University of Nebraska Medical Center


# Role of Genetics in Oncology Personalized Medicine

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# History

GENERAL THERAPEUTIC DEVELOPMENT FOR GENERAL CANCER GROUPS

# More Current Trends

DESIGN THERAPEUTICS DIRECTED TOWARD SPECIFIC GENETIC TARGETS

# Comprehensive Testing Services

**Prenatal & Pregnancy Loss**

**Postnatal**

**Oncology**

Chromosome Analysis (Conventional Cytogenetics)

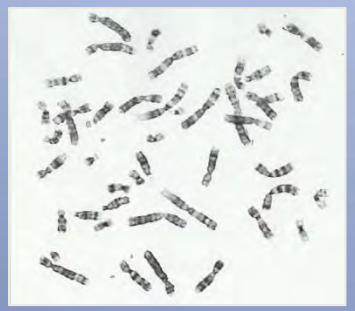
Fluorescence *In Situ H*ybridization (FISH)

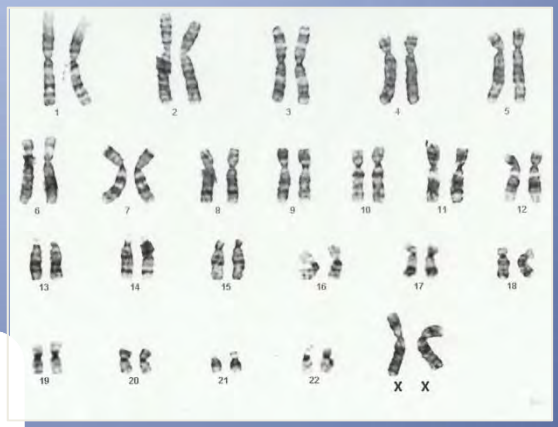
Indication-Specific Gene Panel Testing
using Next Generation Sequencing & High Resolution Del/Dup Analysis

Microarray Analysis

Targeted Deletion/Duplication Analysis

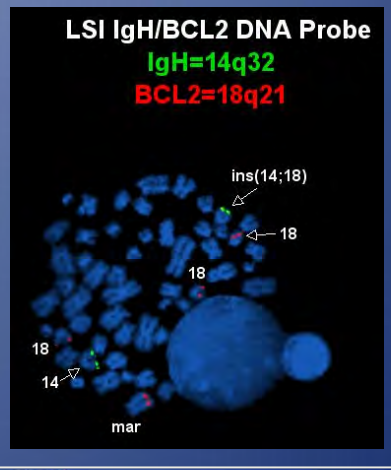
Targeted Gene Sequencing (Sanger)



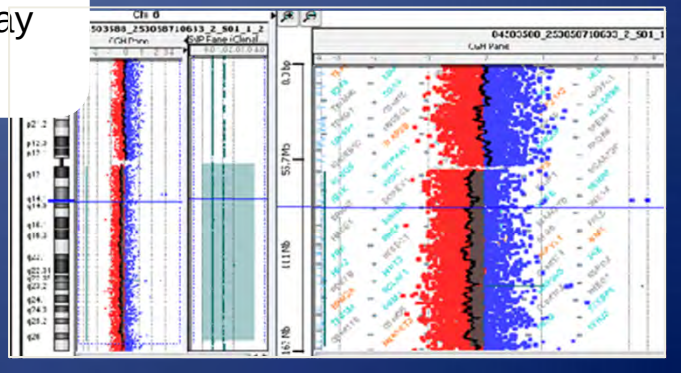


Conventional Cytogenetic Analysis (G-banding)

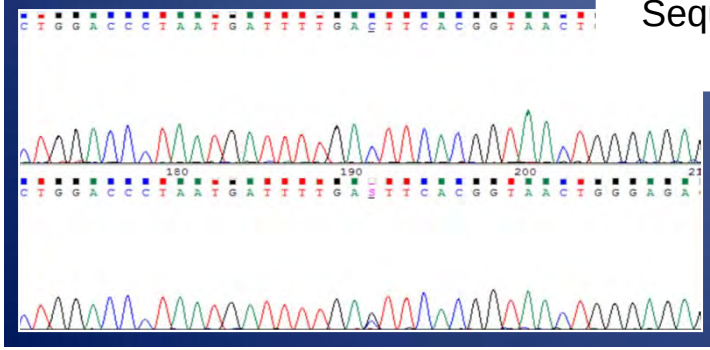
Fluorescence *in situ* hybridization (FISH)



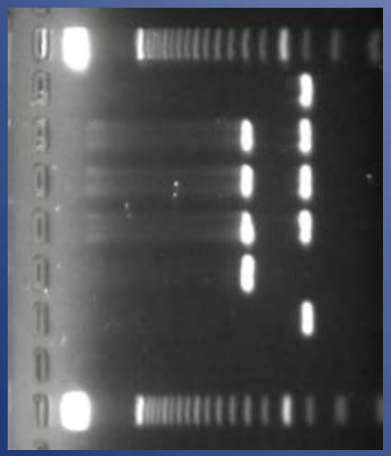

Microarray



DNA Sequencing

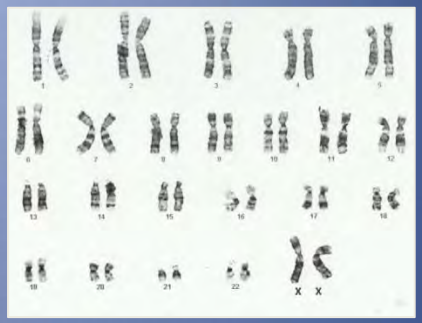


Epigenetic modifications



# Testing Approach for Cancer Specimens

# Conventional Cytogenetics



- Diagnostic specimens
  1. Global assessment of genome
  2. Identification of chromosomal aberrations and delineation of multiple cell lines (if present)
- Follow-up specimens
  1. Response to therapy
  2. Disease progression
  3. Treatment-related secondary malignancies

# Fluorescence *in situ* hybridization (FISH)

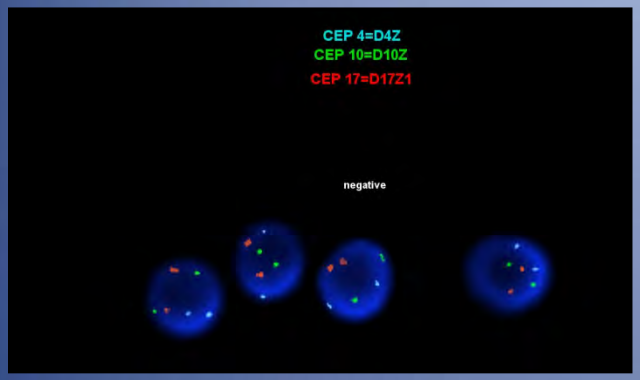
*A technique that utilizes fluorescently-labeled molecules to identify chromosomal abnormalities in dividing and non-dividing cells.*

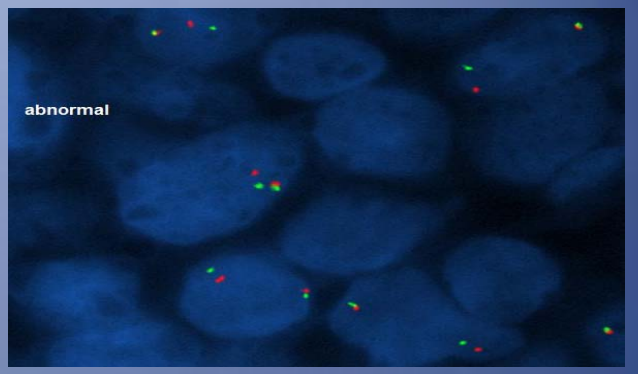
- **Targeted approach** (focused; locus specific) based on differential diagnosis
  - ✓ Information only about the locus (region) the probe is interrogating
  - ✓ Resolution of ~100-150Kb
- Detection includes **numerical** and **structural** chromosomal aberrations *of interest*

# FISH

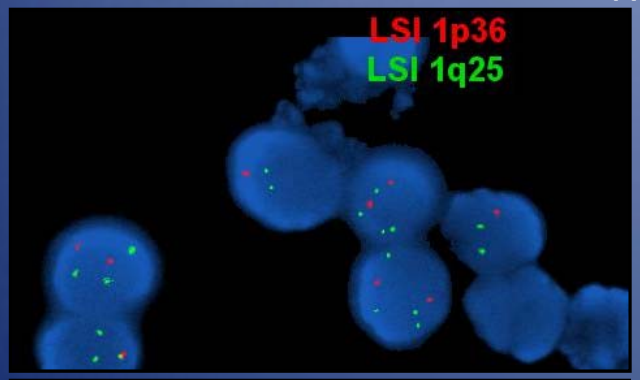
- Utility
  1. Detection of diagnostic and prognostic aberrations
  2. Monitoring response to therapy
  3. Opposite sex transplant
- Indication-specific FISH testing approach
  1. Single gene/region of interest (e.g., *ALK* in ALCL)
  2. Multiple-gene panels (e.g. hyperdiploidy, 13q, p53, *IGH*, and 6q in multiple myeloma

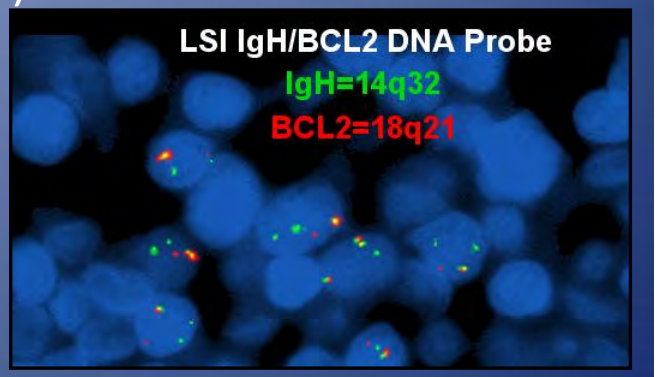
# Types of FISH Probes

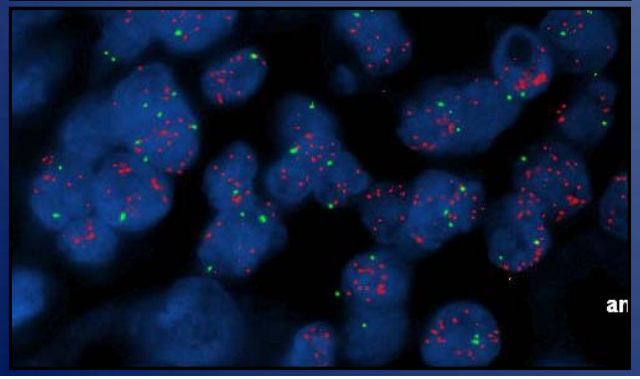


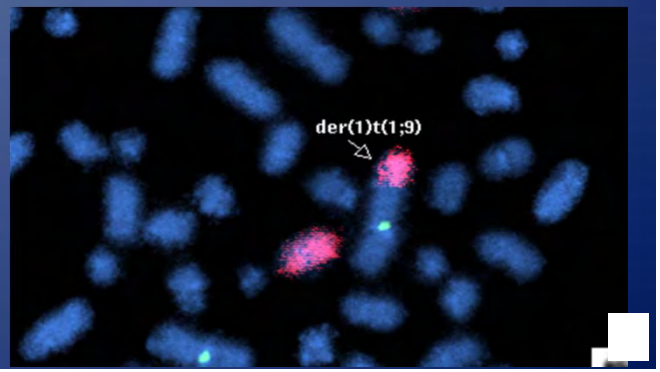



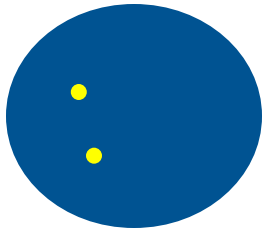
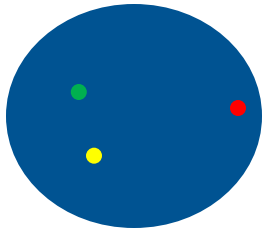
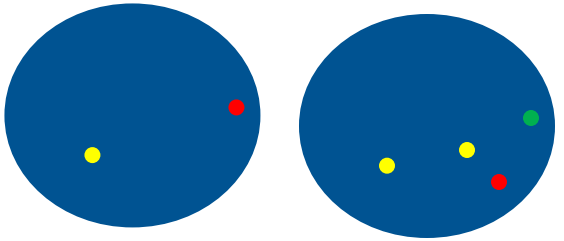
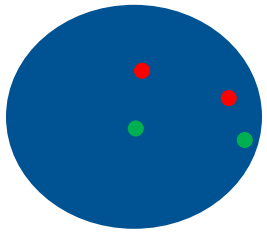
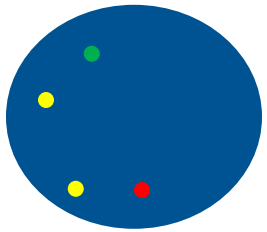
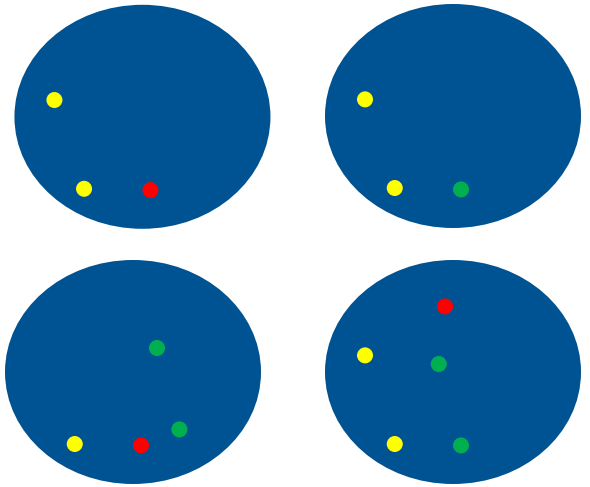

- Enumeration
- Amplification
- Rearrangement ("breakapart")
- Fusion
- Chromosome paint



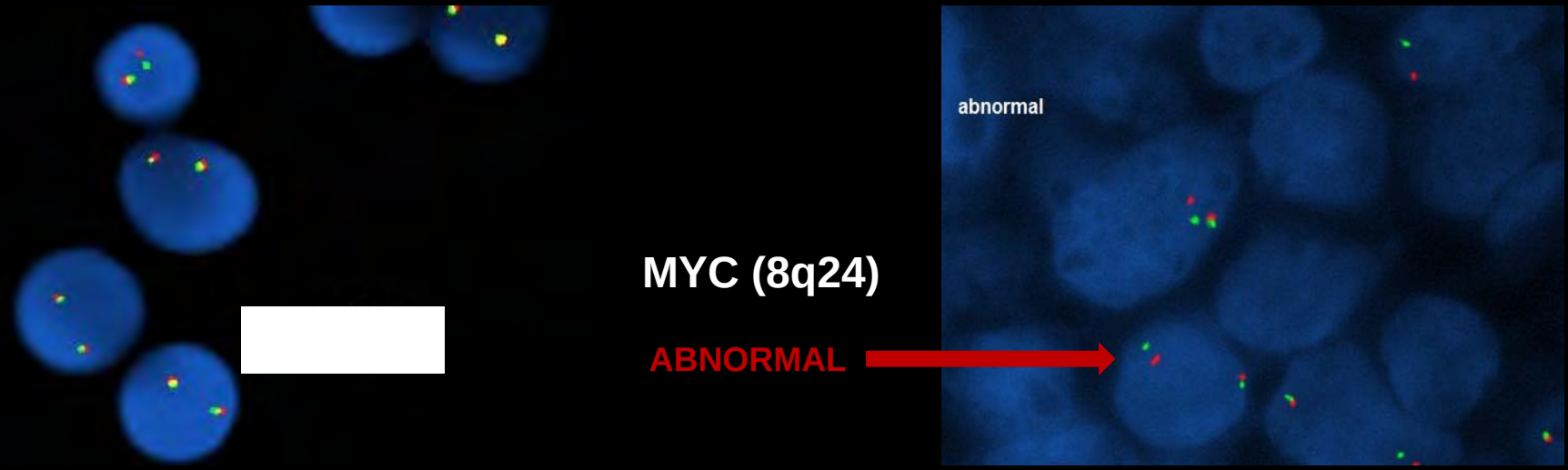





| | Normal | Typical Abnormal | Variant Abnormal |
|---|---|---|---|
| Rearrangement ("Breakapart") Probes |  |  |  |
| Fusion Probes |  |  |  |

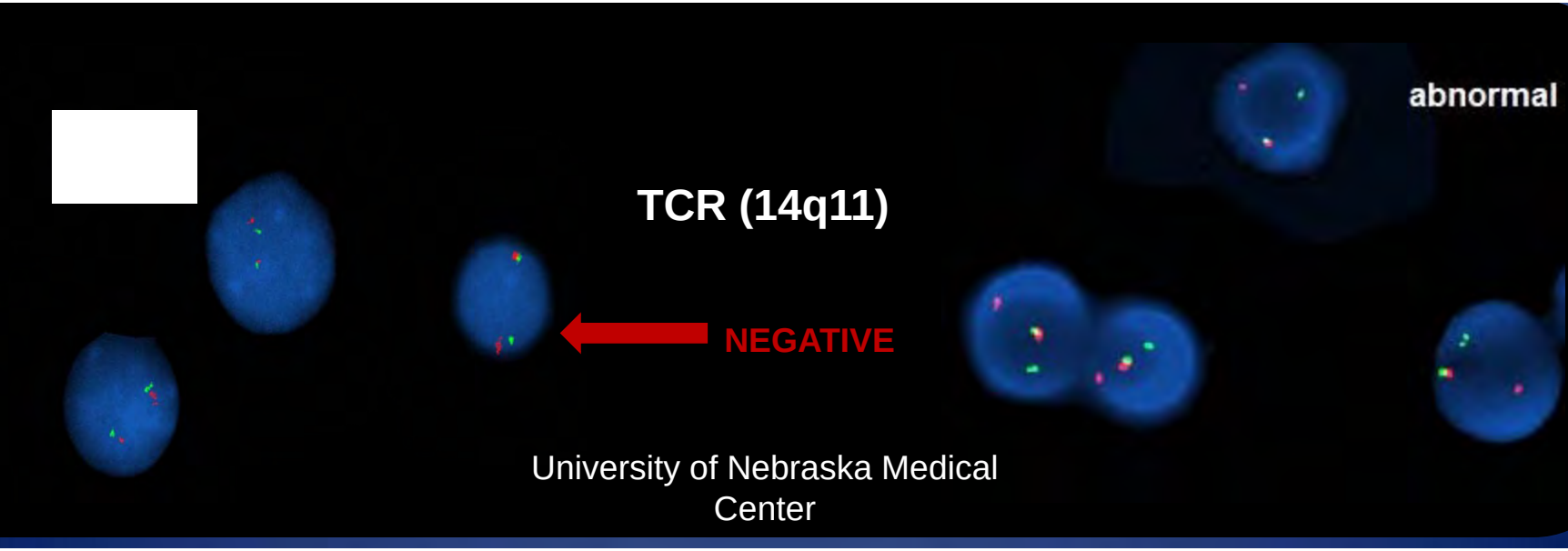
NEGATIVE

ABNORMAL




MYC (8q24)

ABNORMAL




TCR (14q11)

NEGATIVE

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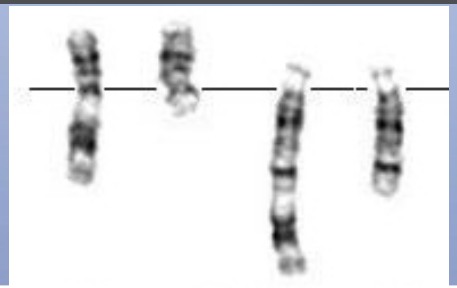
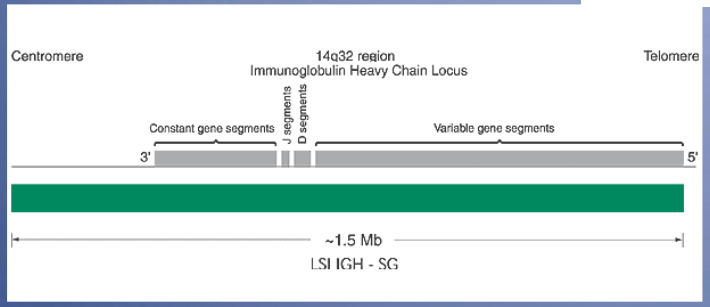
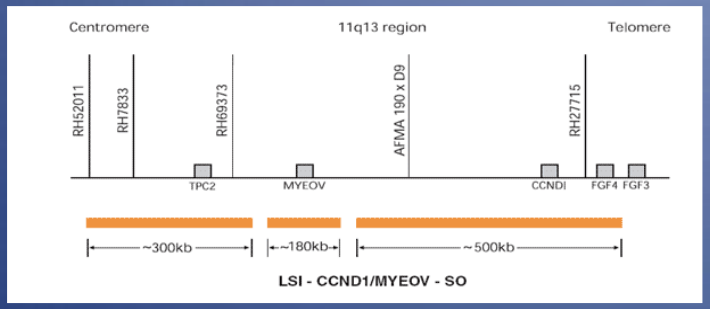
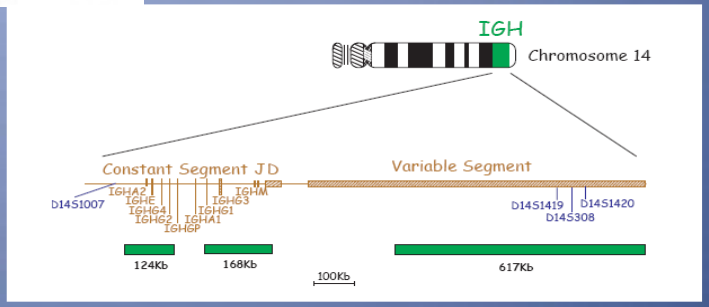


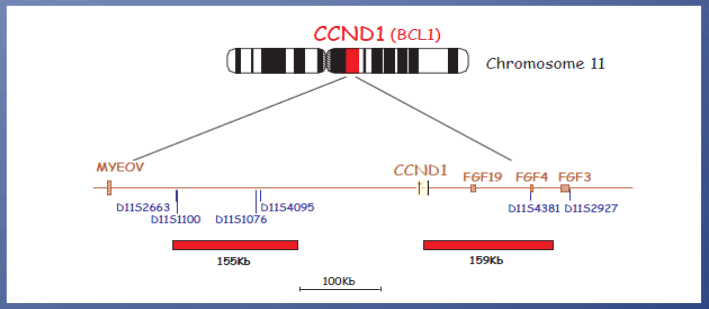
## Abbott Molecular
## IGH/CCND1 XT Dual Fusion Probe

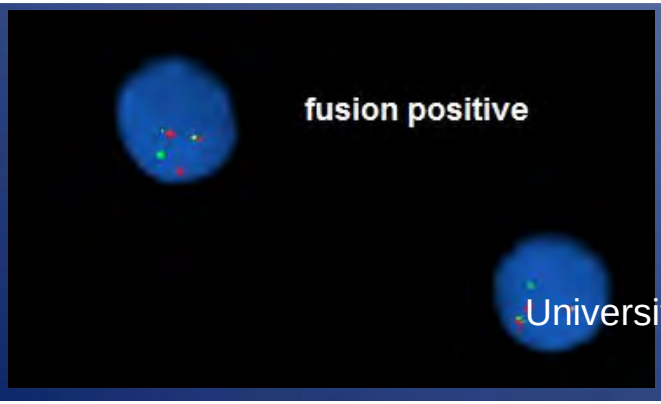


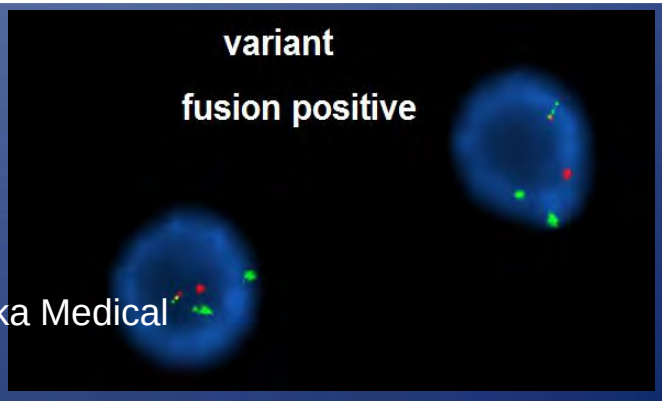




## Cytocell
## IGH/CCND1 Dual Fusion Probe

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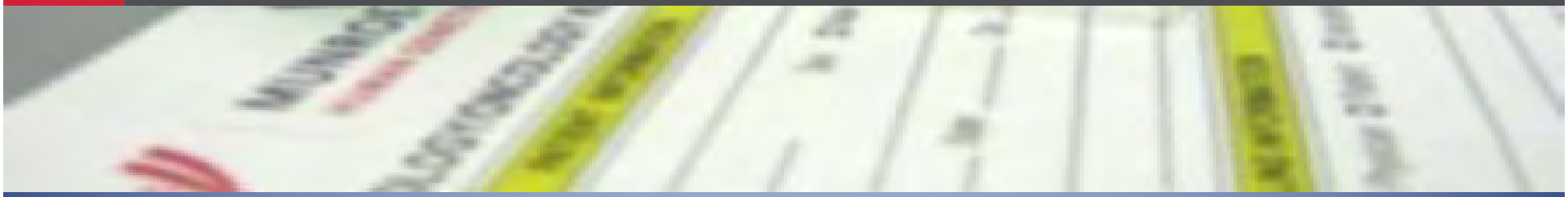
# HGL's FISH Test Menu



•Our test menu includes hundreds of **clinically-validated** FISH probes for both constitutional and acquired disorders.

- **Commercially-available probes**
  - Our laboratory has validated protocols to utilize probes from all of the major vendors.
  - Our laboratory has and continues to serve as a beta-testing site for several commercial vendors.
- **Custom probes**

# HEMATOLOGY/ONCOLOGY/ LYMPHOMA FISH

Adult ALL

Pediatic ALL

T-cell ALL

AML

CLL

CML

Eosinophilia

Lymphoma

Marginal Zone

MM

MDS

MPD

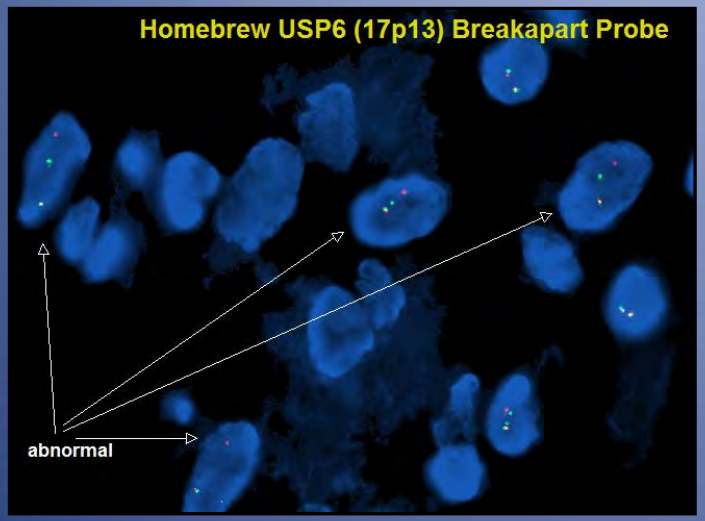
NHL

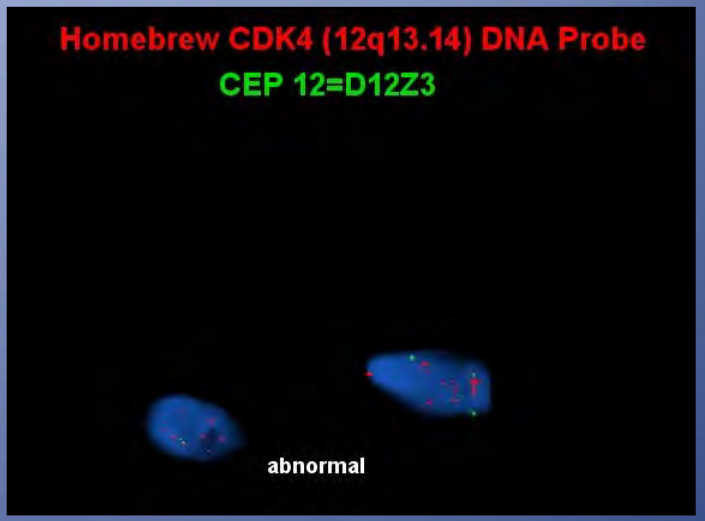
# SOLID TUMOR FISH

| | | |
|---|---|---|
| ARMS | EMC | MBD |
| ASPS | Gastric Cancer | Midline Carinoma |
| ABC | IFS | MLS |
| AFH | IMT | NB |
| Bladder Cancer | Lipoblastoma | Neurological Cancer |
| Breast Cancer | Lipoma | NSCLC |
| CCS/Malignant Melanoma of Soft Parts | WDLS/ALT;DDLS | RCC |
| DFSP | LGFMS | AR/RT;MRT |
| ES/PNET | MASC | SS |

# Custom FISH at HGL

- **Over 300 custom FISH probes**

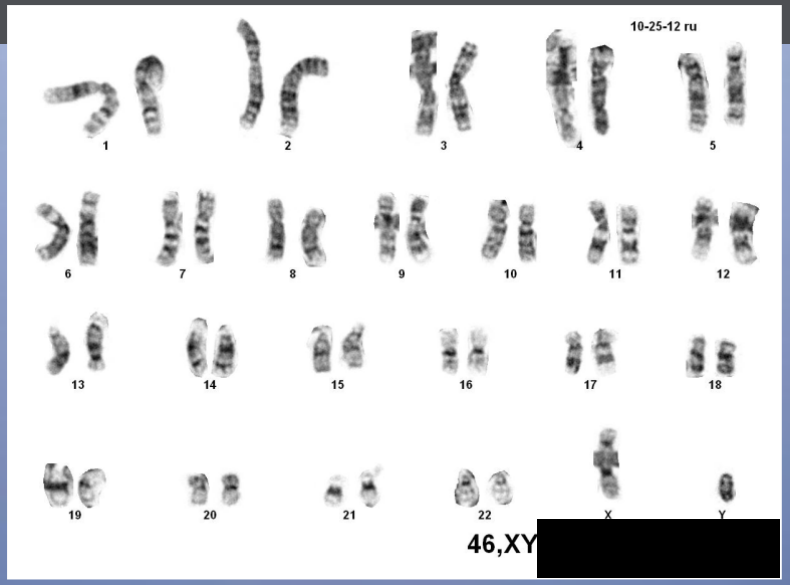


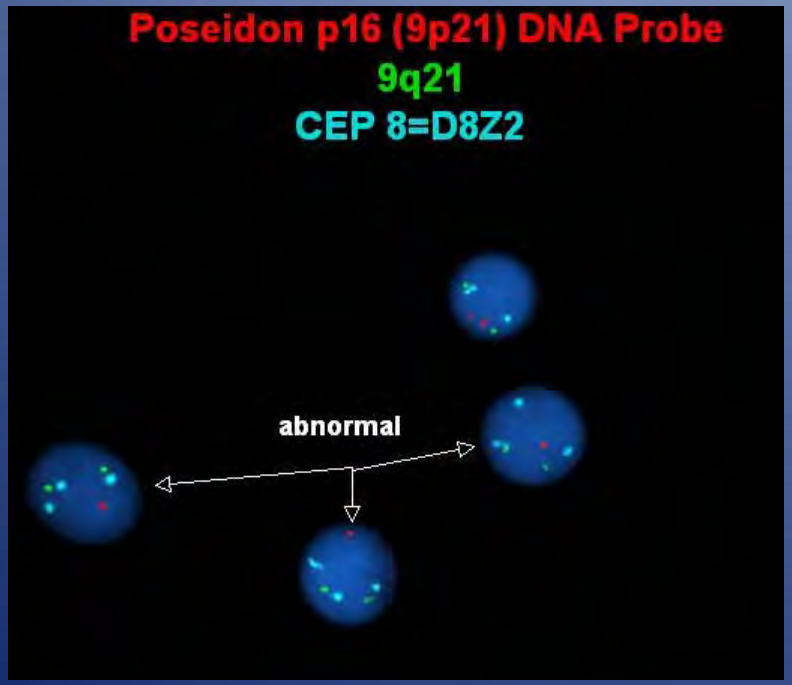



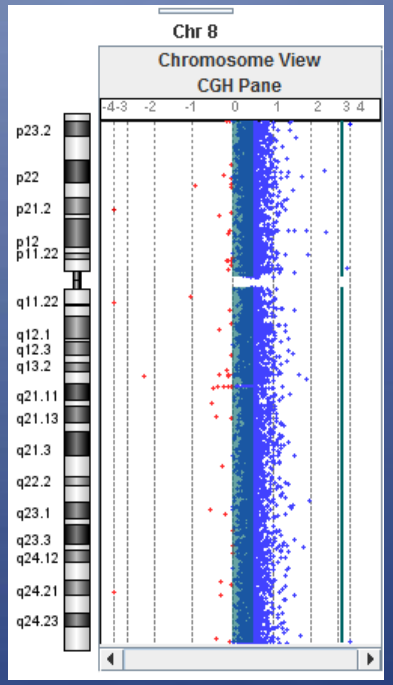

- **Custom FISH Process**
  - Determine region of interest
  - Determine type abnormality
  - Design probe system (based on region & type of abnormality)
  - Make probe by growing, isolating, & labeling the clone(s) of interest
  - Validate probe for clinical use

# Microarray

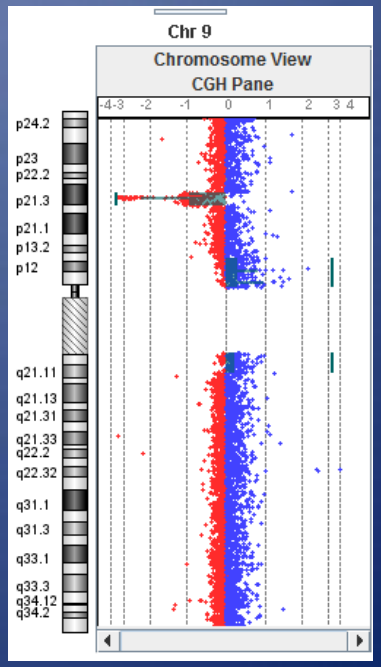
- Utility
  1. Global assessment of genome at high resolution
  2. Detection of diagnostic and prognostic aberrations
- Indication-specific testing approach
  - More comprehensive approach than ordering multiple FISH tests
  - Allows for testing on limited amounts of specimen or on specimens of poor quality
  - Can be performed on both fresh and fixed samples




Trisomy 8




Deletion 9p21 (*CDKN2A*)

# Testing Approach for Cancer Specimens

## Key Points

- Every assay has defined detection abilities and limitations, and FISH testing is only one tool in a clinical genetic laboratory's test menu.

- Commonly, adjunct testing, such as chromosome analysis and/or microarray, is necessary for proper characterization, interpretation, and diagnosis.

# Achieving personalized medicine through identification of genetic aberrations

- Continuing work to advance knowledge of molecular mechanisms that drive transformations from normal to aberrant
- Exploit knowledge of newly discovered biomarkers
  - Provide more efficacious therapies where conventional chemotherapy may provide little or no benefit
  - Reduce unnecessary treatment and reduce enormous health care expenditures
  - Avoid toxic effects of other therapeutic regimens
  - Decrease morbidity

# Predictive vs. prognostic biomarkers

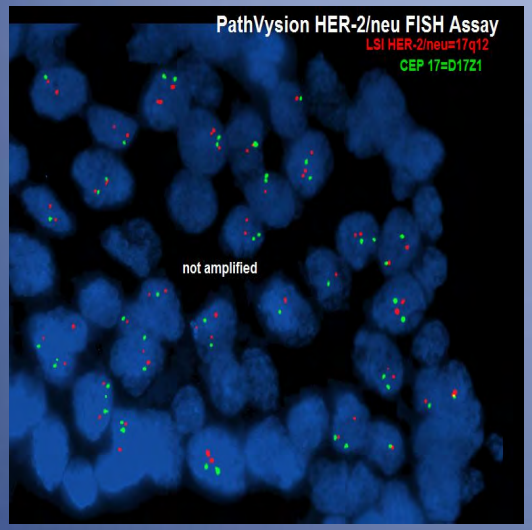
- Predictive biomarkers:
  - Biomarkers that can be used to identify patients who are most likely to respond to a specific therapy
- Prognostic biomarkers:
  - Biomarkers that indicate the likely course of a disease in untreated individuals

# FISH and current commonly used predictive biomarkers

- *ERBB2 (HER/neu)* in breast cancer
- *EML4-ALK* in non-small cell lung carcinoma (NSCLC)
- *BCR-ABL* in chronic myelogenous leukemia (CML)
- *PML-RARα* in acute promyelocytic leukemia (APL)
- *COL1A1-PDGRB* in dermatofibrosarcoma protuberans

# *ERBB2 (HER2/neu)* in breast cancer

- *ERBB2*- more commonly known as *HER2* or *HER2/neu*
- HER2 positivity observed in 20-25% of breast cancers
- Detected by amplification of locus specific DNA FISH probe
- Prognostic marker of poor outcome
- Improved survival with treatment specifically targeted at *HER2* including monoclonal antibodies and tyrosine kinase inhibitors



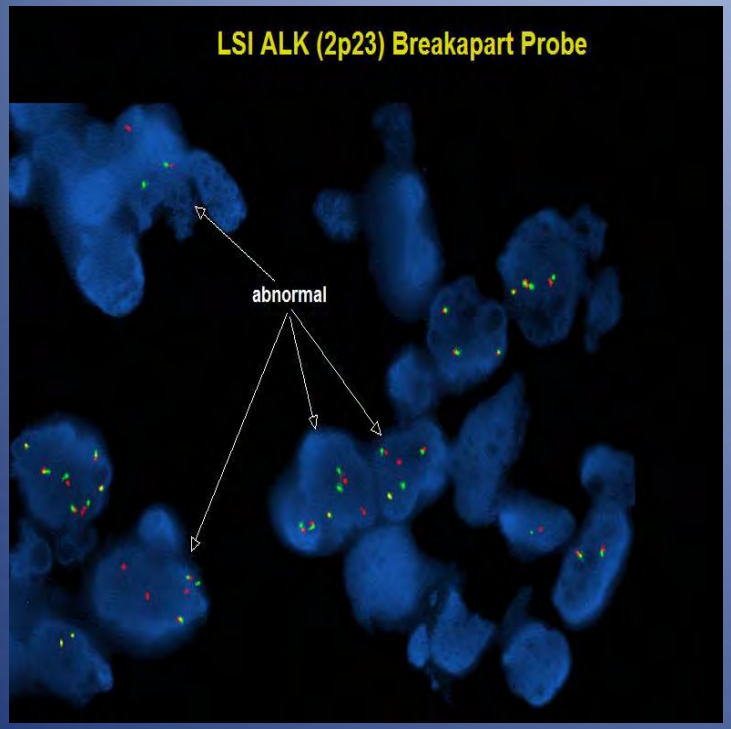


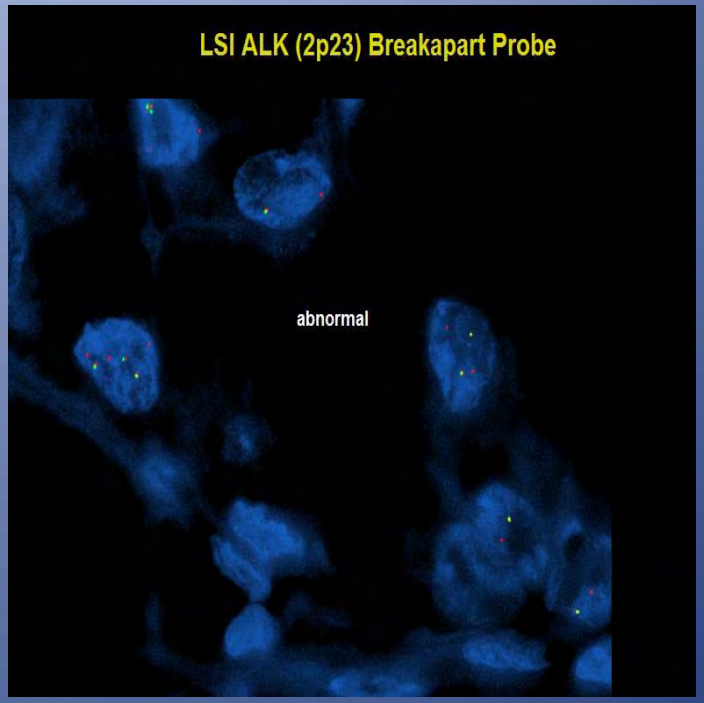
**Interpretation Criteria for Amplification of *ERBB2* using a Dual-Probe Assay**

| Interpretation | *ERBB2*:CEP 17 ratio | Average Number of *ERBB2* signals per cell |
|---|---|---|
| Negative | <2.0 | <4.0 |
| Equivocal | <2.0 | ≥4.0 and <6.0 |
| Positive | ≥2.0 | ≥4.0 |
| | ≥2.0 | <4.0 |
| | <2.0 | ≥6.0 |

# *EML4-ALK* in non-small cell lung carcinoma

- Subset of NSCLC driven by rearrangement in receptor tyrosine kinases (RTKs)
- *EML4–ALK* fusion arises from inversion on chromosome 2, inv(2)(p21p23),
  - Overall incidence ~5%
  - In light / never smokers incidence ~22%
  - In EGFR negative light / never smokers incidence ~33%
- Detected by dual color breakapart locus specific FISH probe
- Improved response rates with targeted tyrosine kinase inhibitor (crizotinib)


LSI ALK (2p23) Breakapart Probe
abnormal

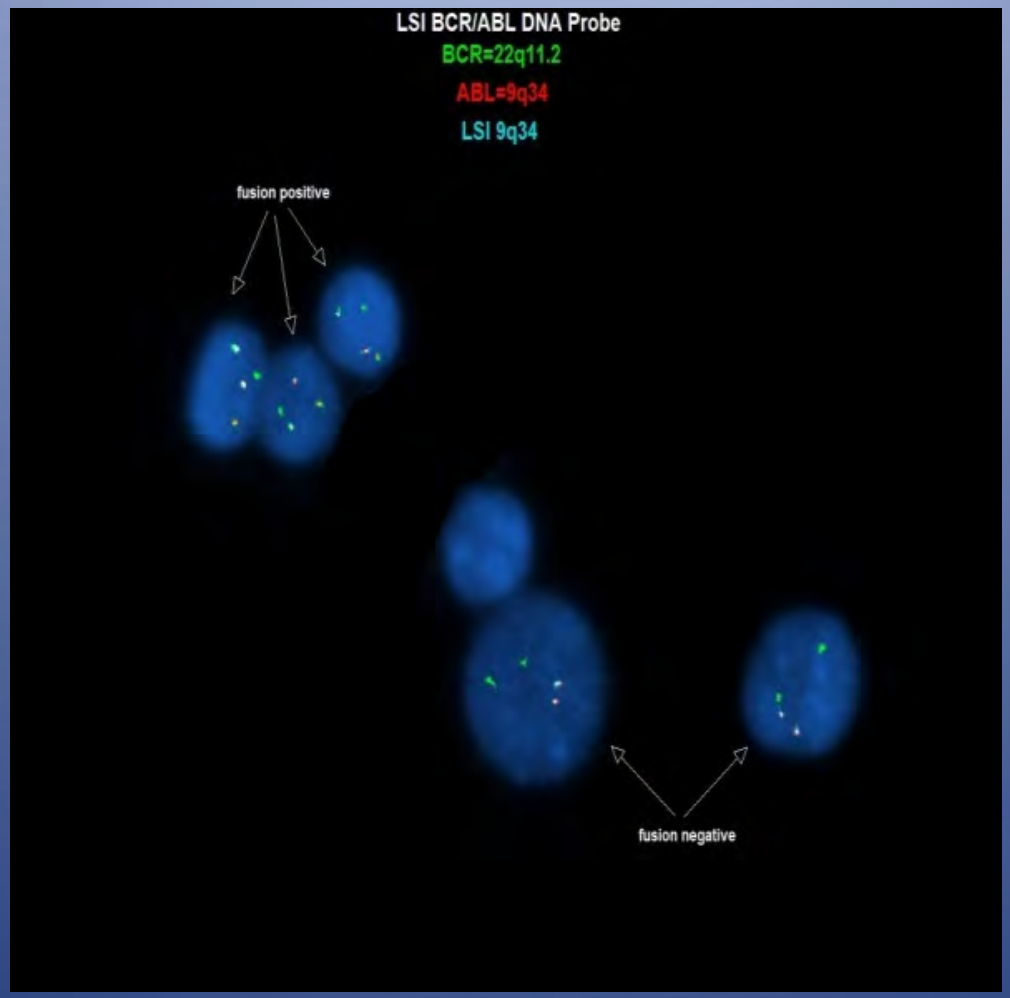

LSI ALK (2p23) Breakapart Probe
abnormal

# Additional predictive biomarkers in NSCLC

- *ROS1*
  - Overall incidence ~2%
  - Rearrangements of *ROS1* detected by breakapart FISH probe
  - Also shown significant clinical response with ALK inhibitor due to homology between kinase domain
- *RET*
  - Overall incidence ~2%
  - Rearrangements of *RET* detected by breakapart FISH probe
  - Several RET inhibitors to be further explored
- *NTRK1*
  - Rearranged in ~3% in *ALK, ROS1, RET* negative NSCLC
  - No specific FISH probe currently available

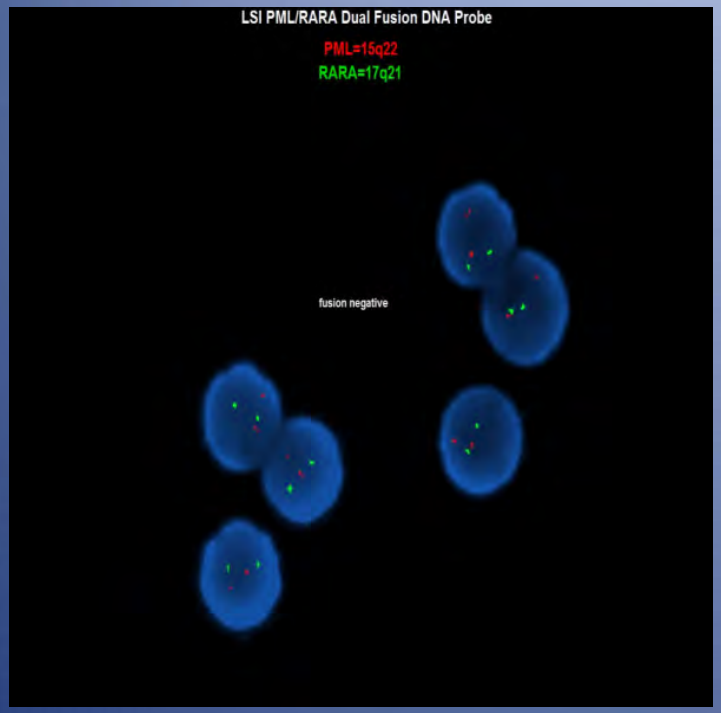
# *BCR-ABL* in CML

- *BRC-ABL* fusion resulting from t(9;22)(q34;q11.2) detectable in 98% in CML and 5-20% acute lymphoblastic leukemia (ALL)
- Detected by dual color translocation FISH probe
- Understanding of abnormal signaling in CML led to first successful target for enhance tyrosine kinase activity of *BCR-ABL*
- In the absence of therapy patients eventually progress from chronic phase to transformed phase
- Development of second generation compounds to circumvent resistance to TKIs resulting from emergence of subclones


LSI BCR/ABL DNA Probe
BCR=22q11.2
ABL=9q34
LSI 9q34
fusion positive
fusion negative

# *PML-RARα* in APL

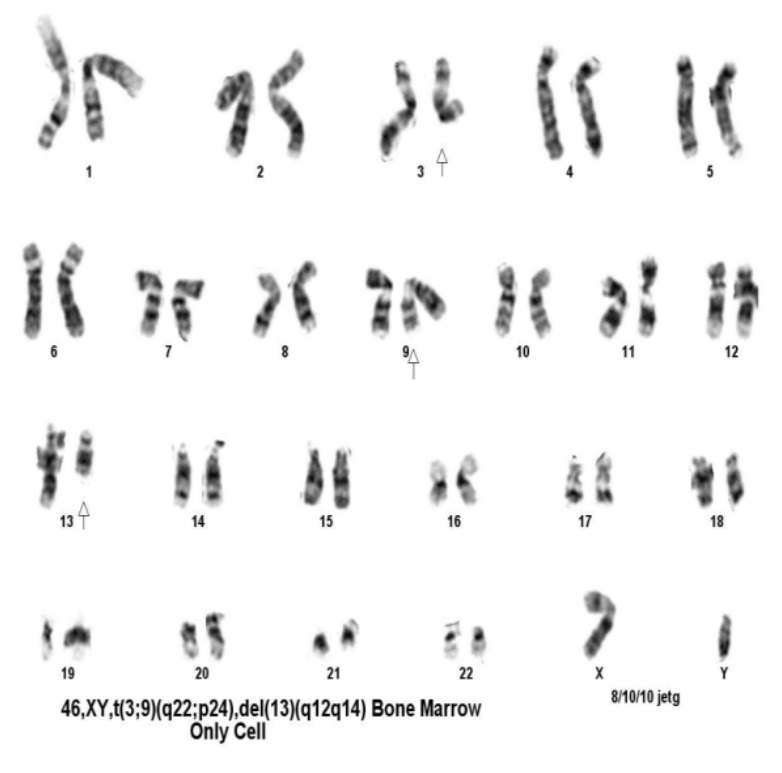
- APL constitutes 5–8% of acute myeloid leukemia (AML) cases
- *PML-RARα* fusion resulting from t(15;17)(q22;q12) detectable in >95% APL
- Detected by dual color translocation FISH probe
- High frequency of life-threatening disseminated intravascular coagulation
- The blasts are highly sensitive to anthracycline-based chemotherapy and differentiate in response to all-trans-retinoic acid and arsenic trioxide treatment.


LSI PML/RARA Dual Fusion DNA Probe
PML=15q22
RARA=17q21
fusion negative


LSI PML/RARA Dual Fusion DNA Probe
PML=15q22
RARA=17q21
fusion positive

# *JAK2* rearrangements indicating a role for JAK2 inhibitors in hematopoetic disorders

- *ETV6/TEL-JAK2* fusions in T-ALL, B-ALL and atypical CML
- *PMC1-JAK2* fusions in AML, CML, TCL
- *NF-E2-JAK2* and AML1-JAK2 fusions in MDS
- *BCR-JAK2* fusions in CML
- *RPN1-JAK2* fusions in CIMF
- *SSBP2-JAK2* fusions in Pre-B cell ALL
- *PAX5-JAK2* fusions in childhood ALL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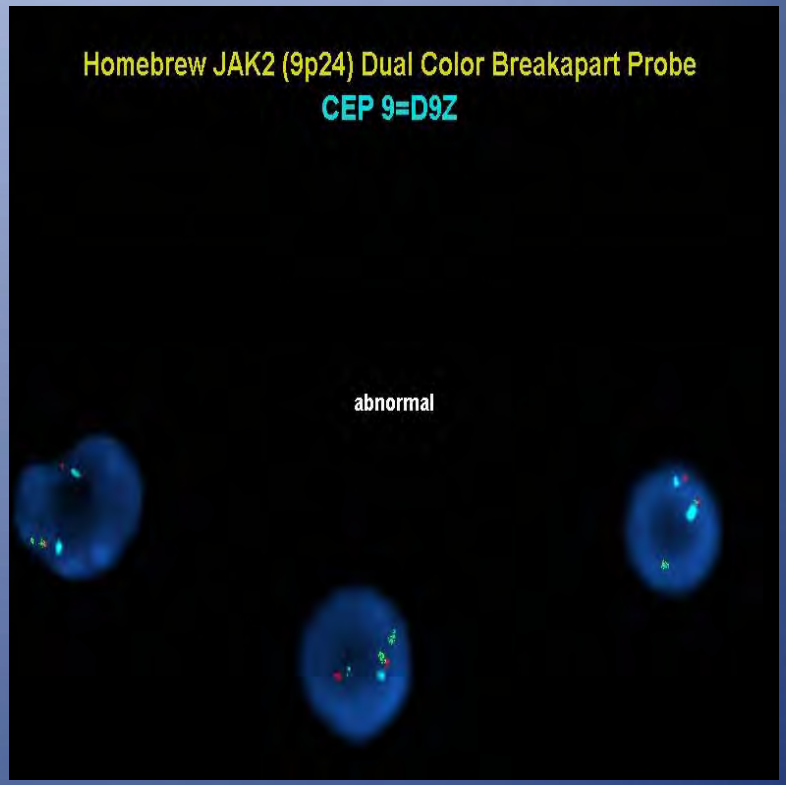
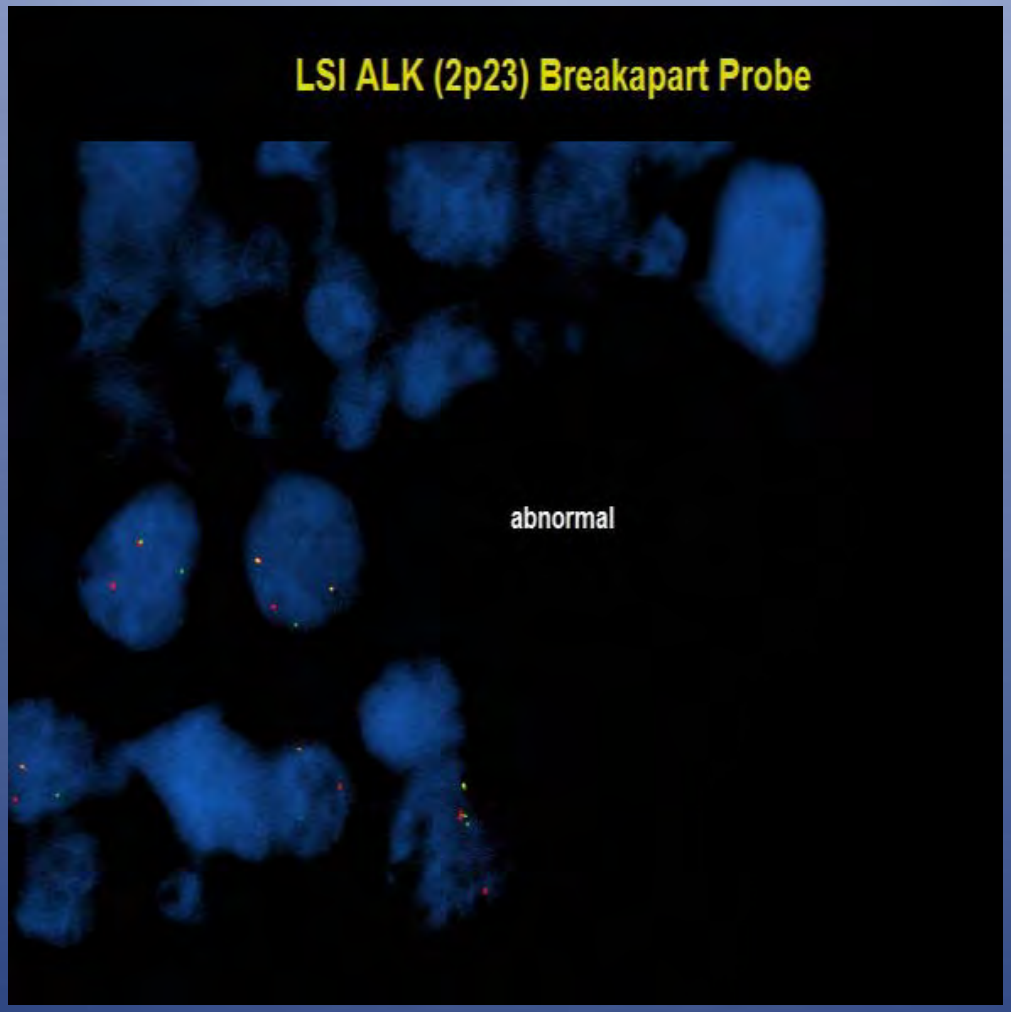
Y
46,XY,t(3;9)(q22;p24),del(13)(q12q14) Bone Marrow Only Cell
8/10/10 jetg


Homebrew JAK2 (9p24) Dual Color Breakapart Probe
CEP 9=D9Z
abnormal

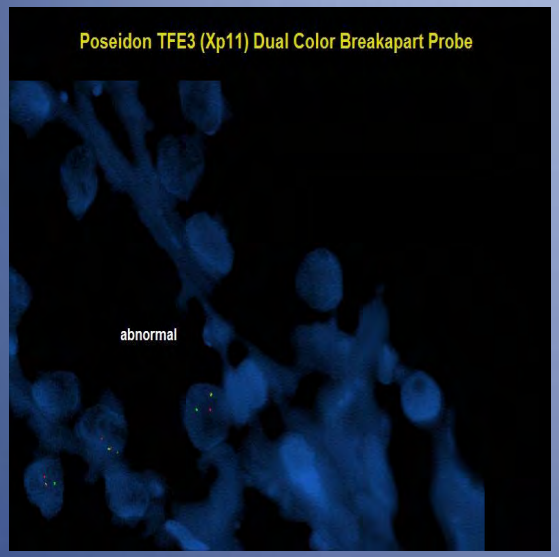
# *ALK* rearrangement in anaplastic large cell lymphoma

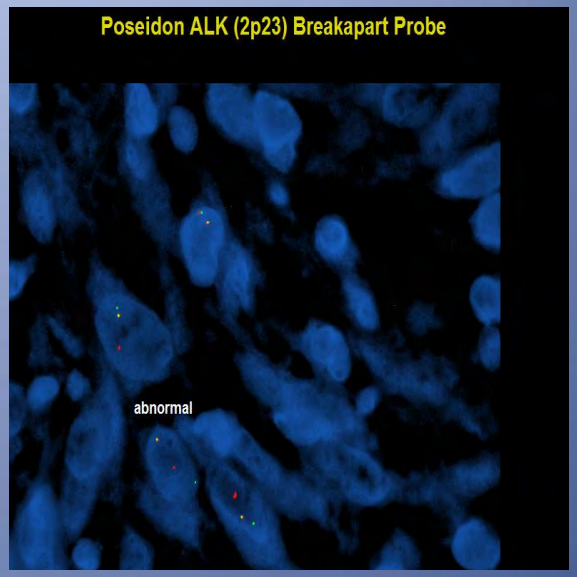
- 3% of adult non-Hodgkin lymphomas and 10-20% of childhood lymphoma
- *ALK-NPM* fusion resulting from t(2;5)(p23;q35) or *ALK* rearrangement with other 2p23 translocations
- Detected by dual color breakapart FISH probe
- Good response with multi-agent, anthracycline-containing regimen in adult ALCL
- No established standard treatment in patients with refractory or relapsed ALCL
- Ongoing clinical trials for TKIs in ALK-positive ALCL

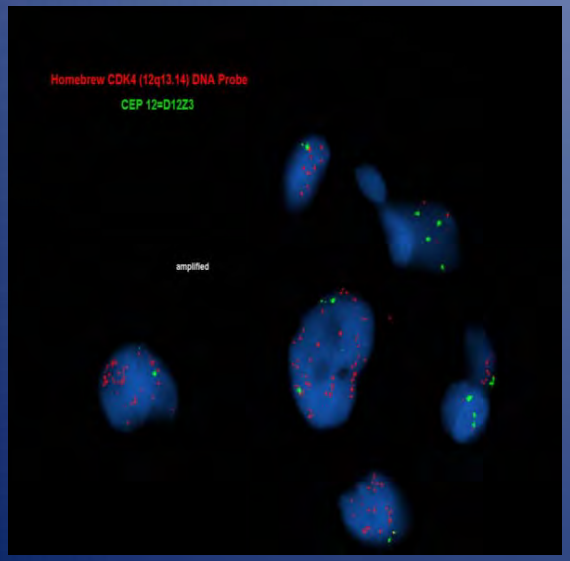

LSI ALK (2p23) Breakapart Probe
abnormal

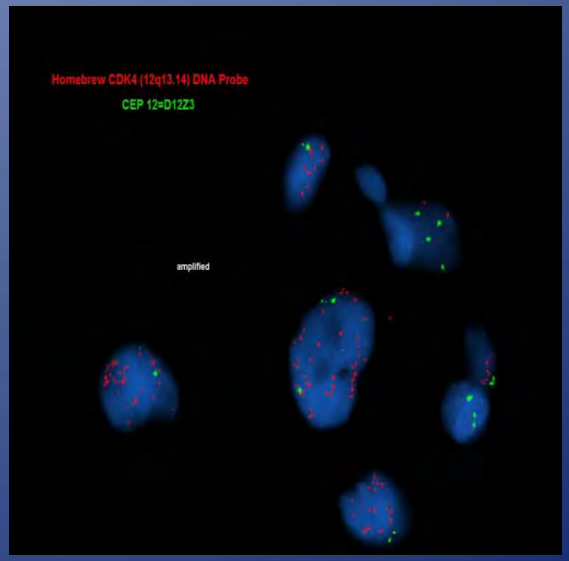
# Additional FISH targets for predictive biomarkers in sarcoma

- *TFE3* in aveolar soft part sarcoma (ASPS)
  - *ASPL-TFE3* fusion resulting from unbalanced t(X;17)(p11;q25) leads to MET transcriptional up-regulation
- *ALK* in inflammatory myofibroblastic sarcoma (IMT)
  - *ALK* rearrangement detected in ~50% of IMT
- *CDK4* in liposarcoma
  - *CDK4* and *MDM2* amplified in most well-differentiated/dedifferentiated liposarcoma
  - PD0332991 is a CDK4 inhibitor
- *CSF1* in pigmented villonodular sarcoma (PVS)
  - *CSF1-COL6A3* fusion resulting from t(1;2)(p13.3;q37) or CSF1 up-regulation with other 1p13.1 rearrangement
  - Both imatinib and nilotinib have demonstrated activity in this disease


Poseidon TFE3 (Xp11) Dual Color Breakapart Probe
abnormal


Poseidon ALK (2p23) Breakapart Probe
abnormal


Homebrew CDK4 (12q13.14) DNA Probe
CEP 12=D12Z3
amplified

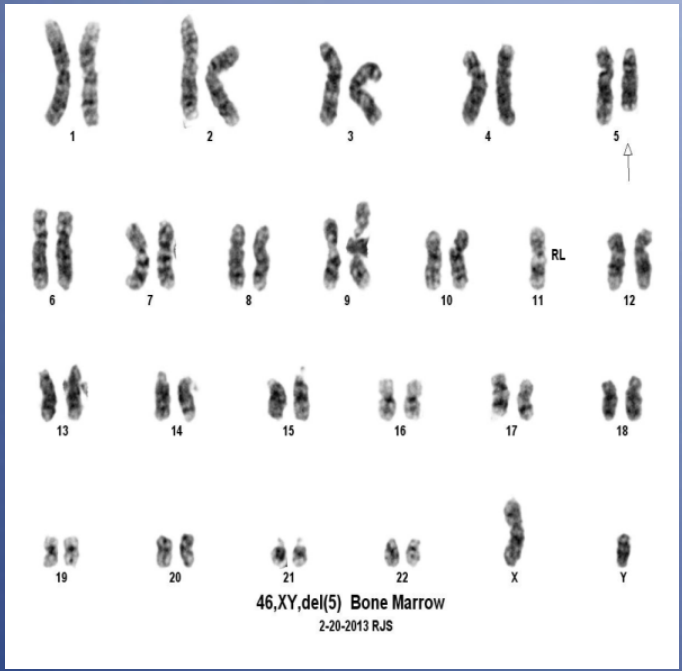

Homebrew CDK4 (12q13.14) DNA Probe
CEP 12=D12Z3
amplified

# Use of FISH for prognostic biomarkers and tailored therapy

- 5q deletion in myelodysplastic syndrome (MDS)
- *MYCN* amplification in neuroblastoma
- Medulloblastoma
  - Stratification of subgroups
    - WNT subgroup: Very good prognosis – Monosomy chromosome 6
    - SHH subgroup: Good prognosis for infants, intermediate for others – *GLI2* and *MYCN* amplification
    - Group 3: Poor prognosis – *MYC* amplification
    - Group 4: Intermediate prognosis – *CDK6* and *MYCN* amplification

# 5q deletion in MDS

- The del(5q) population accounts for approximately 15% of MDS
- Detected with locus specific DNA FISH probe
- Three karyotypically defined subsets:
  - isolated del(5q), including patients with the 5q– (minus) syndrome,
  - del(5q) with one additional chromosome abnormality,
  - and del(5q) with two or more cytogenetic abnormalities (ie, a complex karyotype)
- Overall survival decreases with increasing karyotype complexity
- Lenalidomide : dual biological effects
  - Cytotoxicity to and suppression of del(5q) MDS progenitors
  - Cytogenetic response complemented by the promotion of effective erythropoiesis in nondel(5q) MDS clones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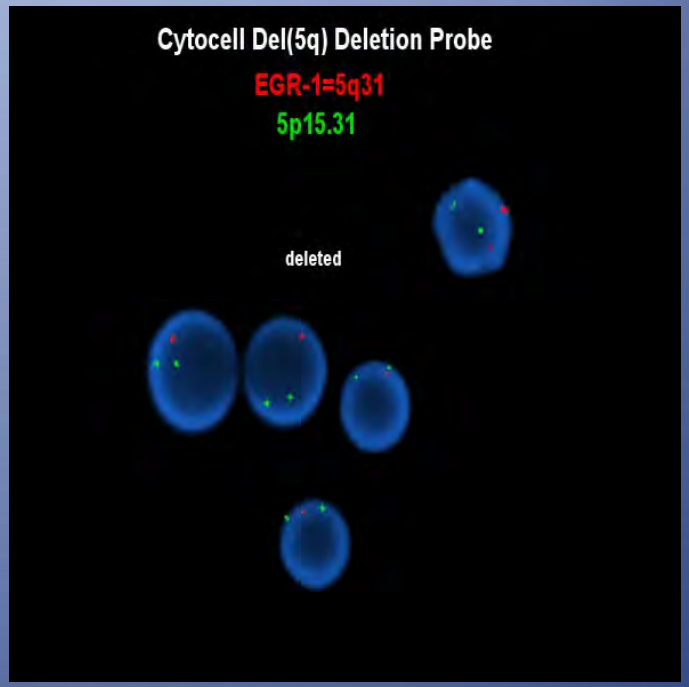
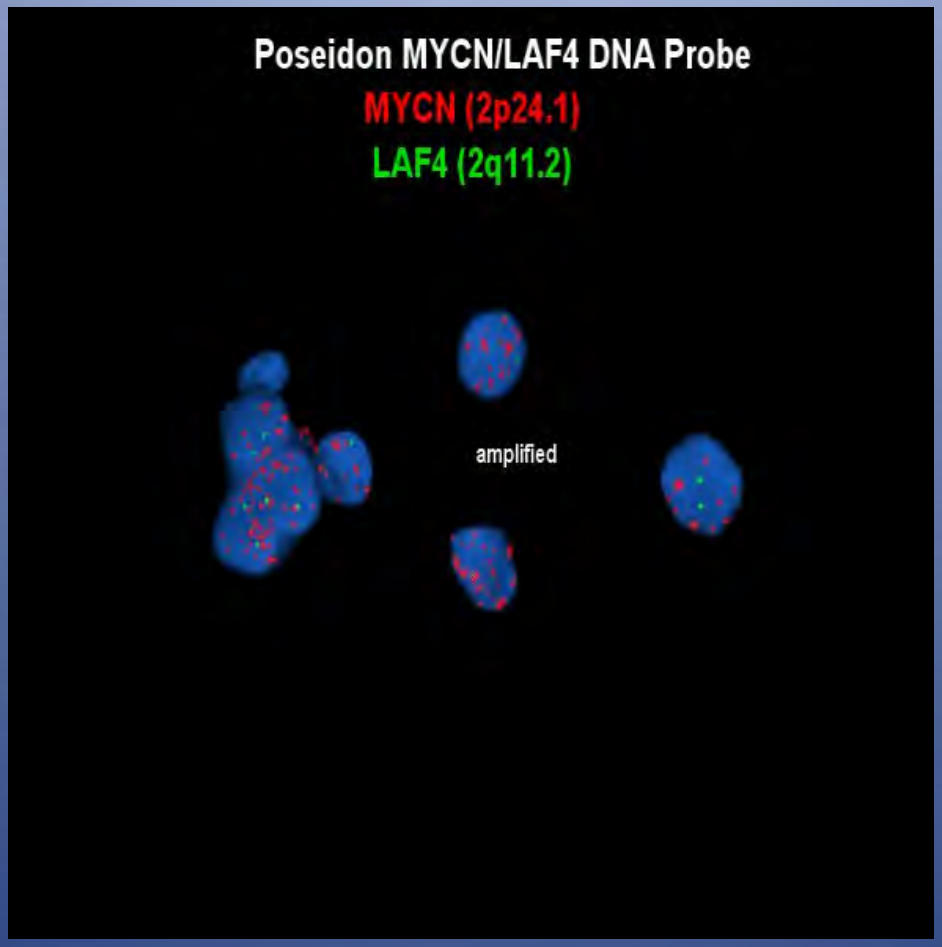
46,XY,del(5) Bone Marrow
2-20-2013 RJS


Cytocell Del(5q) Deletion Probe
EGR-1=5q31
5p15.31
deleted

# *MYCN* in neuroblastoma

- *MYCN* amplified in ~20% of NB
- One of clearest markers for identifying high-risk NB patients no matter disease stage
- Current treatment involves very intensive approach, often combining chemotherapy, radiation, surgery and immunotherapy
- Currently no *MYC* inhibitor for targeted therapy
- Ongoing studies for potential of targeting genes directly or indirectly downstream of *MYCN*


Poseidon MYCN/LAF4 DNA Probe
MYCN (2p24.1)
LAF4 (2q11.2)
amplified

# Next-generation sequencing and predictive/prognostic biomarkers

- *KIT* mutations in gastrointestinal stromal tumors
  - Mutation hotspots involving exon 11 (70-75%) and exon 9 (10-15%)
  - *KIT* mutations also seen in AML, melanoma, seminoma, mastocytosis
- *BRAF* mutations in melanoma
  - Present in 40-60% of advanced melanoma
  - Activating mutation in 80-90% cases is V600E
  - V600E mutation also seen in hairy-cell leukemia
- BRCA1/2 germline mutations in breast and ovarian carcinoma
  - Ongoing studies involving PARP (poly(ADP-ribose) polymerase) inhibitors for high-risk *BRCA* mutation patients
- TSC1/2 germline mutations in perivascular epithelioid cell tumors (PEComas)
  - PEComas may be associated with tuberous sclerosis complex by inactivating mutations in *TSC1* or *TSC2*
  - Ongoing studies for mTOR inhibitors targeting hyperactivation of mTOR pathway with *TAC1/2* mutaions

# HGL Custom FISH Lab

- Actively developing custom DNA probes for fluorescence in situ hybridization since 2000
- Over 250 specific BAC clones currently in-house
- 13 clinically validated custom oncology probe sets in current use

# Hereditary Cancer – NGS

| Panel | # of Genes |
|---|---|
| Breast6 | 6 |
| Breast/Ovarian/Endometrial | 26 |
| Lynch/Colorectal | 18 |
| Pancreatic | 13 |
| Brain/CNS/PNS | 19 |
| Endocrine | 14 |
| Renal | 19 |

# Pretest Counseling

- 3-4 generation pedigree
  - Cancer dx
  - Ancestry/ethnicity
  - Consanguinity
- Evaluation of Patient's risk
  - Risk of cancer
  - Risk to carry cancer susceptibility gene
- Psychosocial assessment
- Education about Hereditary Cancer syndrome
  - Genes, DNA
  - Inheritance patterns
  - Pentrance, expressivity, heterogeneity
- Obtain Informed consent
  - Risk, benefits, limitations

# Informed Consent:

## Potential Benefits of Genetic Testing

- Improved cancer risk management
- Relief from uncertainty and anxiety about cancer risk
- Information for individual and family members
- Lifestyle decision making

# Post-test Counseling

- Disclosure of Results
  - Interpretation
    - Positive, negative, uncertain variant
  - Significance for patient
    - Risks
    - Medical management
    - Resources
  - Implication for family
    - Risks
    - Duty to contact

# University of Nebraska Cancer Genetics Services

- Staffed by 2 Licensed and Board Certified Genetic Counselors:

  Gwen Reiser MS, LCGC  Amber Carter MS, LCGC

- More than 25 years of shared experience providing genetic counseling and risk assessment for Hereditary Cancer

# University of Nebraska Cancer Genetics Services

- Clinics
  - UNMC
  - MMI
  - Olson Center
  - Cowdery Cancer Care Center
  - Bellevue Medical Center
  - Village Point Cancer Center

# University of Nebraska Cancer Genetics Services

- Statewide Genetic Clinics
  - Clinics 4 times per year

# 40 years of dedicated service

- **Comprehensive Genetic Testing**
- **Specialized Personal Consultation with *Board Certified* Genetics Professionals**
- **Cutting Edge Technology**
- **Clinically Relevant Testing**
- **Accurate and Expert Analysis**
- **Prompt and Precise Reporting**
- **Competitive Pricing**

# Reference Laboratory

- Children's Oncology Group (COG)
  - National Reference Laboratory for Lymphoma
  - Reference Laboratory for Leukemia
  - Dr. Sanger is a member of the COG Cytogenetics Committee.
- Cancer and Leukemia Group B (CALGB)
  - Genetics Reference Laboratory
- Laboratory of choice for local, national, and international health professionals

# *Leading through research*

2013-2014

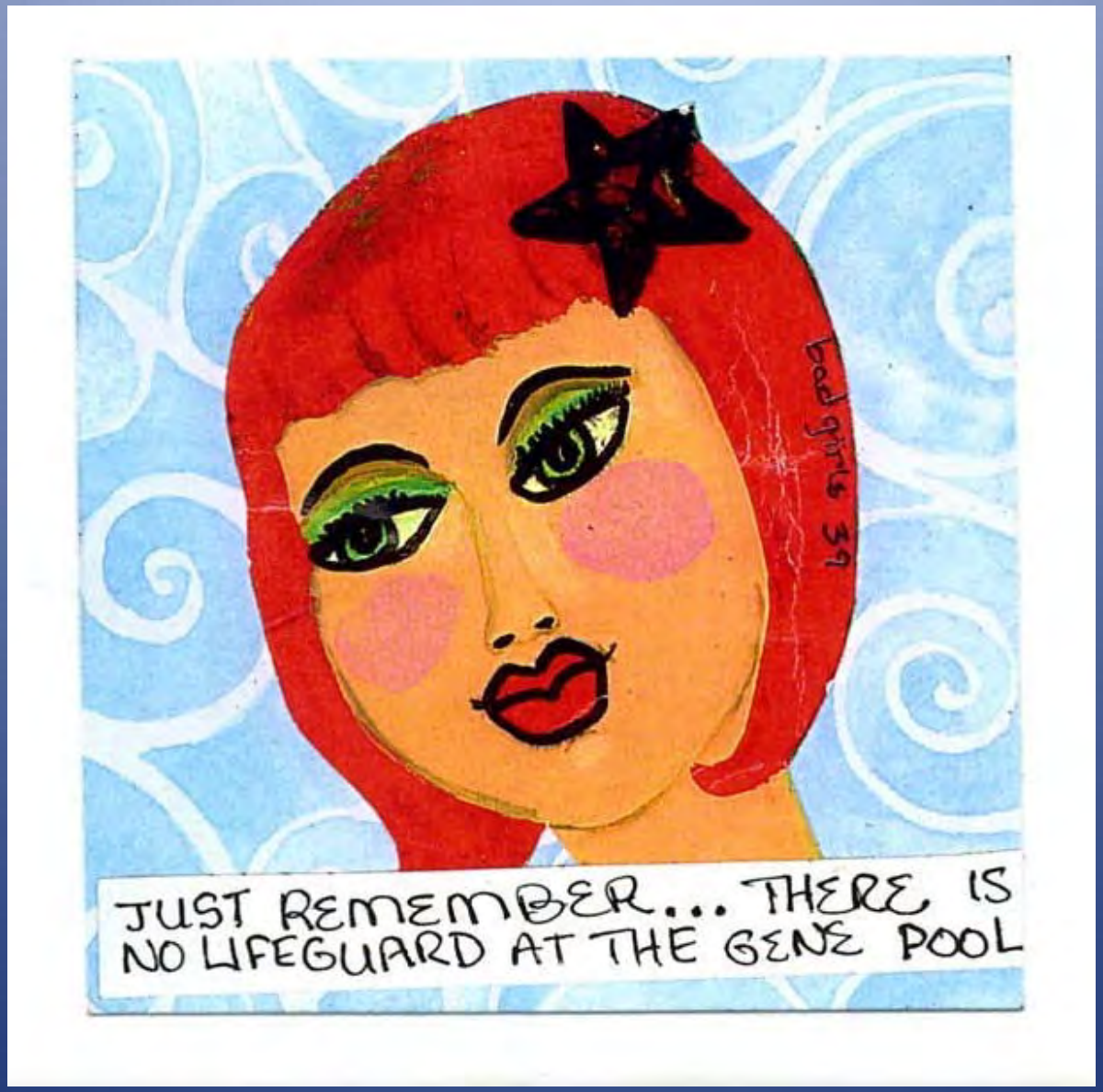
- Over 40 Articles and Publications

- Over 100 Abstracts and Presentations

  including lectures and posters presented at national meetings and educational institutions


# Leading through research Collaborations *within UNMC*

| Division | General Project Details |
|---|---|
| Pathology/Microbiology & Lymphoma Study Group | Numerous lymphoma research projects & collaborative publications (NHL, BL, PTLD, HD) |
| Hematology/Oncology | Numerous hematological malignancy projects involving cytogenetics & FISH (MDS, MM, leukemia) |
| Nebraska Public Health Laboratory (NPHL) & Radiation Safety | Biodosimetry |
| Eppley Institute | Cancer epidemiology & genetics |
| Neurology | Projects associated with ataxia, ALS, and frontotemporal dementia |
| Genetics, Cell Biology, & Anatomy (GCBA) | Whole exome sequencing in familial autism |
| College of Nursing | Smoking cessation |


bad girls 39
JUST REMEMBER... THERE IS
NO LIFEGUARD AT THE GENE POOL

# University of Nebraska Medical Center


***Human Genetics Laboratory…***

combining comprehensive genetic testing with personalized clinical consultation to provide the very best in genetic medicine to every client and patient served

University of Nebraska Medical Center