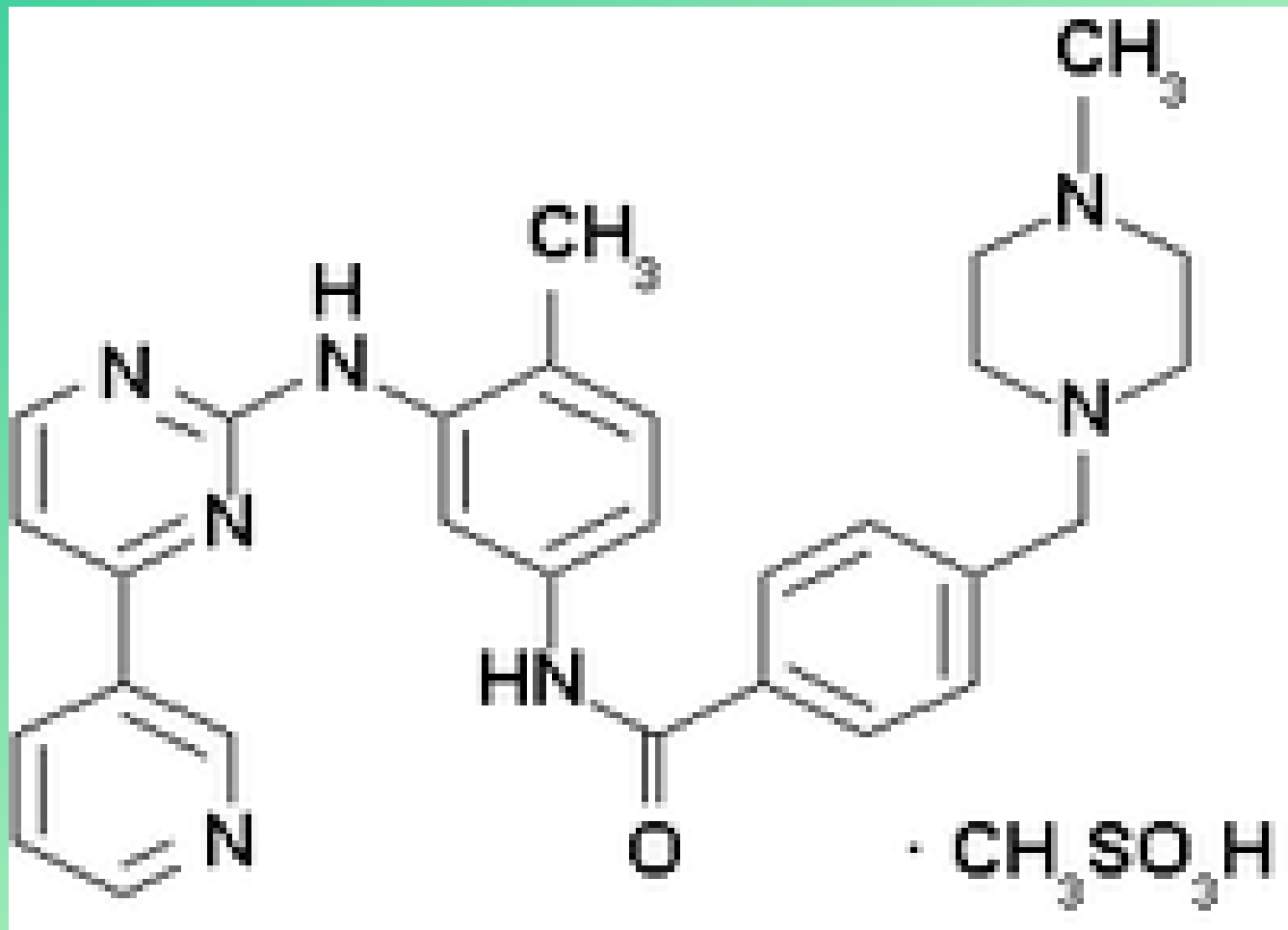
Chemical Structure of Imatinib



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Biochemistry

What happens when Imatinib stops working?

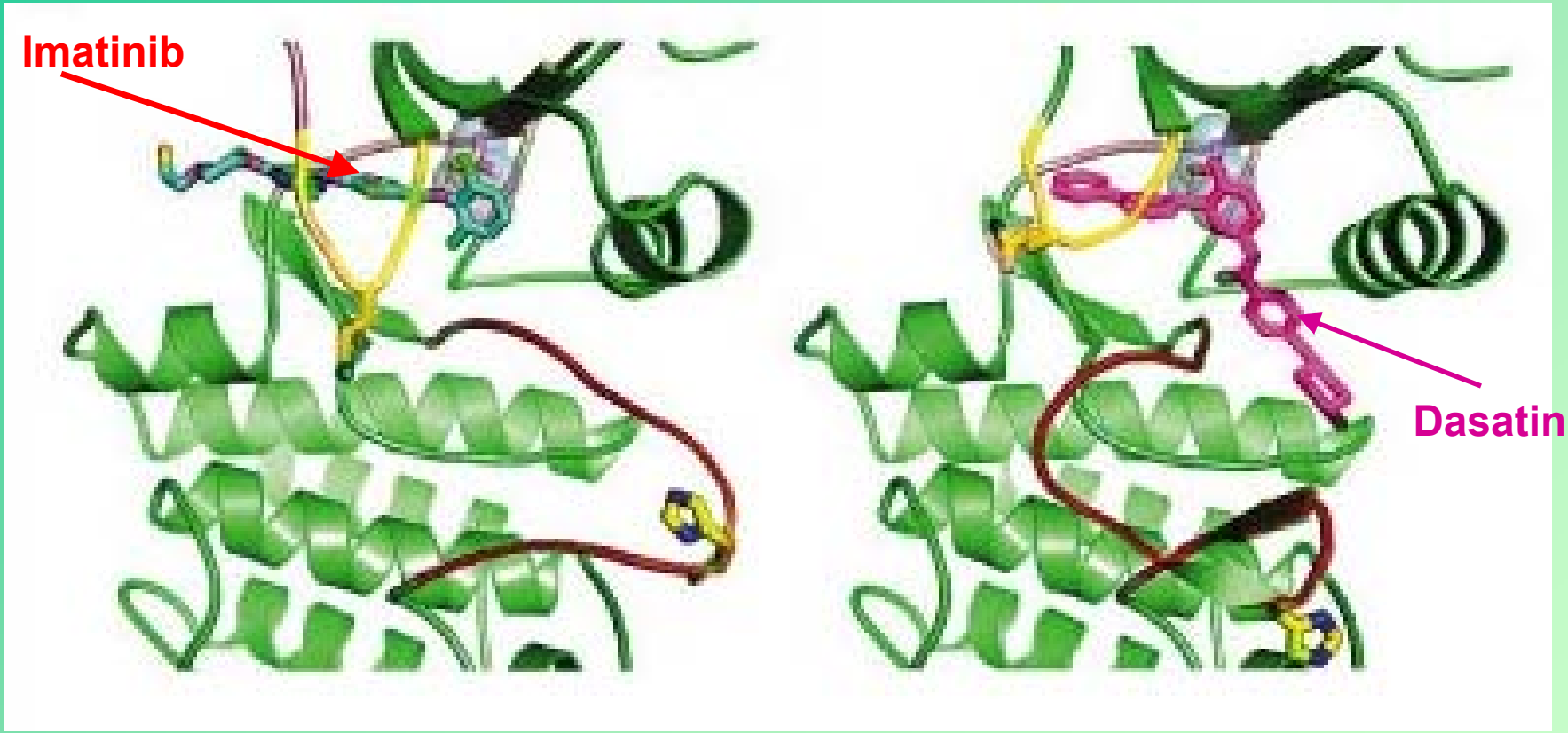
- Clinically patients stop responding
- Is associated with additional point mutations in the novel BCR-ABL gene sequence
- Theory of biochemical basis includes two possibilities:
  - Mutation of tyrosine kinase so that Imatinib no longer fits neatly in the groove
  - Overproduction of TK to overwhelm the drug
- Thus far relatively few patients have become Imatinib resistant but investigators fear that the clock is running
- Scientists at big pharma have been working to overcome this problem

“Sons of Gleevec”: Dasatinib and Nilotinib

- Two drugs have come on the scene which work after Imatinib resistance has become established
  - Dasatinib
  - Nilotinib

- Both featured recently in issue of NEJM devoted to Imatinib resistance (June 15, 2006)

Imatinib and Dasatinib intertwined with TK



From: Talpaz, M. et al. N Engl J Med 2006;354:2531-2541

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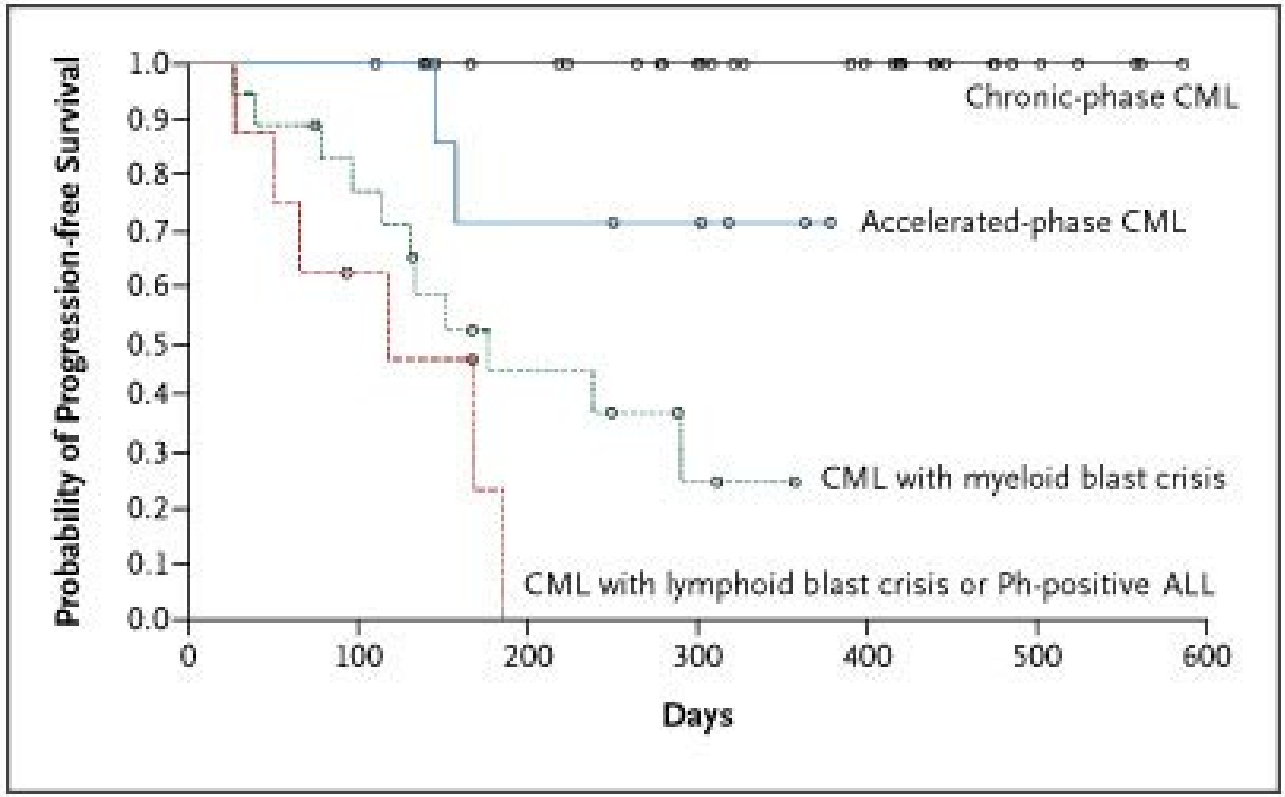
Kaplan-Meier Analysis of Progression-free Survival among Patients with CML or Ph+ALL Treated with Dasatinib most of whom were Imatinib resistant



Talpaz, M. et al. N Engl J Med 2006;354:2531-2541

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Nilotinib

- Only Phase I (dose-toxicity relationship) study completed
- Activity in Imatinib-resistant CML seen in the trial (not the purpose of the study)
  - 9/33 patients in blast crisis had cytogenetic response (8 with < 35% + cells)
  - 22/46 patients with accelerated CML had cytogenetic response (20 with <35%)
  - 9/12 patients in chronic phase CML had cytogenetic response (6 with <35%)

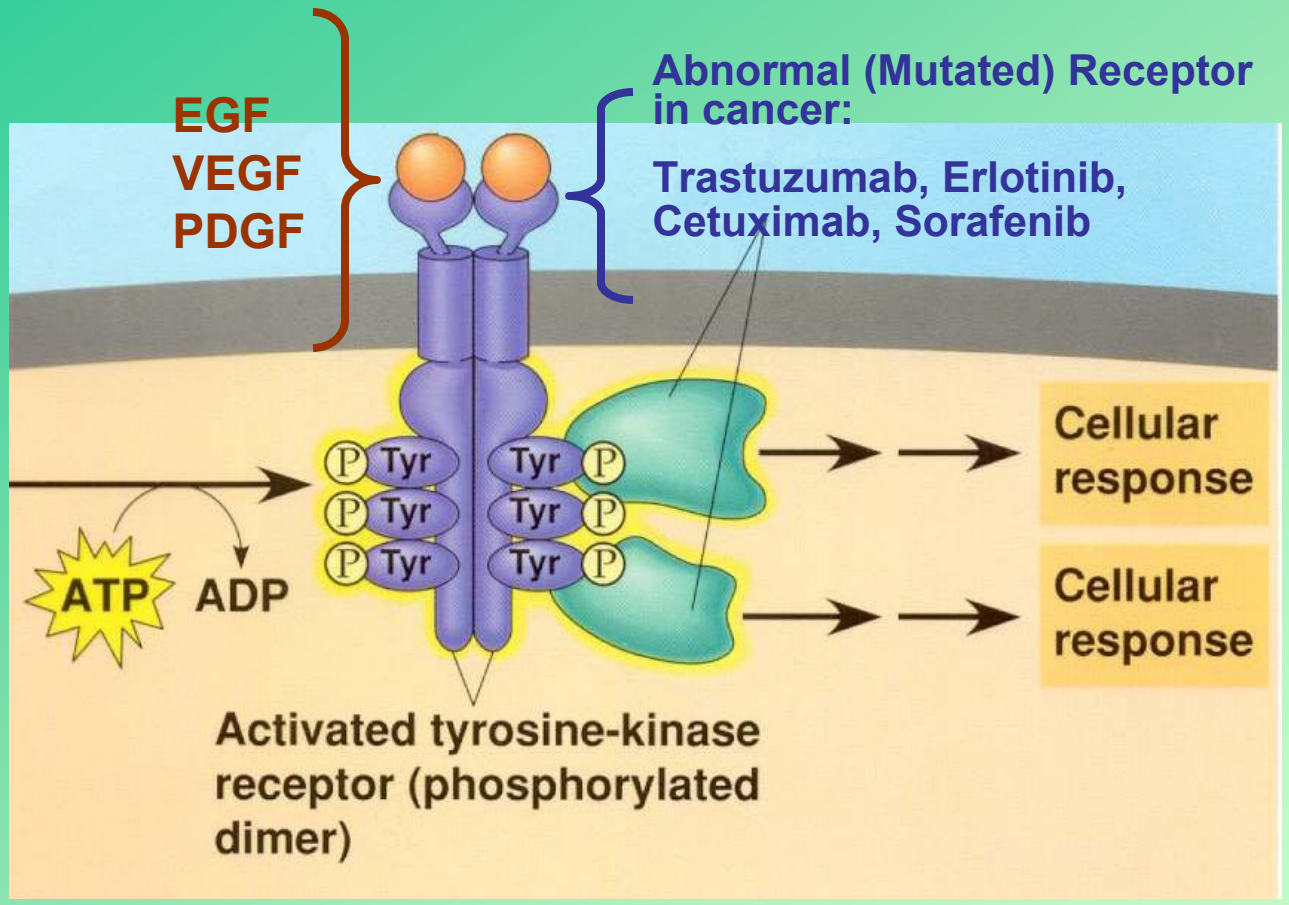
Case #2, post script

- Relation of clonal evolution (e.g., new 7/11 translocation) to Imatinib resistance and potential efficacy of second-line therapy not established
- What clonal evolution contributes to three-dimensional configuration of tyrosine kinase of interest is unclear at the moment
- Talpaz article addresses additional mutations in BCR-ABL domain but not the impact of additional downstream chromosomal translocations (did not do complete cytogenetics on their study patients)

Other tyrosine kinase inhibitors in malignant disease

- Erlotinib (Tarceva®) for non-small-cell lung cancer and pancreatic cancer
- Trastuzumab (Herceptin®) for metastatic and locally advanced breast cancer
- Sorafenib and Sunitinib in metastatic renal-cell carcinoma
- Cetuximab (Erbitux®) in head-and-neck cancer
- What do this diverse group of drugs have in common???

Other TK inhibitors, cont.



All these drugs ultimately work to modulate the phosphorylation of tyrosine

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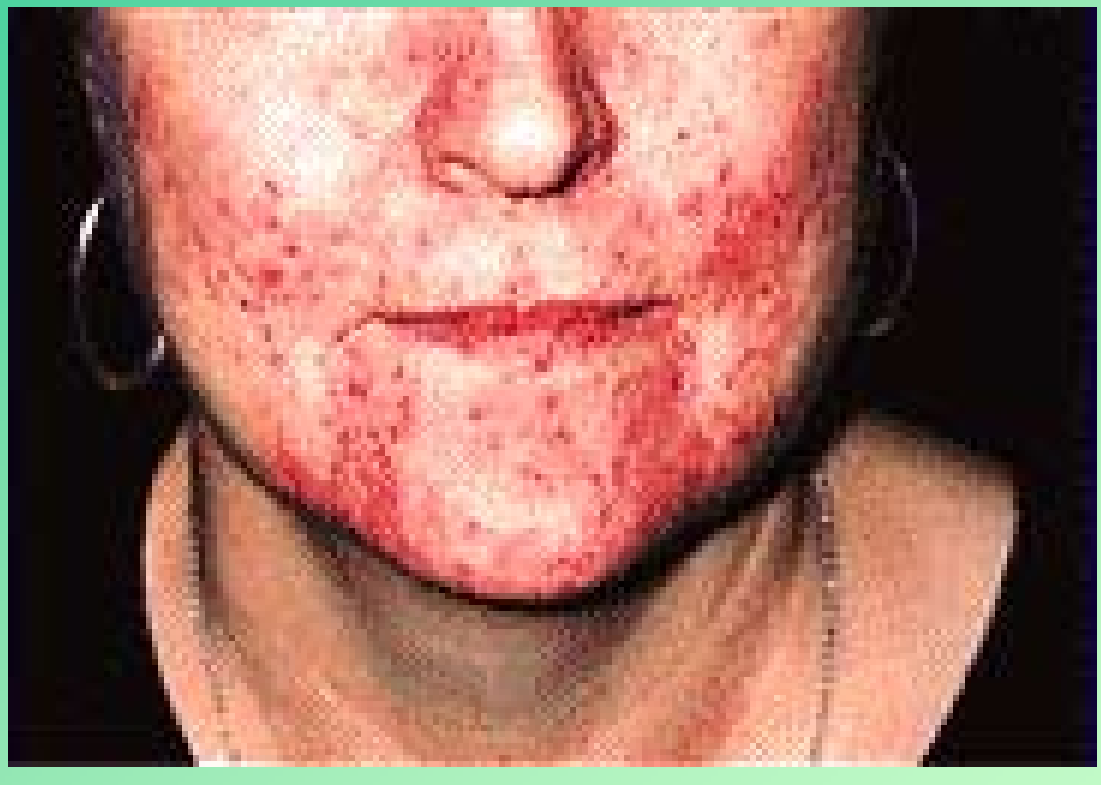
Unique toxicities of TK inhibitors

- Depend to some extent on which ligand binding to the extracellular domain is affected
  - EGF and EGFR antagonists create skin toxicity….

Tarceva Skin Rash



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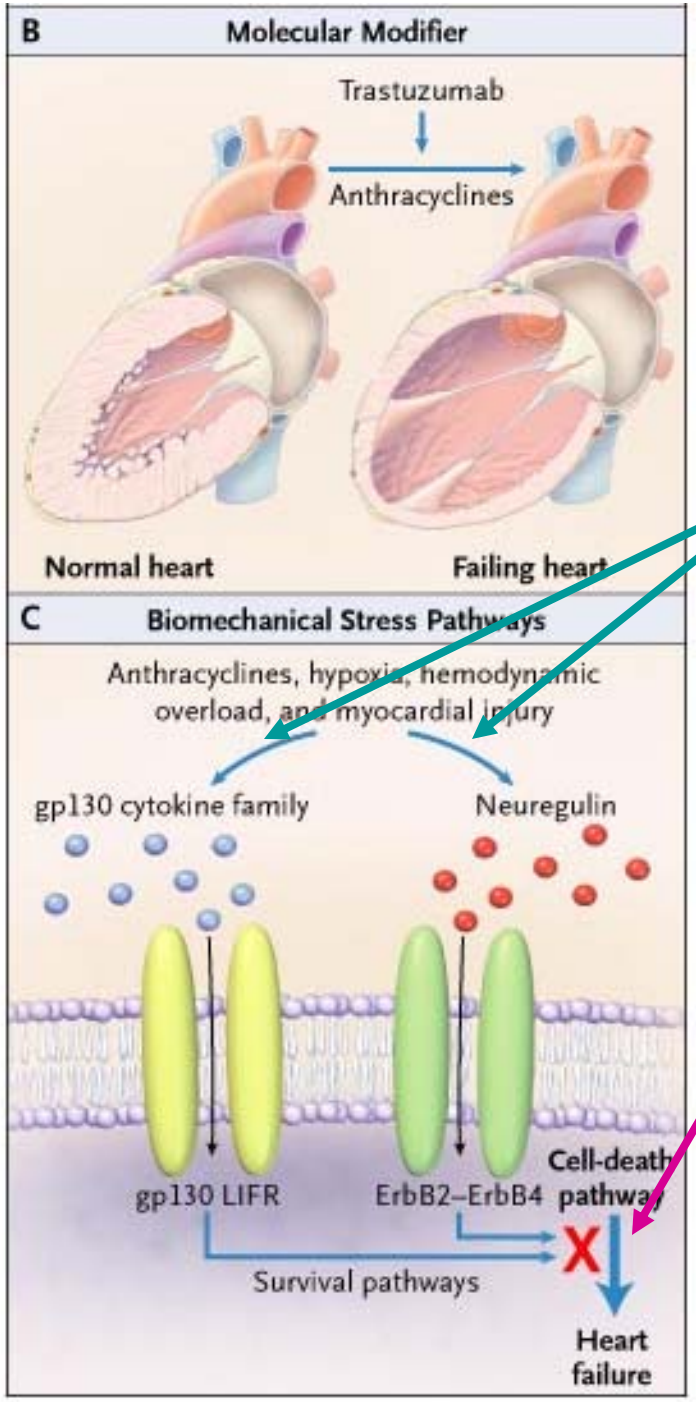
Other TK inhibitors

Unique toxicities of TK inhibitors

- Depend to some extent on which ligand binding to the extracellular domain is affected
  - EGF and EGFR antagonists create skin toxicity….
  - Trastuzumab causes cardiotoxicity probably because of Her-2 signaling in cardiac myocytes

Trastuzumab and the Heart



Myocyte injury triggers recovery process

Cardiac survival pathways blocked by Trastuzumab

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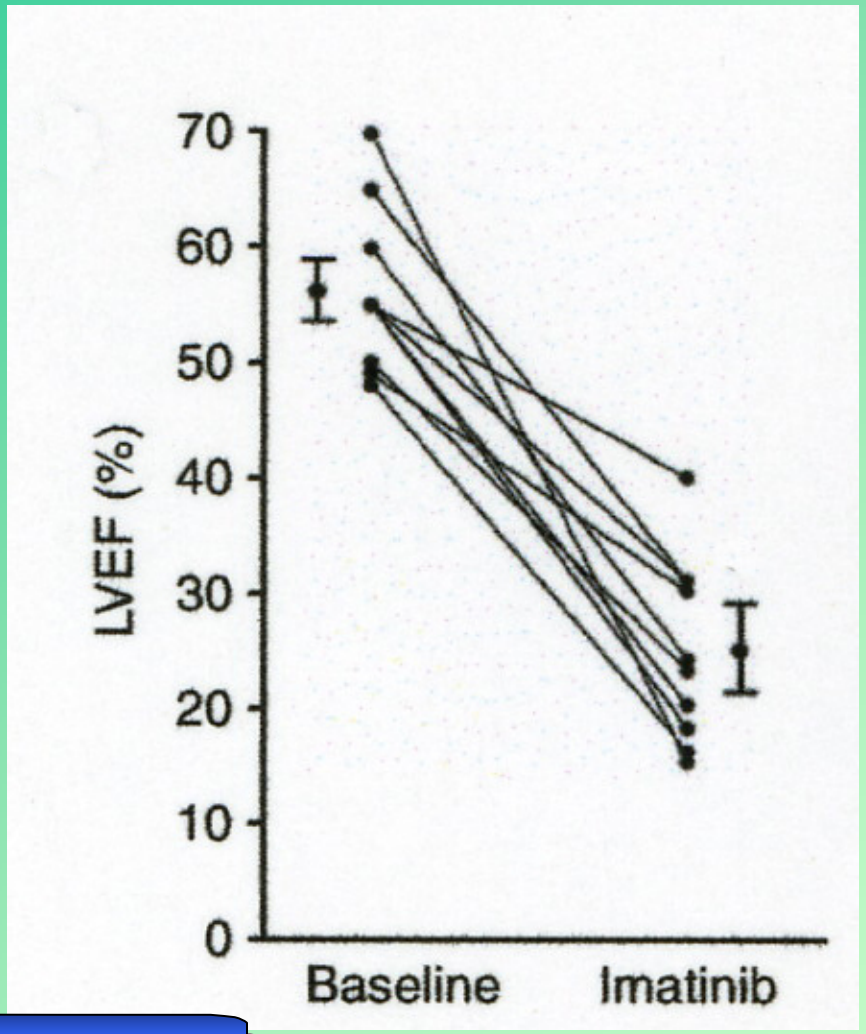
Other TK inhibitors

Imatinib and the Heart

- Recent report of ten patients on Imatinib who developed severe CHF*
- Were part of original clinical trials, so LVEF was measured pre-treatment…
- All patients had pre-existing health issues which predicted for eventual development of CHF (e.g., hypertension, CAD)

***Nature Medicine* online edition 7/23/06**

LVEF pre- and post-treatment in patients developing CHF



Despite normal pretreatment EF's all patients had pre-existing risk factors for development of CHF

Denominator not stated but very large (100's)

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Imatinib and the heart

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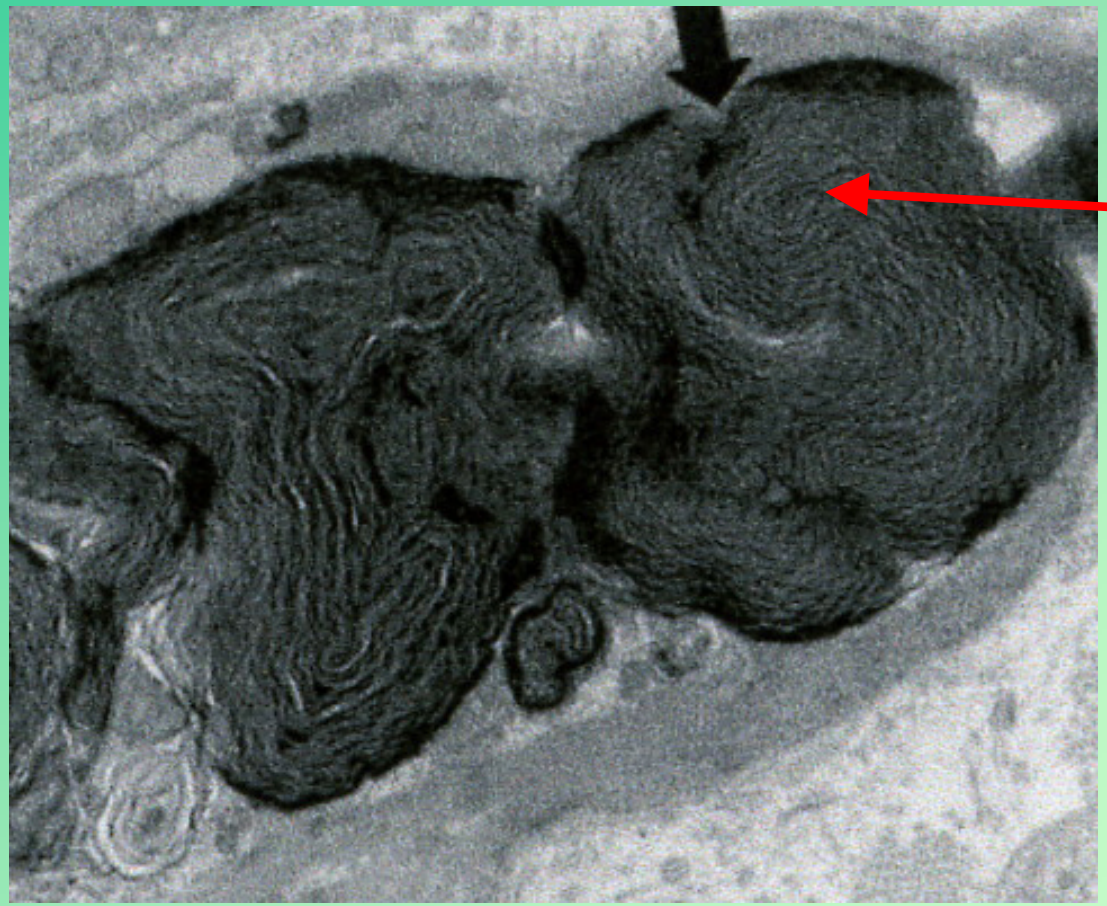
Details of Patient Characteristics*

Patients	1	2	3	4	5	6	7	8	9	10	Avg
Age	72	76	61	59	45	69	75	75	62	49	64.3
Gender	M	M	F	F	M	M	M	M	F	F	6M/4F
Dose	600	800	800	400	600	800	400	400	600	800	620
Duration (mos)	14	14	1	9	1	1	1.5	14	8	8	7.15
Prior CAD	Y	N	N	N	N	BG‡	N	BG	N	N	3/10
Diabetes	N	Y	N	N	N	N	Y	Y	N	Y	4/10
HBP	Y	Y	N	Y	N	Y	Y	Y	N	Y	7/10

‡ BG =bypass graft

*Unpublished data from Novartis

Electron Micrograph of Cardiac Biopsy



Dense membrane whorl in myocyte characteristic of toxin-induced cardiomyopathy

***Nature Medicine* online edition 7/23/06**

Imatinib and the Heart

- Recent report of ten patients on Imatinib who developed severe CHF*
- Were part of original clinical trials, so LVEF was measured pre-treatment…
- Mouse model created which duplicates Imatinib lesion…

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Effect of Imatinib on Mouse Hearts

	Vehicle	Imatinib 200 mg/kg (5 weeks)
FS (%)	28.7 ± 3.63	19.9 ± 0.86**
EF (%)	49.0 ± 5.00	35.8 ± 1.43**
LVEDD (mm)	3.79 ± 0.19	4.17 ± 0.24 *
LVESD (mm)	2.76 ± 0.13	3.36 ± 0.17***
LVW/BW (mg/g)	4.68 ± 0.29	3.72 ± 0.27**

FS, fractional shortening; EF, ejection fraction; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVW/BW, left ventricular mass normalized to body weight ($n = 4$–5). Data are mean ± s.d. *$P < 0.03$ versus vehicle; **$P < 0.003$ versus vehicle; ***$P = 0.0005$ versus vehicle.

***Nature Medicine* online edition 7/23/06**

Imatinib and the Heart

- Recent report of ten patients on Imatinib who developed severe CHF*
- Were part of original clinical trials, so LVEF was measured pre-treatment…
- Mouse model created which duplicates Imatinib lesion
  - Basis for studying effect of TK-inhibition on normal form and function

***Nature Medicine* online edition 7/23/06**

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Cardiotoxicity of Imatinib

- Drug so effective that small incidence of CHF is not preventing clinicians from using it
- New data results in new discussion with patients on drug

Balance in Biology

- TK inhibition may not be the only answer to modulation of uncontrolled growth (neoplasia)
- Tyrosine phosphorylation can be balanced by a series of enzymes known as Tyrosine Phosphatases
- These enzymes undo the phosphorylation brought about by TK's

Typical Protein Tyrosine Phosphatase

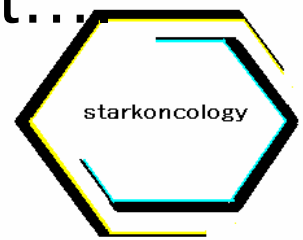
Four α helices

Four-stranded parallel β sheet

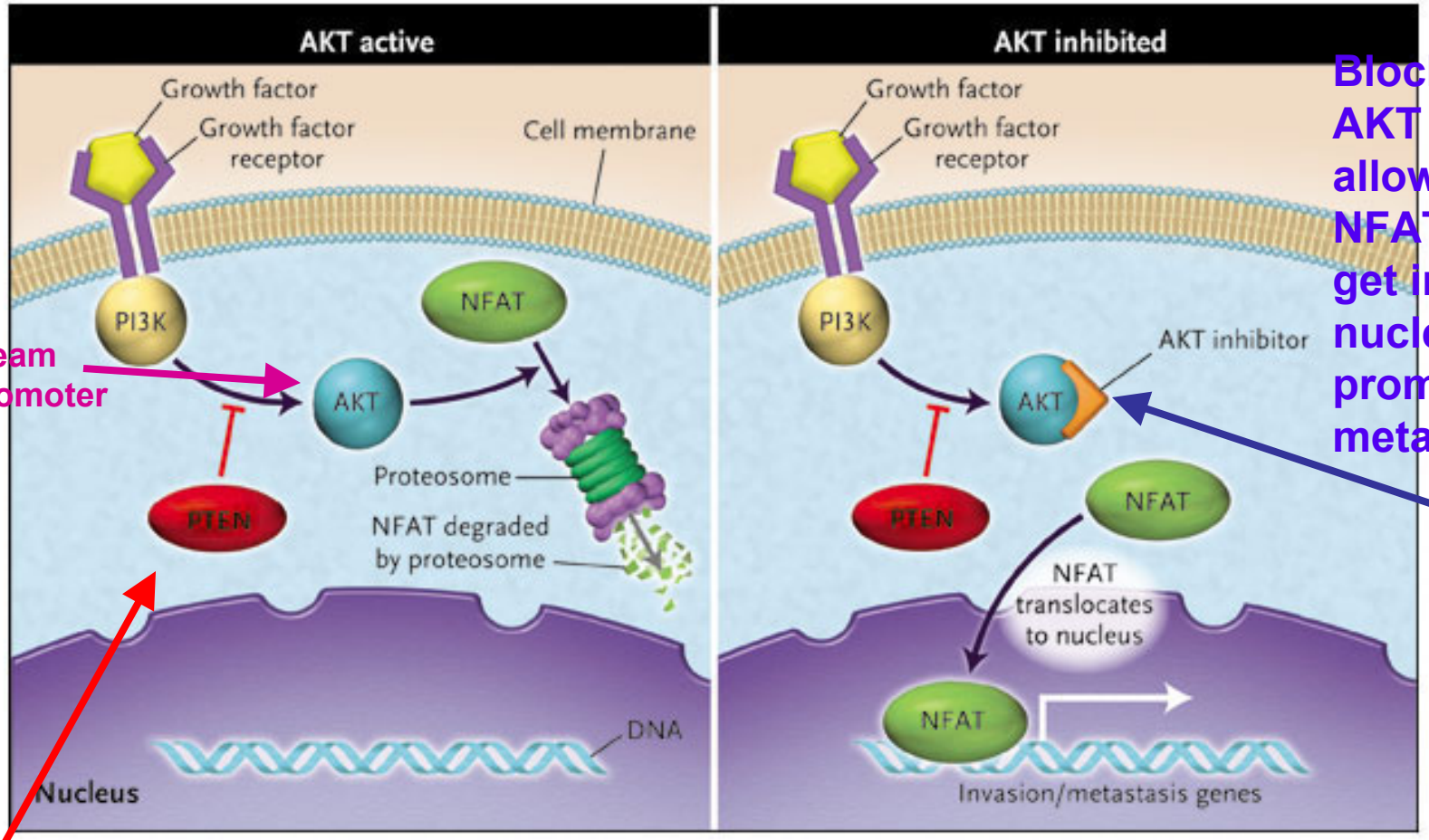
Single 3-10 helix

Protein Tyrosine Phosphatases (PTP's)

- Studied in a variety of non-malignant situations
- Perhaps best studied in the regulation of the insulin receptor
- Possible clue to the regulation of insulin resistance
- Drug development to capitalize on our knowledge of PTP's is still of only theoretical interest; no clinical development yet
- PTP leads to the latest word on this subject…



The Akt Pathway: tumor suppression gone awry



Downstream tumor promoter

Blocking AKT allows NFAT to get into nucleus, promoting metastasis

Product of PTEN, tumor suppressor gene (PTP family)

Sawyers C. N Engl J Med 2006;355:313-315

AKT pathway

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AKT inhibitors, cont.

- AKT inhibition could work to block growth of cancer
- However, increase in metastatic capability is a risk
- Clinical trials to test new AKT inhibitors will be very difficult to evaluate in light of this dichotomy of possible results!
- Stay tuned….

Tyrosine Kinases: Conclusions

- Deeper understanding of the structure and function of this key enzyme has led to better knowledge of how cells proliferate
- Huge implications for understanding and treating cancer
- Spinoffs in the understanding of molecular biology in general abound

Acknowledgements

- Dr. Mark Flemmer
- Drs. Diane Maia and Kevin Green
- Novartis for unpublished information