K562/GM-CSF Vaccination in Combination with Imatinib Mesylate for Chronic Myeloid Leukemia

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Disclosure: Conflict of Interest

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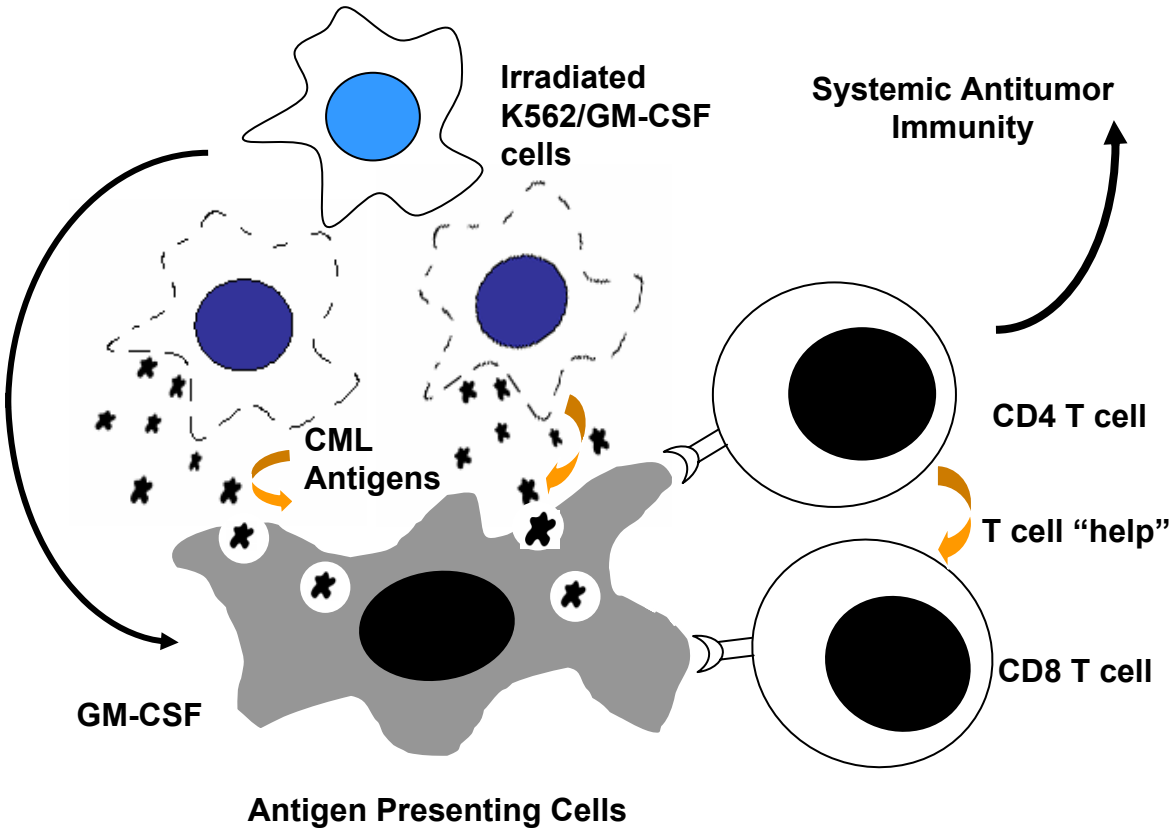
CML is an "Ideal Disease" to Study Impact of Immunotherapy

- CML is "immunoresponsive":
  - Allogeneic BMT = curative:
  - T cell deplete allogeneic BMT = increased relapse
  - Donor lymphocytes are effective salvage

- Imatinib mesylate produces minimal residual state:
  - Cytogenetic remissions ~ 80% newly diagnosed pts

- Tumor cell vaccine = K562/GM-CSF - derived from CML erythroblast cell line offers potential target antigens:
  - BCR/ABL - Survivin - Proteinase-3
  - PRAME - hTERT - WT-1

Proposed Mechanism of T-cell Activation via K562/GM-CSF Immunotherapy



K562/GM-CSF Immunotherapy + Gleevec:
Pilot Study

- **Primary Objectives:**
  - **In pts treated with IM > 1 year, can K562/GM-CSF vaccination:**
    - **↓ tumor burden?**
    - **↑ complete cytogenetic or molecular responses?**
  - **Assess safety and tolerability**

- **Secondary Objectives:**
  - **Characterize T cells → CML associated antigens**
  - **Quantify pre and post vaccine antigen-specific T cells**
  - **Characterize the immunological effects of adding a topical toll-like receptor agonist at vaccination sites**
  - **Characterize the cellular infiltrate at vaccine site**

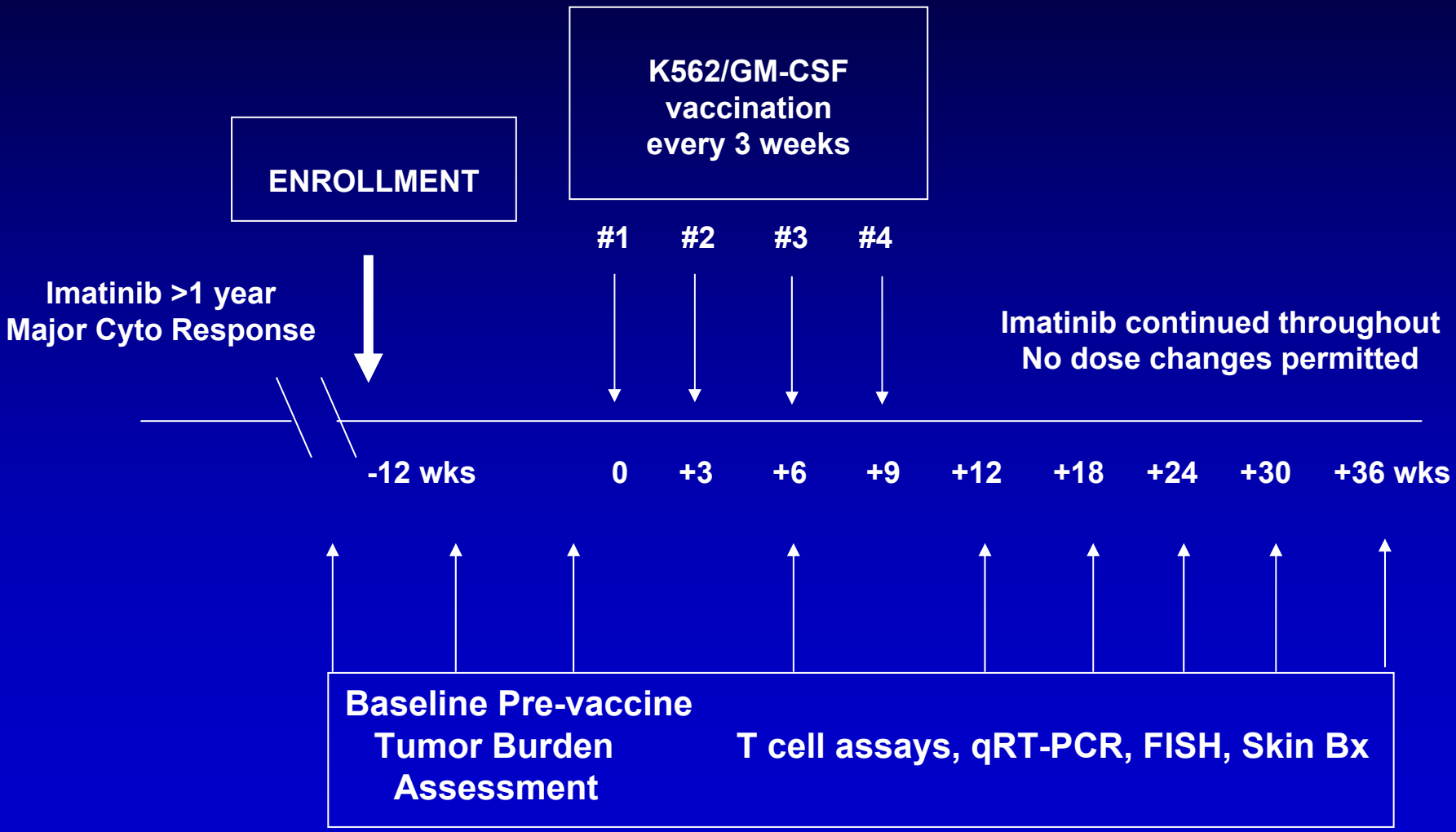
K562/GM-CSF Immunotherapy + Gleevec:
Patient Eligibility

- Adults with chronic phase CML
  - Treated x 12 mos imatinib (minimum)
  - Imatinib dose unchanged throughout trial
- Major cytogenetic response
- Measurable disease burden:
  - peripheral blood = FISH
  - peripheral blood = PCR

PCR Assay: BCR-ABL

- Detection: BCR-ABL fusion mRNA using:
  - Taqman one step quantitative RT-PCR assay
  - Fusion Quant reagents (Ipsogen)
  - ABI Prism 7900 instrument
- Amplification: ABL mRNA = control gene
- Standardization: BCR-ABL and ABL curves generated with each run
- Quantification: ratio = copies BCR-ABL mRNA per 1000 copies ABL mRNA
- Limit of Detection: 1 in 1 x 10^5 to 5 x 10^5 copies
- Resolution: – 0.5 - 1 log

K562/GM-CSF Immunotherapy + Gleevec:
Treatment Schema



K562/GM-CSF Immunotherapy + Gleevec:
Treatment Plan

- Four vaccines administered at 3 week intervals:
  - $1x10^8$ irradiated K562/GM-CSF cells distributed over 10 sites

- 5% imiquimod (Aldara) = TLR-7 agonist:
  - Induction of cytokines from monocytes: IFN-α, TNF, IL-12
  - Enhance antigen presentation
  - Last 14 subjects received topical aldara
    - ~ 85 mg at 9 of 10 injection sites x 8 hours, QOD x 3

- Disease burden measured every 6 wks for 6 mos
  - Measures = 3 pre vaccine, 1 during, 5 post final vaccination

  - Every other sample was split at time of collection:
  - ½ processed = real time ½ batched = processed at end

K562/GM-CSF Immunotherapy + Gleevec:
Patient Characteristics (n = 19)

- Male : Female — 9 : 10
- Median Age — 52 (28-76) years
- Median duration disease — 57 (16-111) months

- Previous Therapy Gleevec (n = 19)
  - Median dose — 400 mg (300-800 mg) daily
  - Median duration — 37 (13-53) months

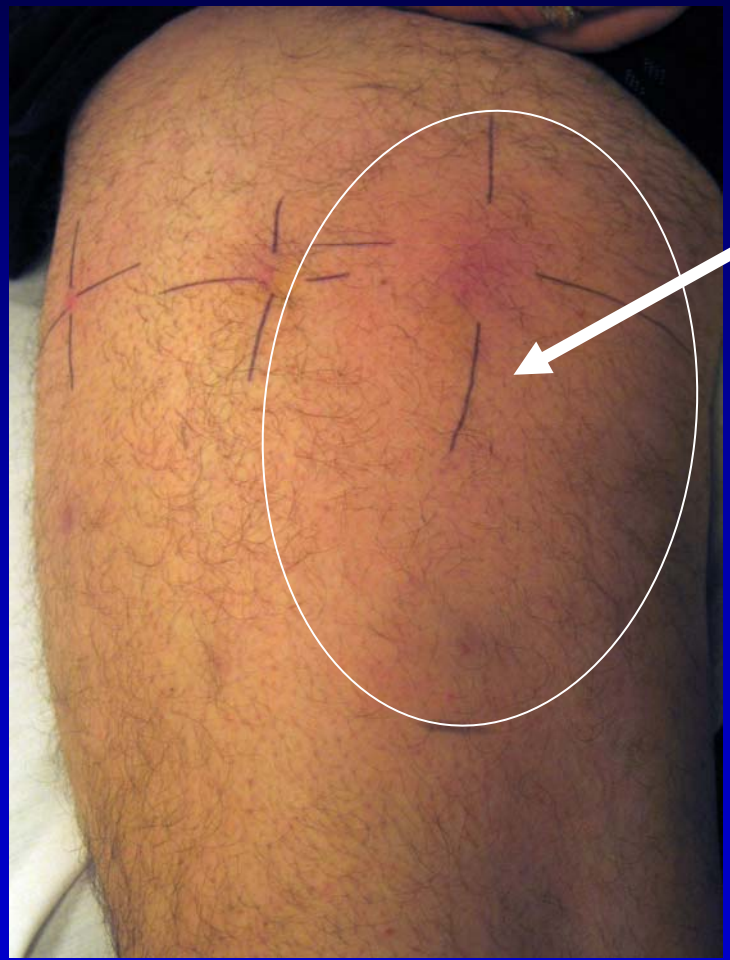
- Previous Therapy Interferon (n = 16)
  - Median dose — (3-13 x 10^6) units daily
  - Median duration — 13.5 (5-75) months

- Best Response to Gleevec at enrollment:
  - FISH Positive = 4
  - FISH Negative / PCR Positive = 15

K562/GM-CSF Immunotherapy + Gleevec:
Immunotherapy Toxicities

- All patients experienced skin reactions at injection sites (grade 1-2)
  - 3 subjects with grade 3 = better with cold packs
- Other adverse reactions possibly related to vaccine, include:
  - Myalgias (grade 1-2) = 4 subjects
  - Temperatures (grade 1) = 3 subjects
  - Flu-like feelings = 2 subjects
  - Fatigue (grade 1-2) = 2 subjects
- No noted hematologic or autoimmune toxicities

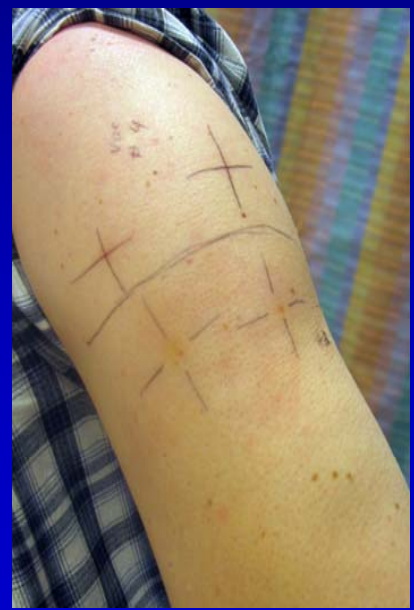
K562/GMCSF Immunotherapy + Gleevec:
Vaccination Site Evaluation



Vaccines administered horizontally across the extremities

Measure area of erythema and induration

Some patients had “recall” at old vaccine sites



K562/GM-CSF Immunotherapy + Gleevec:
Response Summary

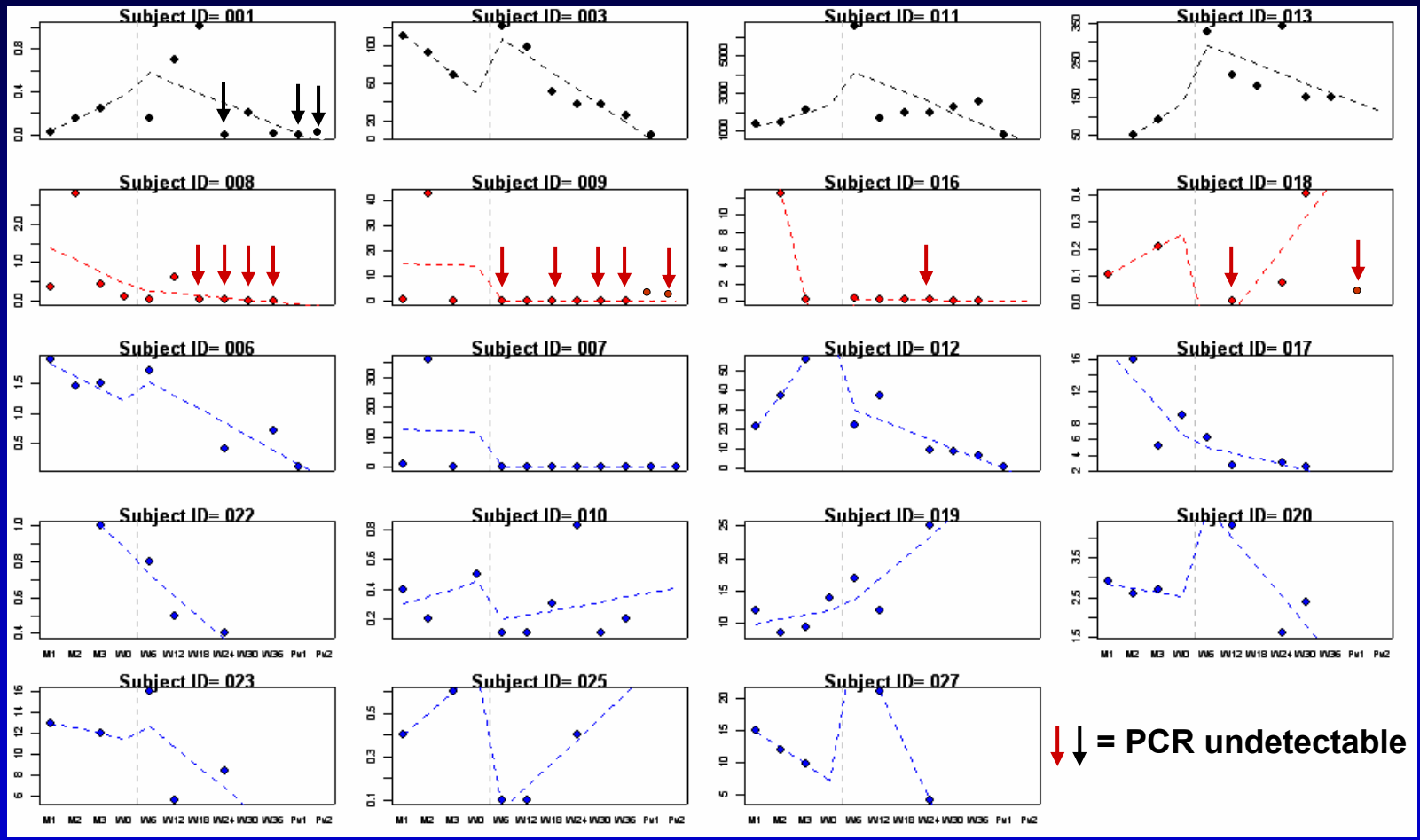
- Overall "response rate" = 10/19 pts (53%)
- FISH pos (n = 4): 2 responses
  - 1 pt = FISH negative → PCR negative
  - 1 pt = FISH negative → PCR positive (> 1.5 log ↓)
- FISH neg / PCR pos (n = 15): 8 responses
  - 4 pts = PCR negative
  - 4 pts = > 1 log ↓ in PCR

K562/GM-CSF Immunotherapy + Gleevec:
Statistical Analysis

- **Analyses of Trends: an analysis was conducted to examine individual trends in PCR assay values over ALL available measures**
- **Generalized Estimating Equations: estimate weighted mean differences in PCR results pre- vs post- vaccination values over ALL available measures**
- **Chi-squared statistics: tested hypotheses that pre- and post- vaccination measures would be different (Type I error = 0.10 due to small sample size)**

Statistical Analysis

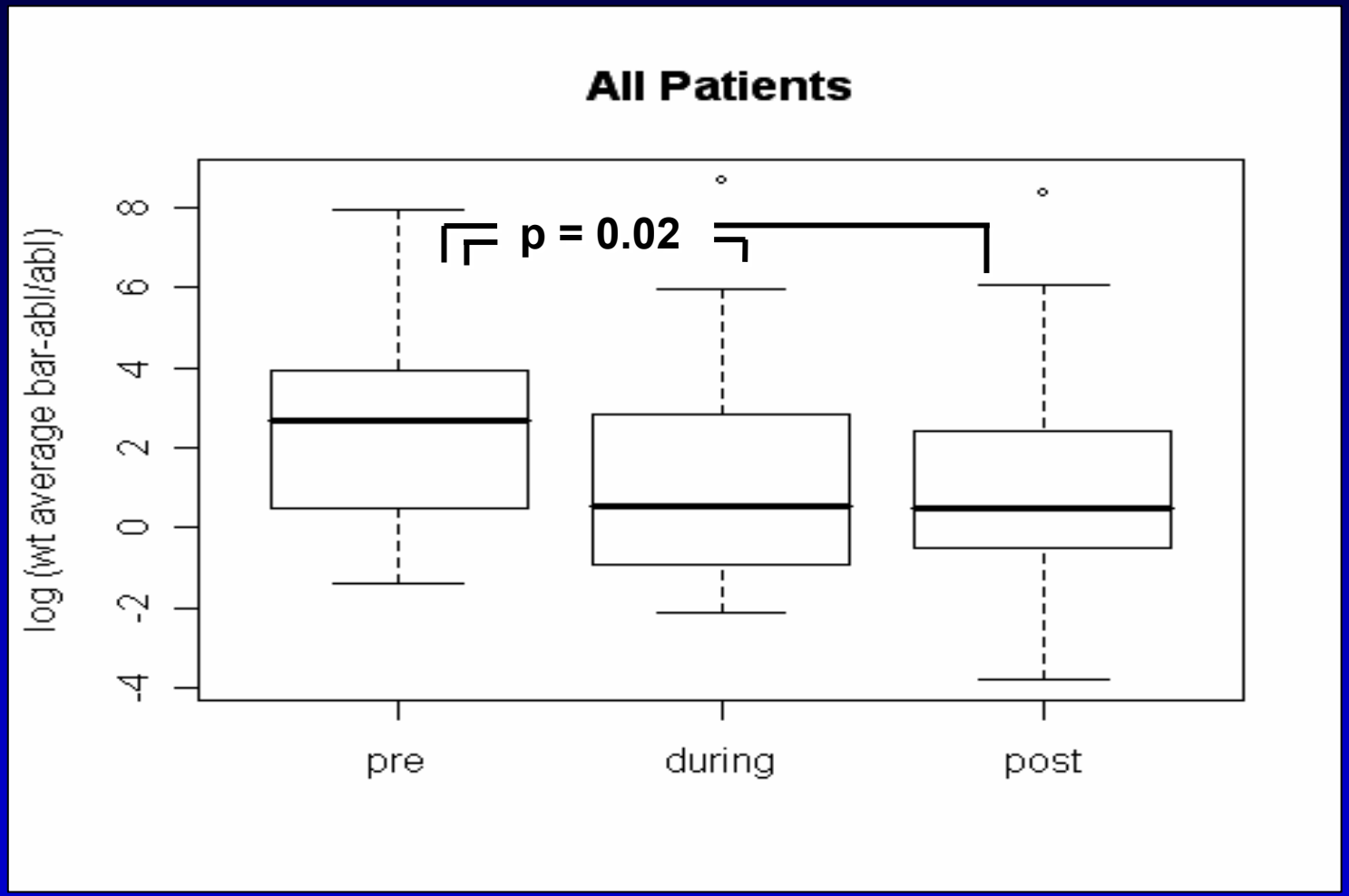
Scatterplots of PCR = Analysis of Trends



- PCR measures – both batched + contemporaneous values used
- Generalized Estimating Equation = mean differences pre- and post-vaccine, p=0.02

K562/GM-CSF Immunotherapy + Gleevec:

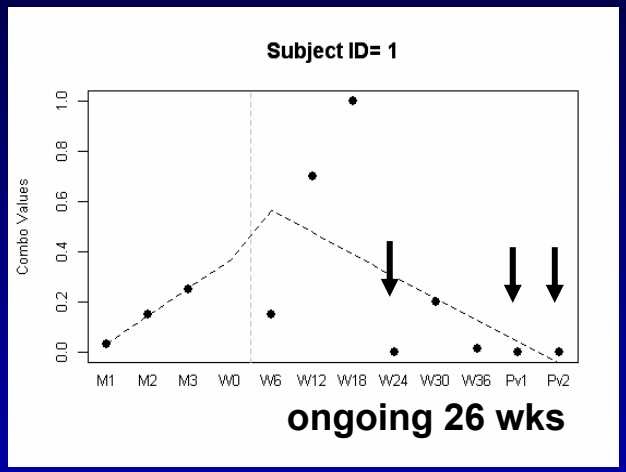
Change in PCR Pre, During, Post Vaccine



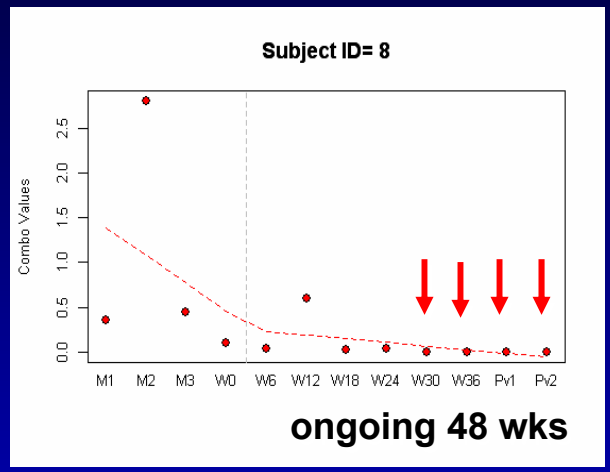
Significant ↓ between pre and post vaccine PCR values, p = 0.02

Statistical Analysis = PCR neg

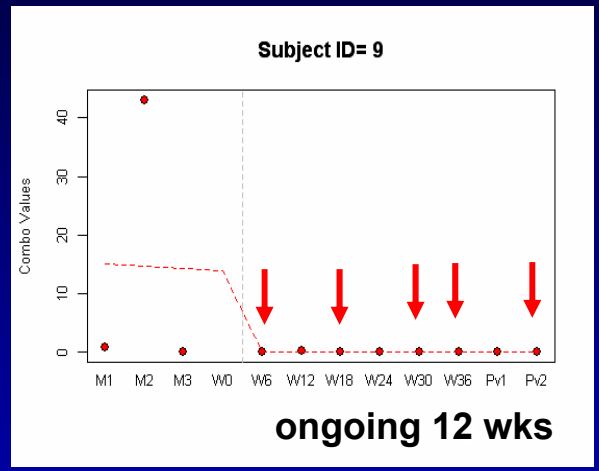
Scatterplots of PCR = Analysis of Trends



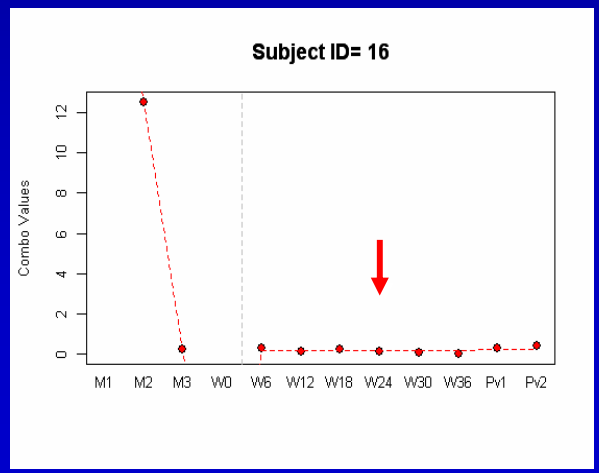
ongoing 26 wks

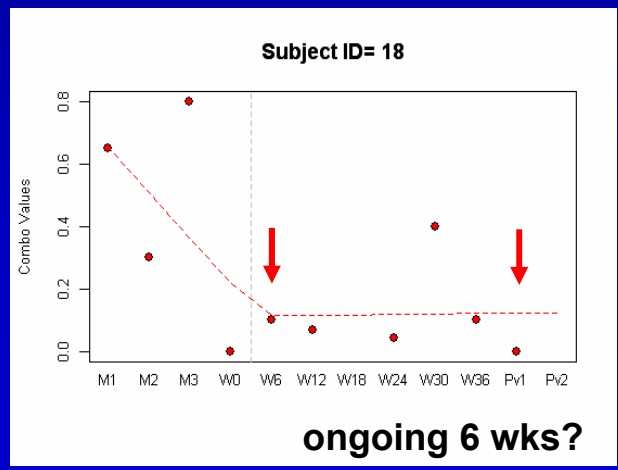


ongoing 48 wks



ongoing 12 wks





ongoing 6 wks?

↓ = PCR undetectable

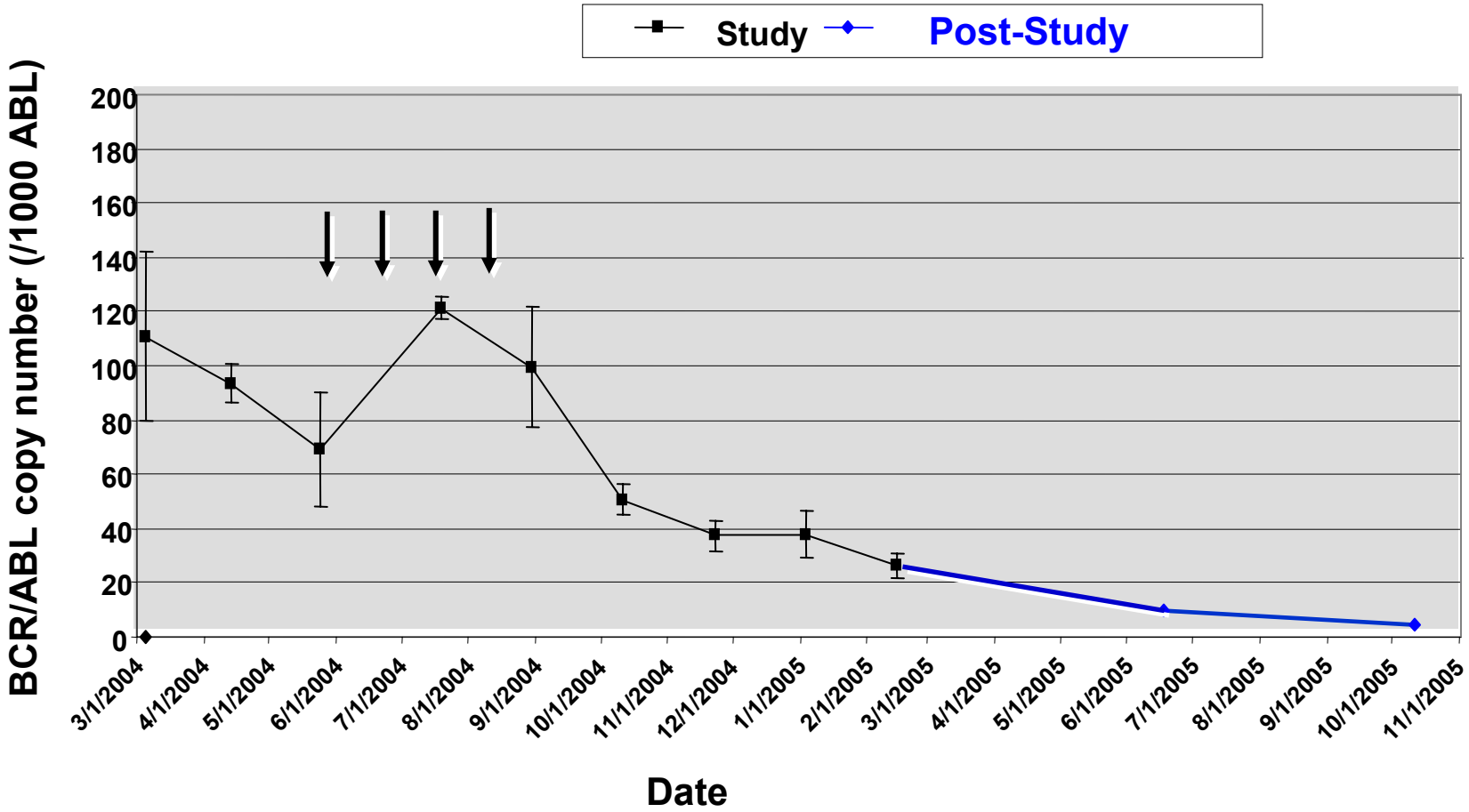
K562/GMCSF Immunotherapy + Gleevec:

Patient 003 Clinical History

- 57 year old with minimal PMH
- Spring 1999, noted ↑ WBC routine evaluation
- Diagnosed with CML-CP
  - June 1999, Interferon x 10 mos = minor cyto response
    - poorly tolerated
  - July 2000, initiated on STI-571 (110) = major cyto response
  - July 2003, lost maj cyto response = ↑ to 600 mg daily
    - Returned to major cyto response (never complete)
- March 2004 – evaluated for vaccine trial

Change in PCR Over Time

Patient 003 – B. K.



K562/GM-CSF Immunotherapy + Gleevec:
Responder Characteristics

	Molecular Remission					log reduction (> 1)				
Duration of Disease	37	16	59	50	78	58	19	86	18	112
Previous Interferon?	+	-	+	+	+	+	+	+	+	+
Duration of Gleevec	24	15	48	25	51	43	13	49	18	37
Gleevec Dose (mg)	800	600	400	800	400	600	600	400	800	400
Dx Burden (FISH)	+	-	-	-	-	+	-	-	-	-

Yellow = Aldara Patients

K562/GM-CSF Immunotherapy + Gleevec:
Conclusions

- **K562/GM-CSF vaccine is safe and improves individual responses in patients on imatinib:**
  - **Including achieving molecular remissions despite long-term imatinib treatment**
  - **Minimal toxicities observed**
- **Vaccine "responses" seen in both:**
  - **patients previously on interferon vs none**
  - **aldara and non-aldara arms**
  - **subjects with FISH (+) and FISH (–) disease burden**
  - **early (during) and late (after vaccination)**
- **Vaccine may eliminate residual leukemia following cytoreduction with imatinib mesylate**

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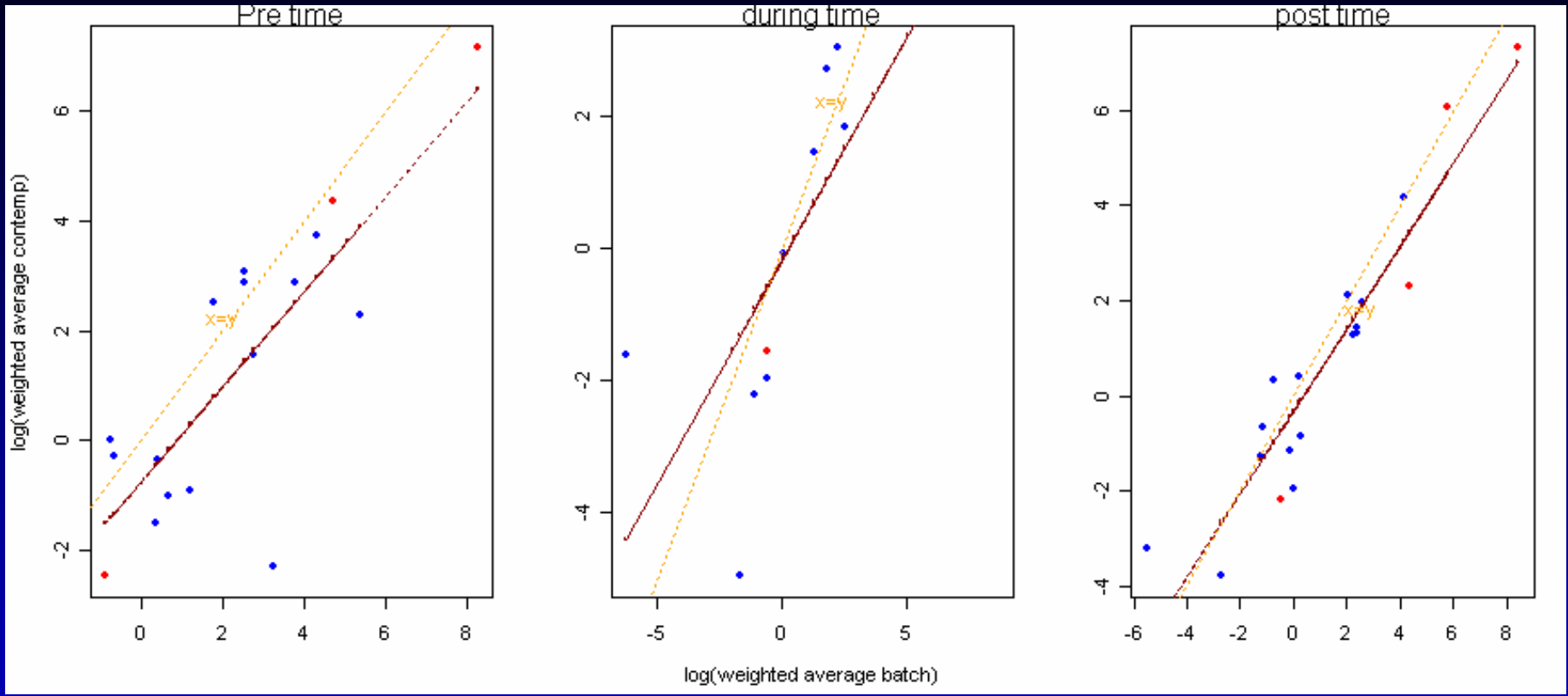
Clinical Response

* Aldara Patients

Sub	Age	Gleevec (Duration)	Fish Status Pre-Vaccine	Best Response Post Vaccine	Time: Vaccine to Response	Current Status (months from vaccine)
001	60	800 (24 mos)	FISH pos	PCR neg x 52 weeks	52 weeks	PCR neg (22 mos)
003	57	600 (43 mos)	FISH pos	FISH neg x 52 wks, > 1 log PCR	36 weeks	FISH neg (23 mos)
011 *	65	800 (40 mos)	FISH pos	progression	6 weeks	Off study (17 mos)
013 *	57	300 (53 mos)	FISH pos	stable disease		FISH pos (15 mos)
008 *	28	600 (15 mos)	FISH$^-$/ PCR$^+$	PCR neg x 48 weeks	18 weeks	PCR neg (17 mos)
009 *	56	400 (48 mos)	FISH$^-$/ PCR$^+$	PCR neg x 30 weeks	30 weeks	PCR neg (14 mos)
016 *	34	800 (25 mos)	FISH$^-$/ PCR$^+$	PCR neg x 8 weeks	24 weeks	PCR pos (14 mos)
018 *	51	400 (51 mos)	FISH$^-$/ PCR$^+$	PCR neg x 6 weeks	12 weeks	PCR neg (12 mos)
006	35	600 (13 mos)	FISH$^-$/ PCR$^+$	> 1 log decrease	36 weeks	PCR pos (18 mos)
007	60	400 (49 mos)	FISH$^-$/ PCR$^+$	> 1 log decrease	36 weeks	PCR pos (15 mos)
012 *	47	800 (18 mos)	FISH$^-$/ PCR$^+$	> 1 log decrease	40 weeks	PCR pos (15 mos)
017 *	77	400 (37 mos)	FISH$^-$/ PCR$^+$	.5 - .99 log decrease	12 weeks	PCR pos (11 mos)
022 *	46	600 (13 mos)	FISH$^-$/ PCR$^+$	.5 - .99 log decrease	30 weeks	PCR pos (9 mos)
010	66	400 (39 mos)	FISH$^-$/ PCR$^+$	stable disease		PCR pos (15 mos)
019 *	59	600 (49 mos)	FISH$^-$/ PCR$^+$	stable disease		PCR pos (11 mos)
020 *	44	400 (17 mos)	FISH$^-$/ PCR$^+$	stable disease		PCR pos (11 mos)
023 *	47	600 (48 mos)	FISH$^-$/ PCR$^+$	stable disease		PCR pos (8 mos)
025 *	45	800 (15 mos)	FISH$^-$/ PCR$^+$	stable disease		PCR pos (11 mos)
027 *	36	400 (14 mos)	FISH$^-$/ PCR$^+$	stable disease		PCR pos (8 mos)

Measuring Bcr-Abl…

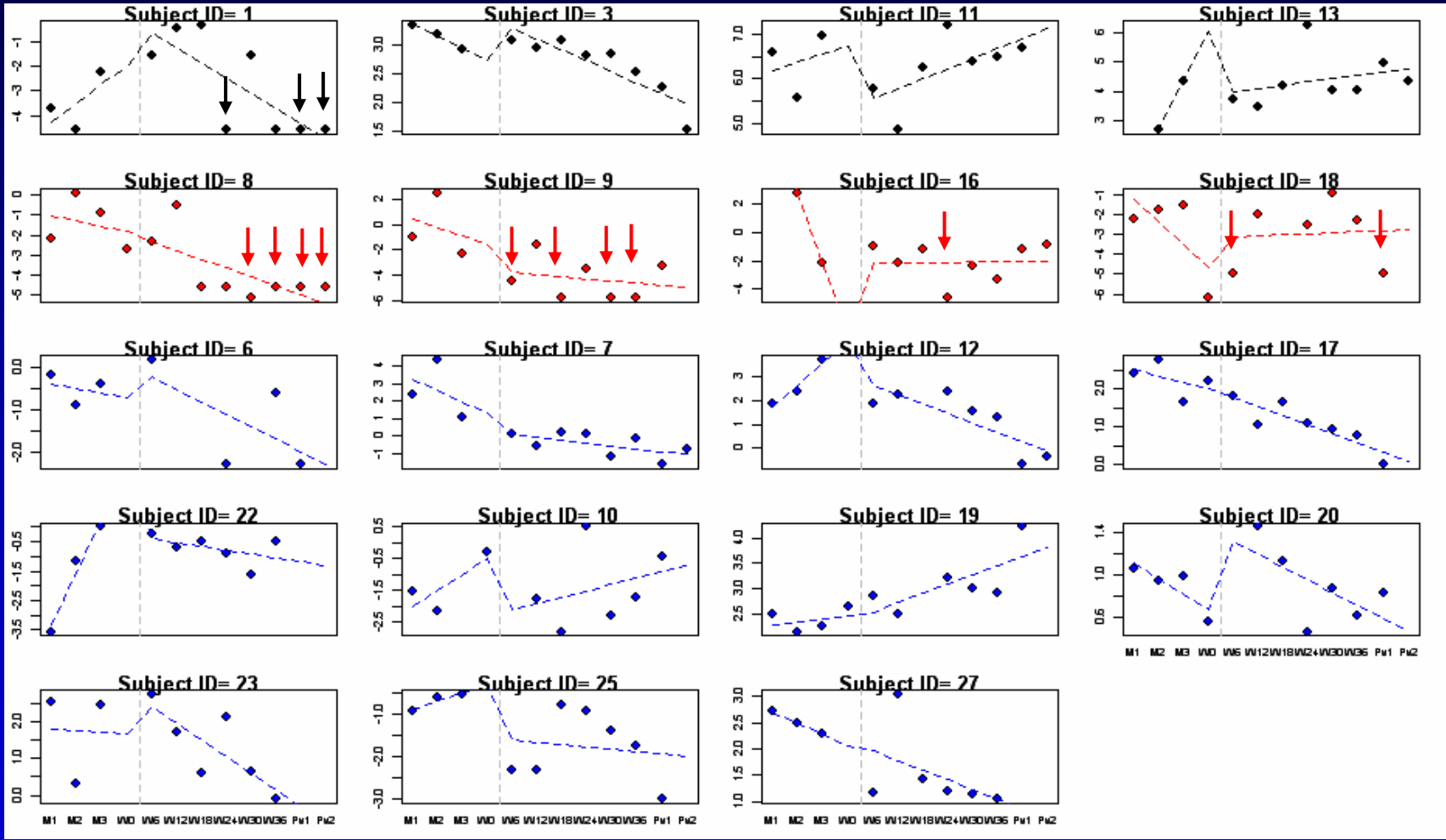
- Results were obtained in real-time (contemporaneous) and batched
- Controls were different between runs
- Some patient-visits contained contemporaneous results, some batch, and some had both available
- All results were placed on a common scale to eliminate scale effects between them by use of a regression model
- Batch results were ‘converted’ to contemporaneous and contemporaneous were left as observed, so all results are on contemporaneous scale
- Contemporaneous was selected as the common scale since such results will continue to be collected on this cohort and are typical of the type collected in practice



Scatterplot of log transformed, batch and contemporaneous (weighted) average over available evaluations within pre-, during and post-vaccination (FISH positive patients are shown in red; FISH negative are shown in blue). The orange line denotes equality (where batch equals contemporaneous weighted averages). The red line is the fitted regression.

Statistical Analysis

Scatterplots of PCR = Analysis of Trends



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- Generalized Estimating Equation = mean difference between pre- and post-vaccine, p=0.02

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Responder Characteristics

	Molecular Remission					log reduction (> 1)				log reduction (0.5 – 0.99)	
Duration of Disease	37	16	59	50	78	58	19	86	18	111	23
Previous Interferon?	+	-	+	+	+	+	+	+	+	+	+
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Gleevec Dose (mg)	800	600	400	800	400	600	600	400	800	400	600
Dx Burden (FISH)	+	-	-	-	-	+	-	-	-	-	-

Yellow = Aldara Patients