Metaphase chromosomes, interphase FISH, and RQ-PCR for early monitoring of CML treatment response.

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Analysis options for monitoring post treatment CML

- Marrow (BM)
  - Conventional cytogenetic metaphase analysis (until CCyR)
  - RQ-PCR for BCR/ABL
- Blood (PB)
  - RQ-PCR for BCR/ABL
  - Dividing cells obtained: metaphase G-banded analysis
  - No dividing cells: Interphase FISH

Concerns

- Observations from monitoring treatment response in CML
  - Serial results from > 400 patients
  - Integrated approach – chromosomes, FISH, RQ-PCR
- Concerns over accuracy of early treatment monitoring….
  - Discrepant results between:
    - Ph +ve metaphase cells
    - Interphase FISH for BCR/ABL
    - RQ-PCR for BCR/ABL
    - Blood (PB) vs marrow (BM)

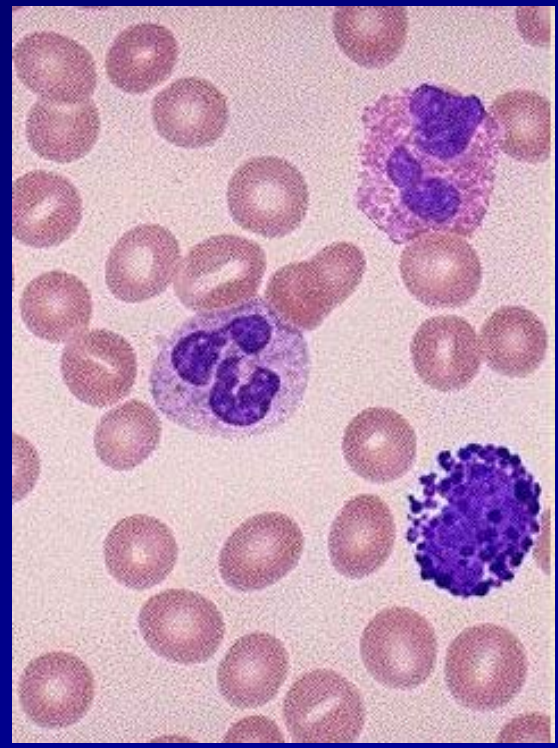
Patient A

- Presented April 1999
- age 57
- 46,XX,t(9;22)[20]
- IFN:
  - Clinical remission but all cells remained Ph+ve cytogenetically. IFN stopped in Feb 2000
- Started on Glivec in August 2001
- Clinician requested interphase FISH analysis **in addition to** metaphase G-banded analysis

Date of BM	Metaphase % Ph+ve	Interphase FISH % BCR/ABL +ve
Aug 2001	100 [30]	78
Oct 2001	100 [40]	67
Feb 2002	100 [45]	56
May 2002	100 [30]	95
Sept 2002	100 [30]	73
Dec 2002	95 [19/20]	49
Mar 2003	100 [25]	35

Why were the marrow metaphase and interphase results discrepant?

- ? Haemo-dilution:
  - Increases proportion of non-CML cells (lymphocytes)
  - Extent varies between samples
    - Sampling procedure effect
    - Treatment effect

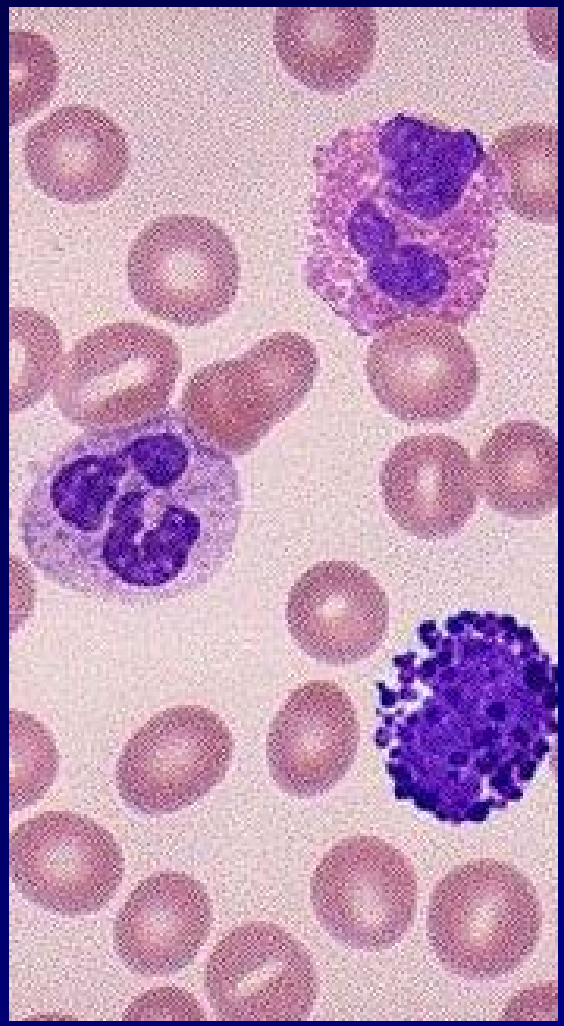


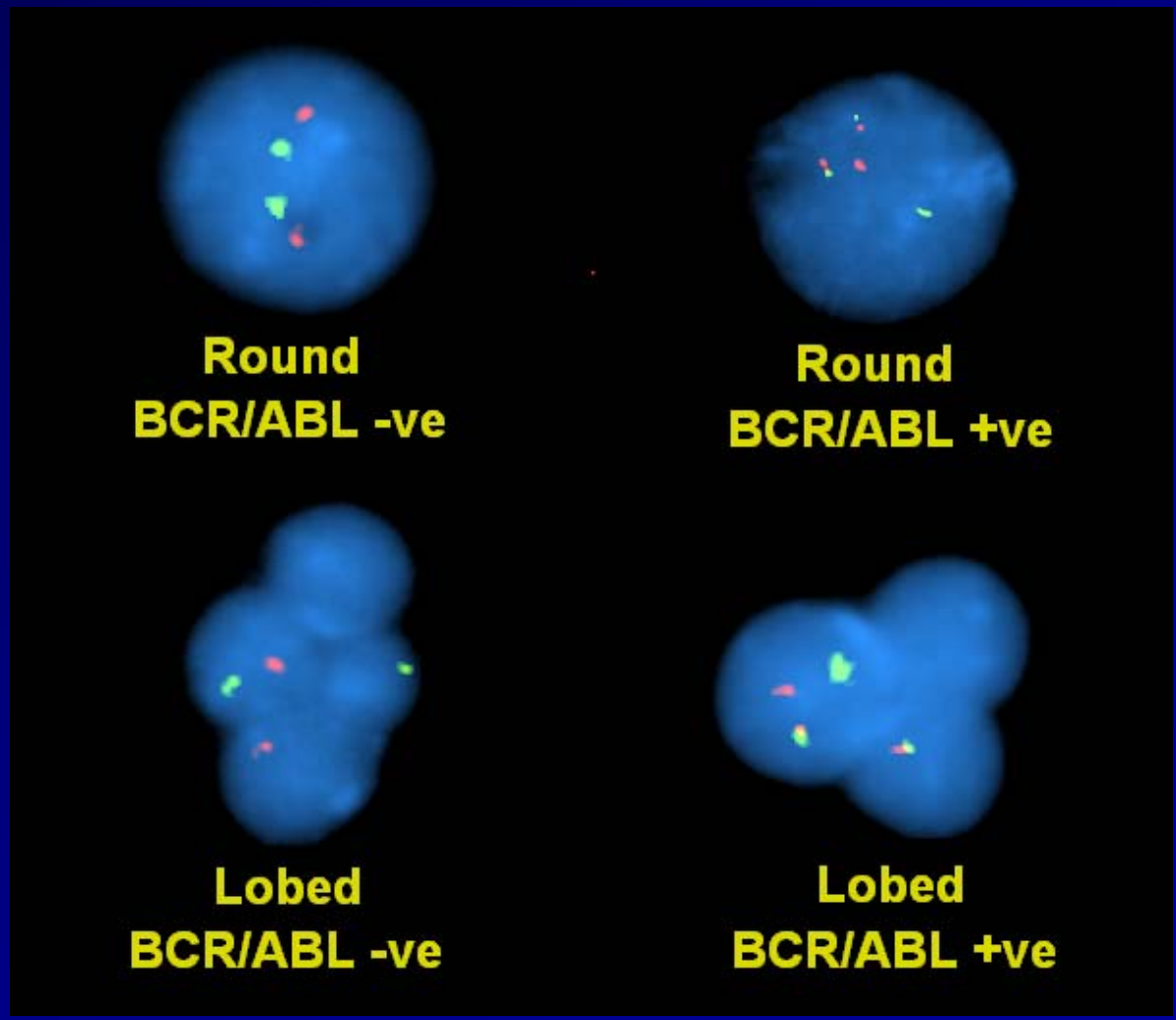
- ? Glivec induced neutropenia:
  - Under-representation of myeloid cells
  - Increases likelihood of haemo-dilution
  - Transient?

- ? FISH scoring artefacts
  - Score only non-overlapping nucleii
  - Ph clone primarily granulocytes (neutrophils, basophils, eosinophils) which have lobed/multi-lobed nuclei and precursors

- Re-scored slides examining ‘lobed’ nuclei alone

FISH scoring: based on nuclear morphology





Patient A. Review of cytogenetic results

- Metaphase analysis:
  - No response
- Whole marrow interphase analysis:
  - ? Response
- Lobed nuclei interphase analysis:
  - No response
  - gIF correlates better with Ph metaphase cells than wIF

Date of marrow	Metaphase % Ph +ve	Interphase analysis Whole marrow wIF % BCR/ABL +ve	Interphase analysis Lobed nuclei gIF %BCR/ABL +ve
Aug 2001	100 [30]	78	
Oct 2001	100 [40]	67	
Feb 2002	100 [45]	56	
May 2002	100 [30]	95	
Sept 2002	100 [30]	73	
Dec 2002	**95 [19/20]**	**49**	**71**
Mar 2003	**100 [25]**	**35**	**98**

- Are these discrepancies reproducible? Also in PB?
  - Significant no. of PBs received for treatment monitoring.
  - Analysis often performed using interphase FISH.

Serial interphase FISH results

- Prospective study comparing results from whole PB and selected lobed nuclei analysis
- Scored 50-100 interphases from whole PB and 50 selected lobed interphase cells

Patient B			Patient C		
Date of sample	% BCR/ABL+ve Whole Blood	% BCR/ABL+ve Lobed nuclei	Date of sample	% BCR/ABL+ve Whole Blood	% BCR/ABL+ve Lobed nuclei
Jan 03	75	95	Aug 03	90	97
May 03	36	100	Nov 03	84	98
Aug 03	66	100	Feb 04	37	86
Jan 04	65	98			

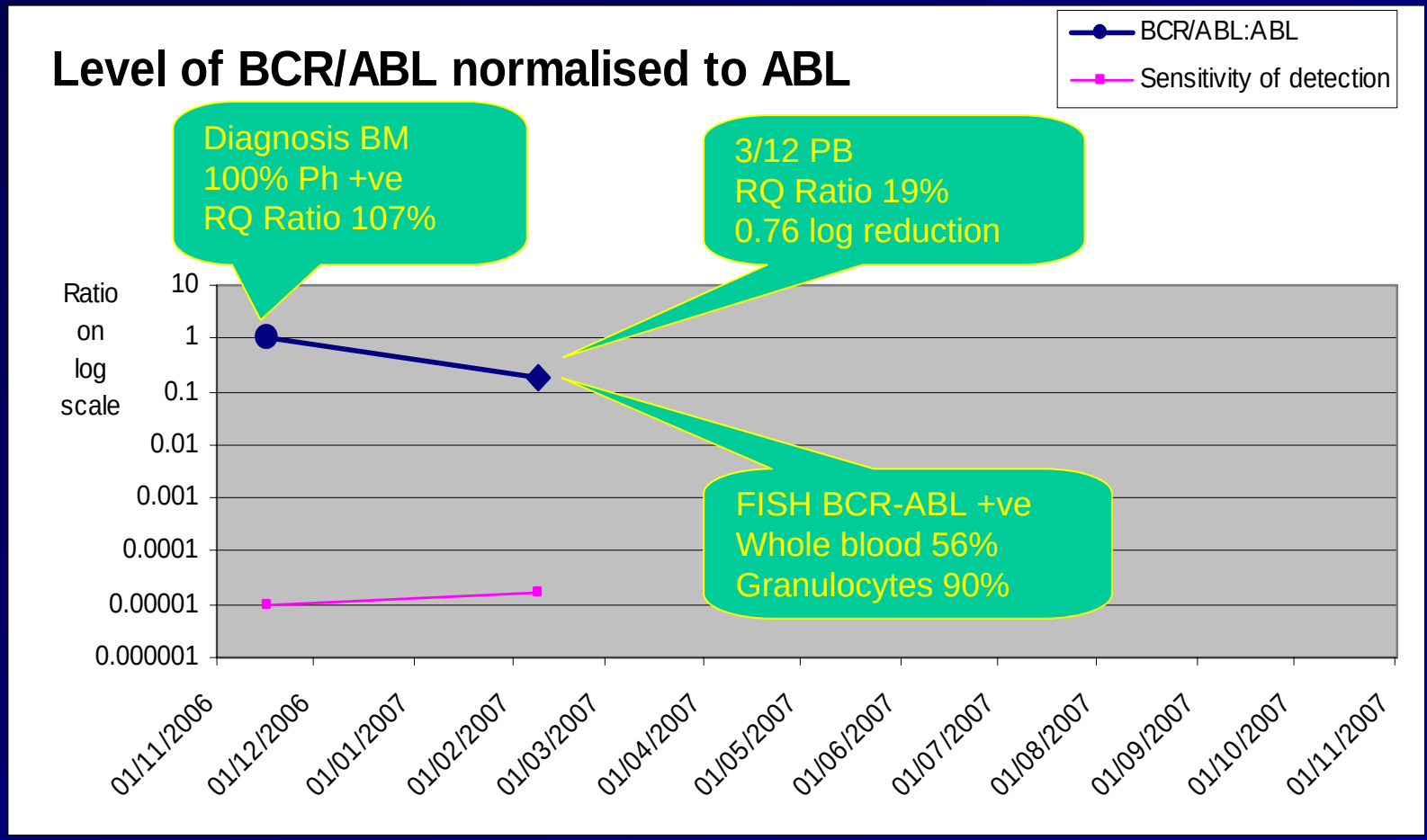
Potentially misleading results from whole sample interphase FISH (wIF)

?Due to neutropaenia & proportion of uninvolved lymphocytes in sample

Seen consistently since.

Are there implications for RQ-PCR on PB?

Patient D. RQ-PCR on PB



Follow up at 3/12. Pattern typical – seen in many patients.

Factors to consider

- What cells are expected in the BM?
- What cells are expected in PB?
- Which cells represent the leukaemia?

- How do CML cells behave?

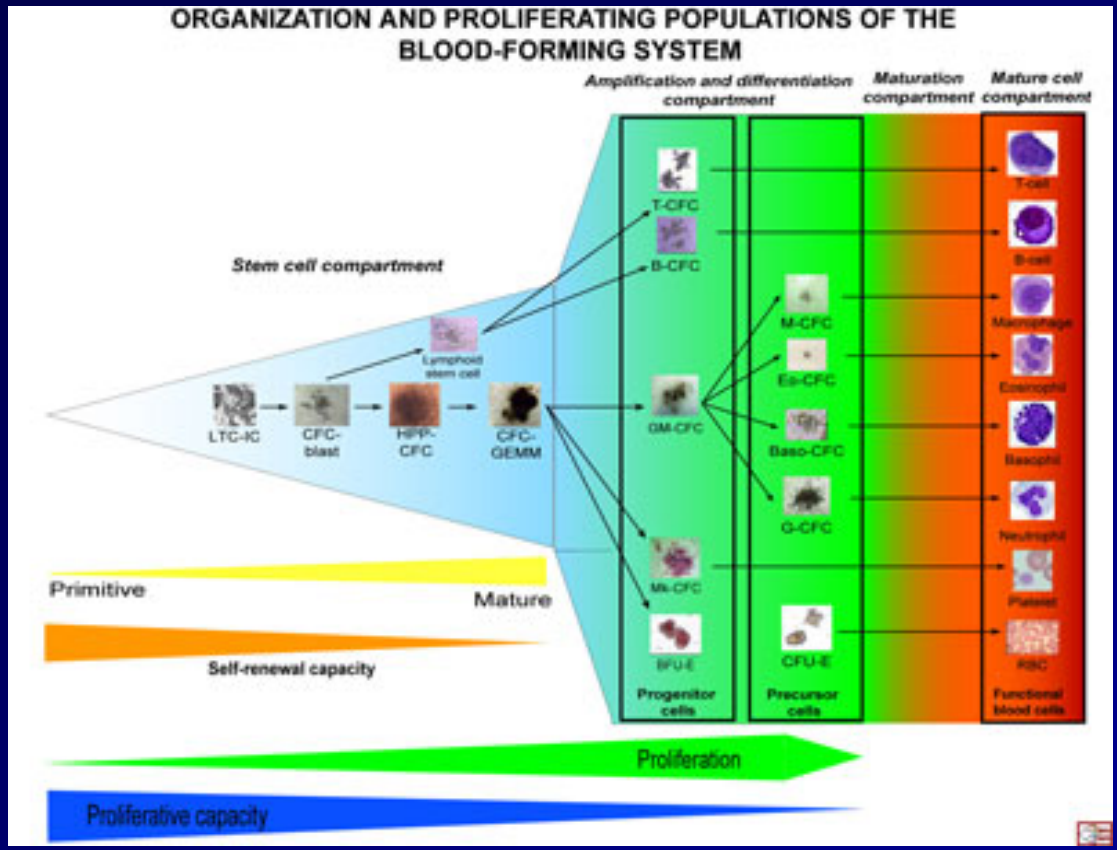
Typical Marrow and Blood cells

Cell types	Marrow %	Blood %
Neutrophils (segmented)	10-30	40-65
Neutrophils (band)	10-30	
Eosinophils	0-6	4
Basophils	0-2	1
Monocytes	0-3	6
Myeloid Precursors	11-48	
Lymphocytes	5-15	20-40
Plasma cells	0-2	
Megakaryocytes	0-2	
Nucleated rbc	18-34	

Data combined from various sources

Is treatment induced neutropaenia due to reduction in Ph cells?
Or a generalised response?
What are the kinetics of response? Do blood mature Ph + grans clear most rapidly?
If neutropaenic - blood virtually all lymphocytes - ? Minimal involvement in CML

Marrow cells mitotic, blood cells are not.



http://www.hemogenix.com/stem_cells_and_the_blood-forming_system/files/Diagrams/Heirarchyv7.jpg

- How do CML cells behave?
  - No differentiation block
  - Early release from marrow
  - Delayed maturation
    - i.e. continue dividing after enter blood
- Normal blood does not contain mitotic cells

Factors to consider - and consequences

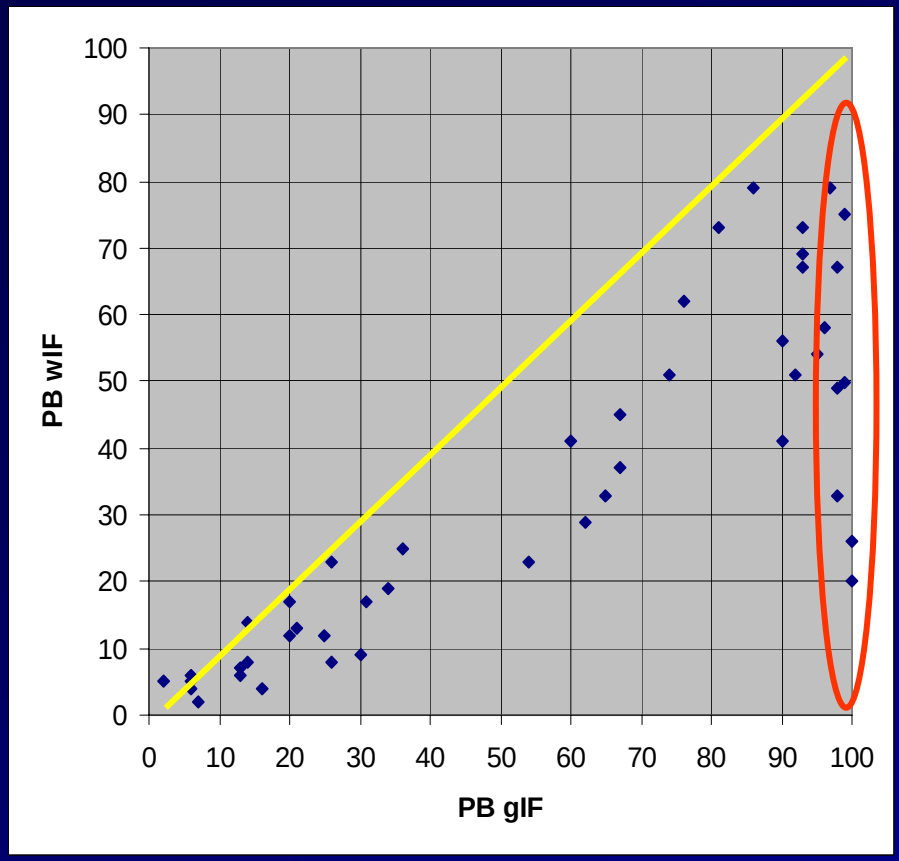
- What cells are expected in the BM?
  - Mostly immature myeloid cells
- What cells are expected in PB?
  - Mostly mature granulocytes & lymphocytes
- How do CML cells behave?
  - No differentiation block
  - Early release from marrow
  - Delayed maturation
    - i.e. continue dividing after enter blood
- Ph +ve metaphase cells
  - Gold standard for level of disease
  - (if there is enough leukaemia)
- BCR/ABL +ve interphase cells
- RQ-PCR BCR/ABL:ABL ratios
  - If neutropaenic - potential to under-represent level of disease
- Ph +ve metaphase cells in blood
  - Over-represent level of disease

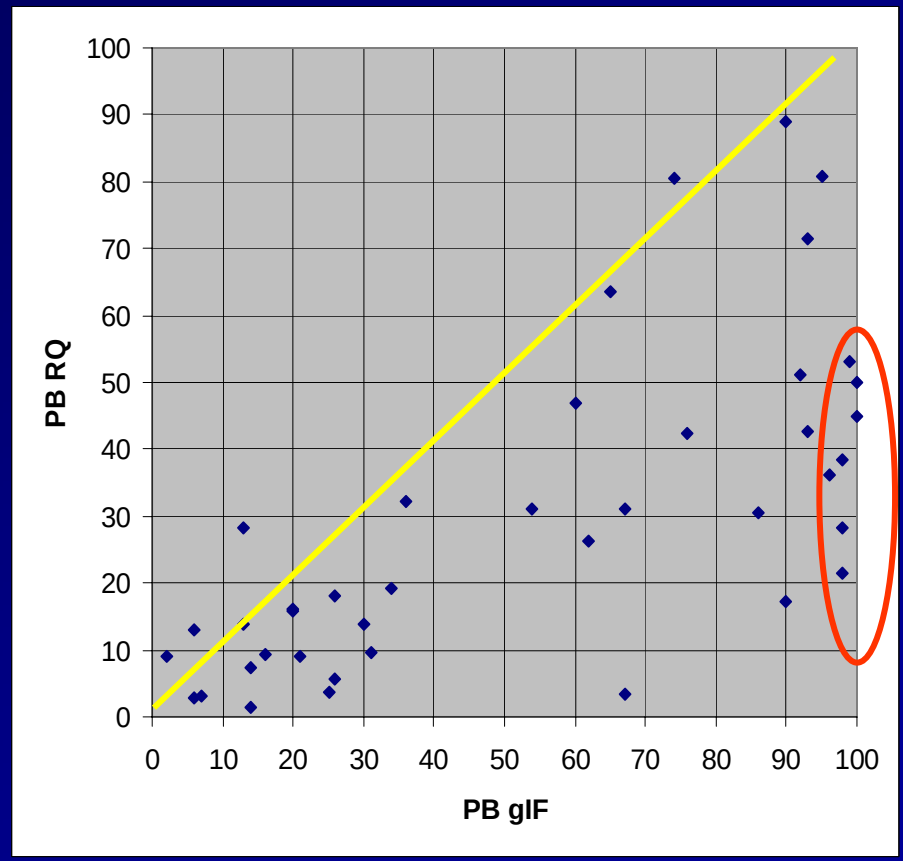
More Prospective data

- Data extracted from diagnostic cases where parallel tests have been performed.
  - Chronic phase CML, within first 2 years of treatment with Glivec
  - Diagnosed since 2002
  - Ph percentage on at least 20 cells (max 60)
  - Interphase FISH on at least 50 nucleii
  - Samples within 2 weeks considered concurrent
  - Prior to any subsequent complete loss of response
  - BCR/ABL to ABL percentage ratio adjusted to % of patient specific diagnostic ratio, or % of mean diagnostic ratio (Diagnosis = 100%).

- Not comprehensive data as resource limited diagnostic series.

PB correlations





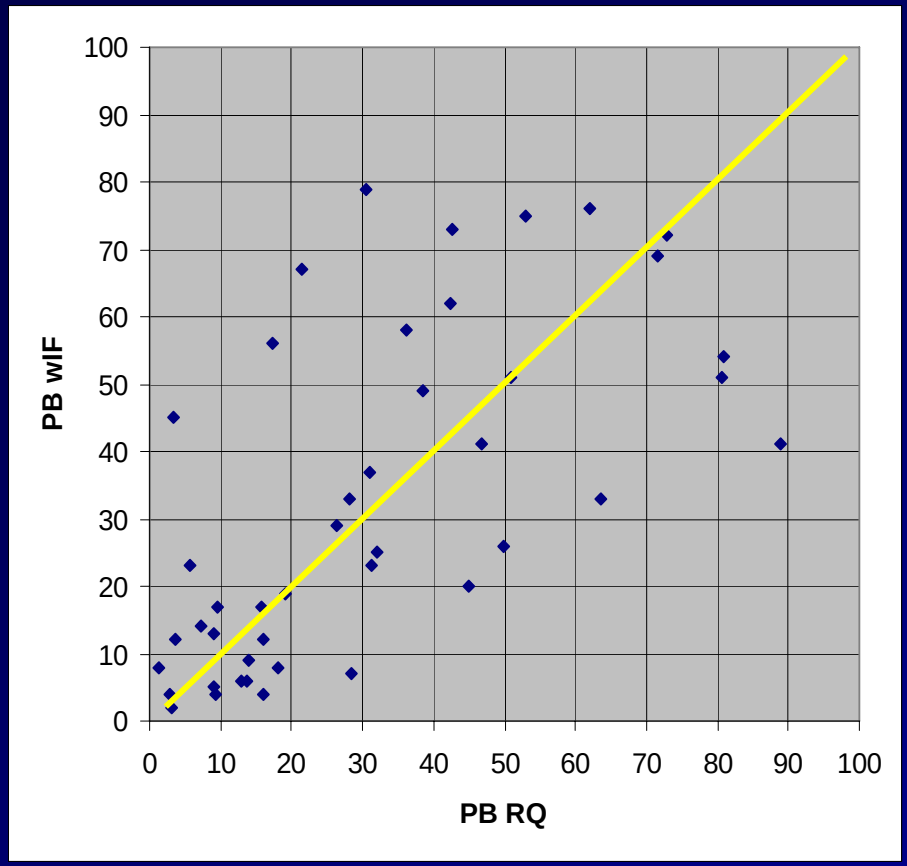
Mean wIF/gIF = 0.61

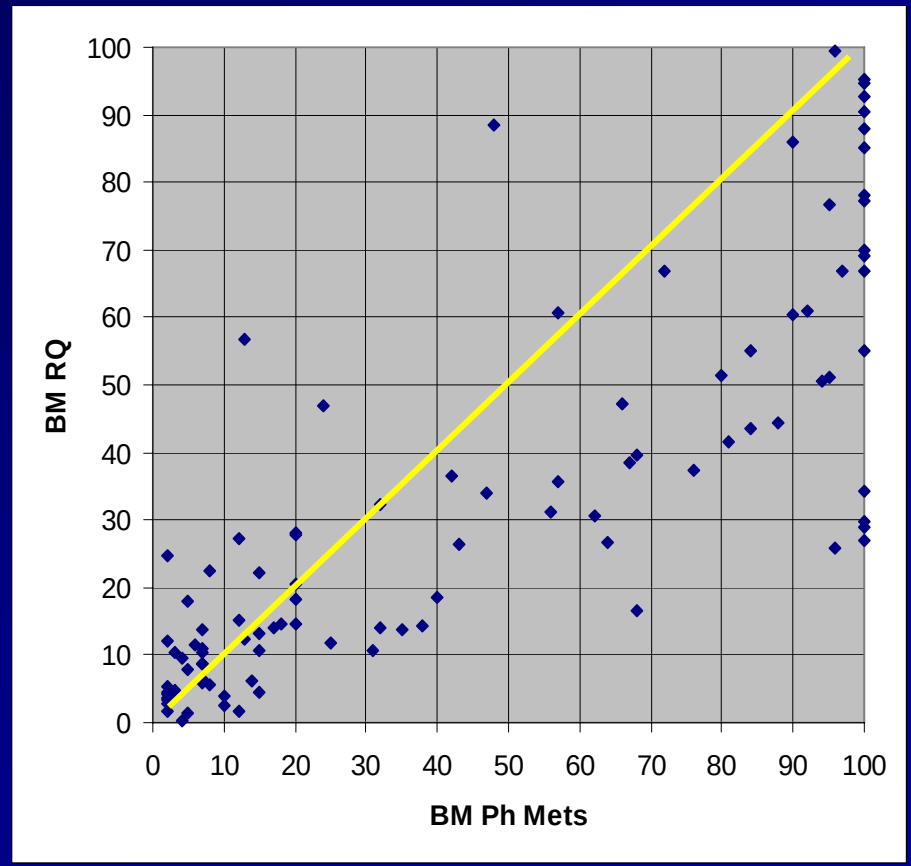
Excludes gIF < 15%

Mean PB RQ/gIF = 0.72

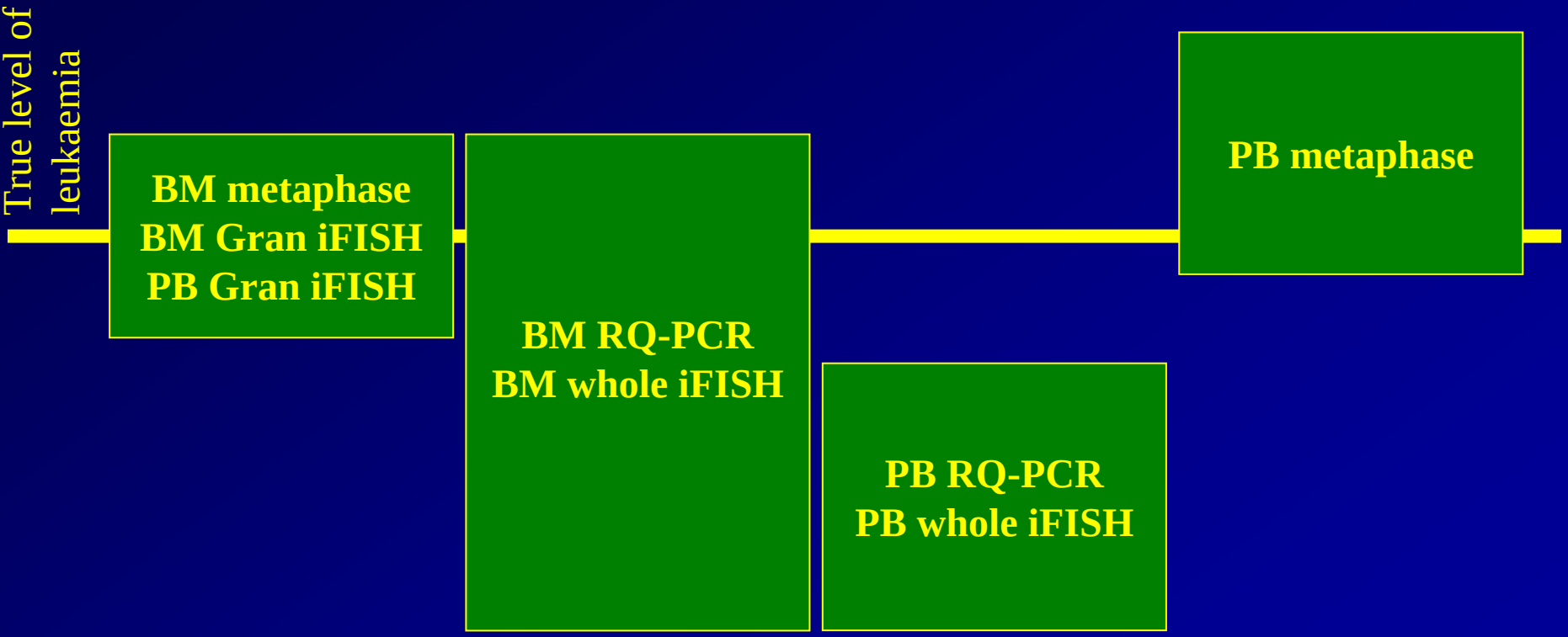
Excludes gIF < 15%

Correlations 2





Summary – Prior to achieving CCyR



Summary – Implications for early treatment monitoring

Our view:

- BM samples every 3/12 until CCyR preferred (then PB every 3/12).
- If PB when not in CCyR:
  - Caution with respect to RQ-PCR & whole interphase FISH
  - Caution with respect to metaphase chromosome analysis
  - Granulocyte iFISH preferred
    - Selective scoring by morphology
    - or selection prior to test (MACS, FACS, lymphoprep)
      - See Reinhold et al. Leukaemia 2003;17:1925-1929
- RQ-PCR at 3/12 on PB likely to be misleading.
  - If no HR at 3/12 then BM preferred to PB
  - If PB:
    - If RQ-PCR shows no response – plausible
    - If RQ-PCR shows response – NOT reliable – confirm granulocyte iFISH