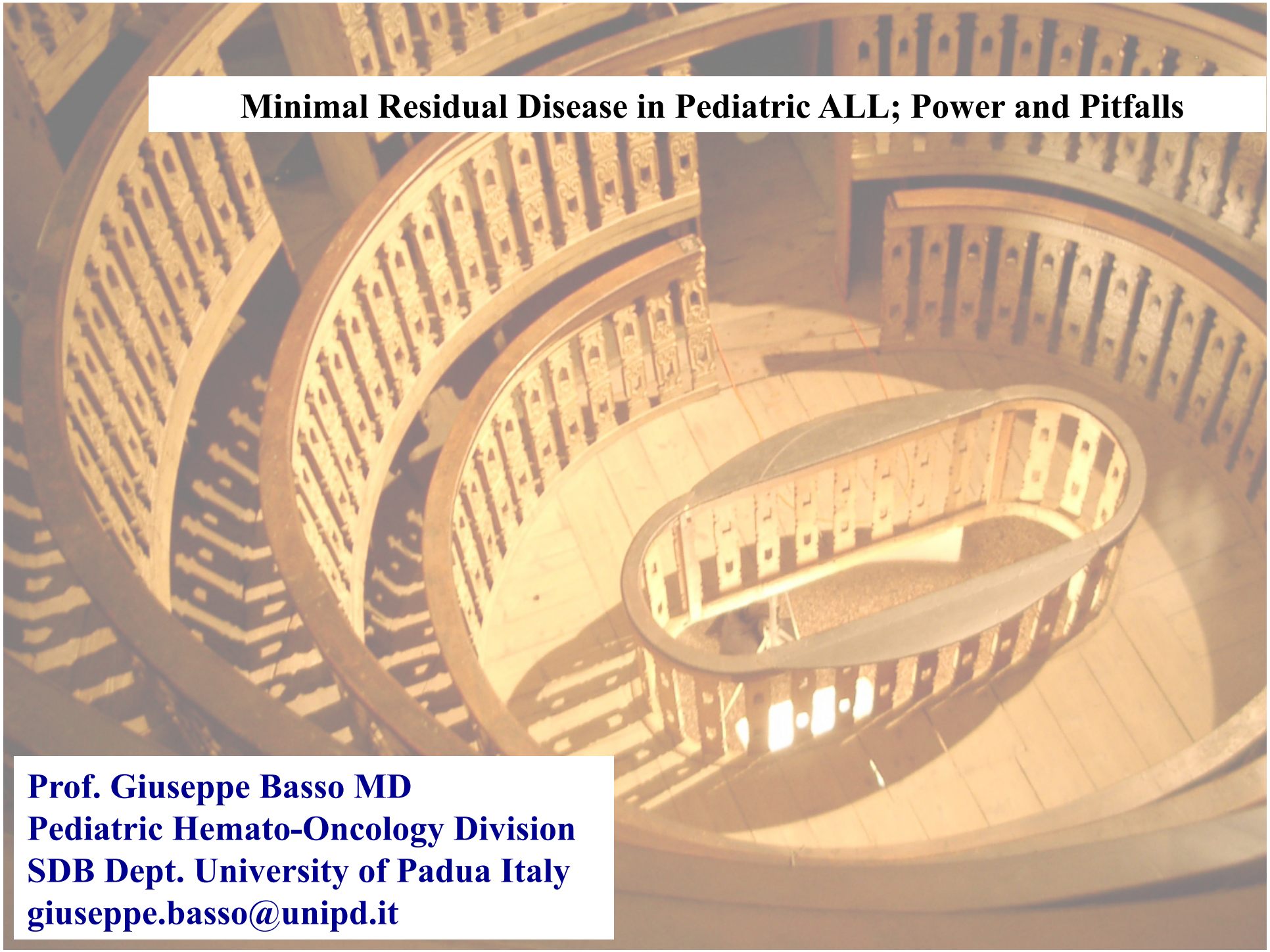

Minimal Residual Disease in Pediatric ALL; Power and Pitfalls

Prof. Giuseppe Basso MD
Pediatric Hemato-Oncology Division
SDB Dept. University of Padua Italy
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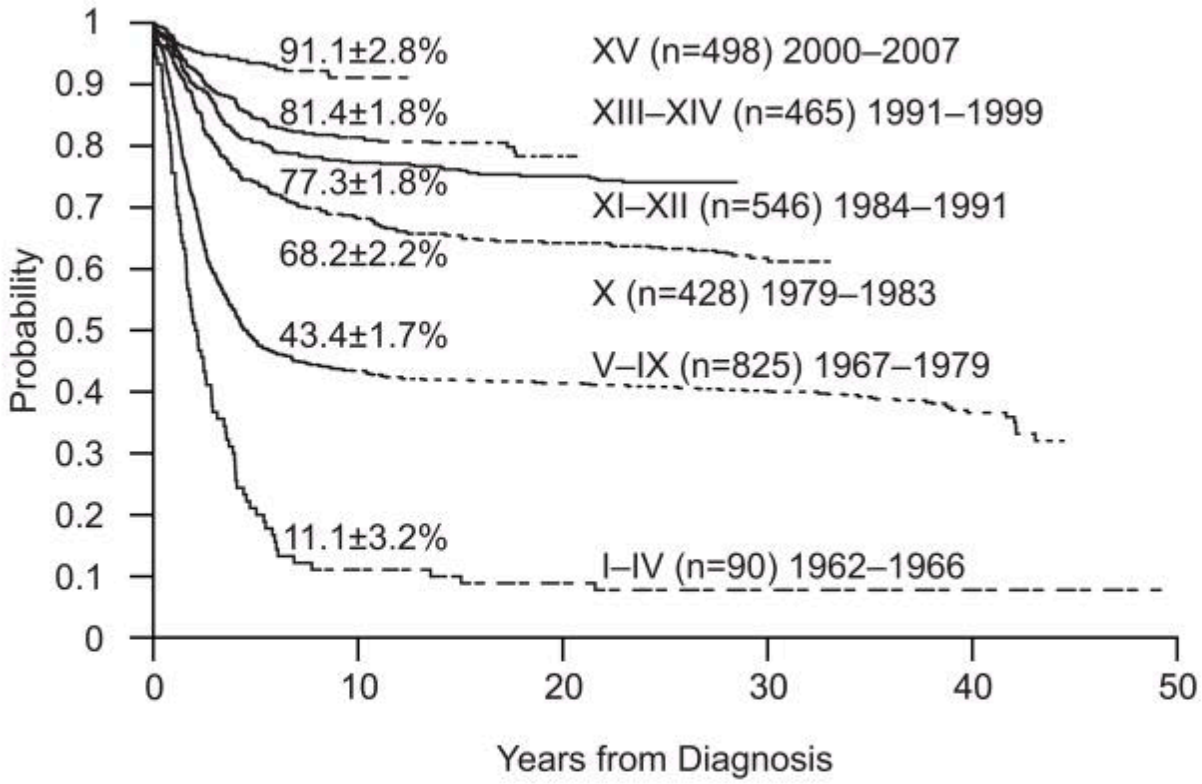
Pediatric ALL
a Success Story

Figure 1



Kaplan-Meier analysis of survival for 2852 children with newly diagnosed acute lymphoblastic leukemia who were enrolled in 15 consecutive Total Therapy studies at St. Jude Children's Research Hospital from 1962 to 2007. Ten-year survival estimates are shown. The results demonstrate steady improvement in outcome over the past half century.

IMPROVEMENT IN THERAPY RESULTS

BIOLOGICALLY DERIVED INFORMATION

- **A well defined and profound diagnostic approach**
- **Risk classification obtained from biological factors**
- ***Development of tailored therapies based on individual therapy response; MRD***
- **New therapeutic approaches(BMT, biological drugs…)**

MRD
a long story

Minimal Residual Disease in Childhood Acute Lymphoblastic Leukemia: Analysis of Patients in Continuous Complete Remission or with Consecutive Relapse

Andrea Biondi[1], Shouhei Yokota[2], Thomas E. Hansen-Hagge[2], Vincenzo Rossi[1], Giovanni Giudici[1], Oscar Maglia[1], Giuseppe Basso[3], Christel Tell[2], Giuseppe Masera[1], and Claus R. Bartram[2]

[1]Clinica Pediatrica Università di Milano, H. 'S. Gerardo', Monza, Italy, [2]Section of Molecular Biology, Department of Pediatrics II, University of Ulm, Germany, and [3]Clinica Pediatrica Università di Padova, Italy

LEUKEMIA, Vol 6, No 4 (April), 1992: pp 282–288

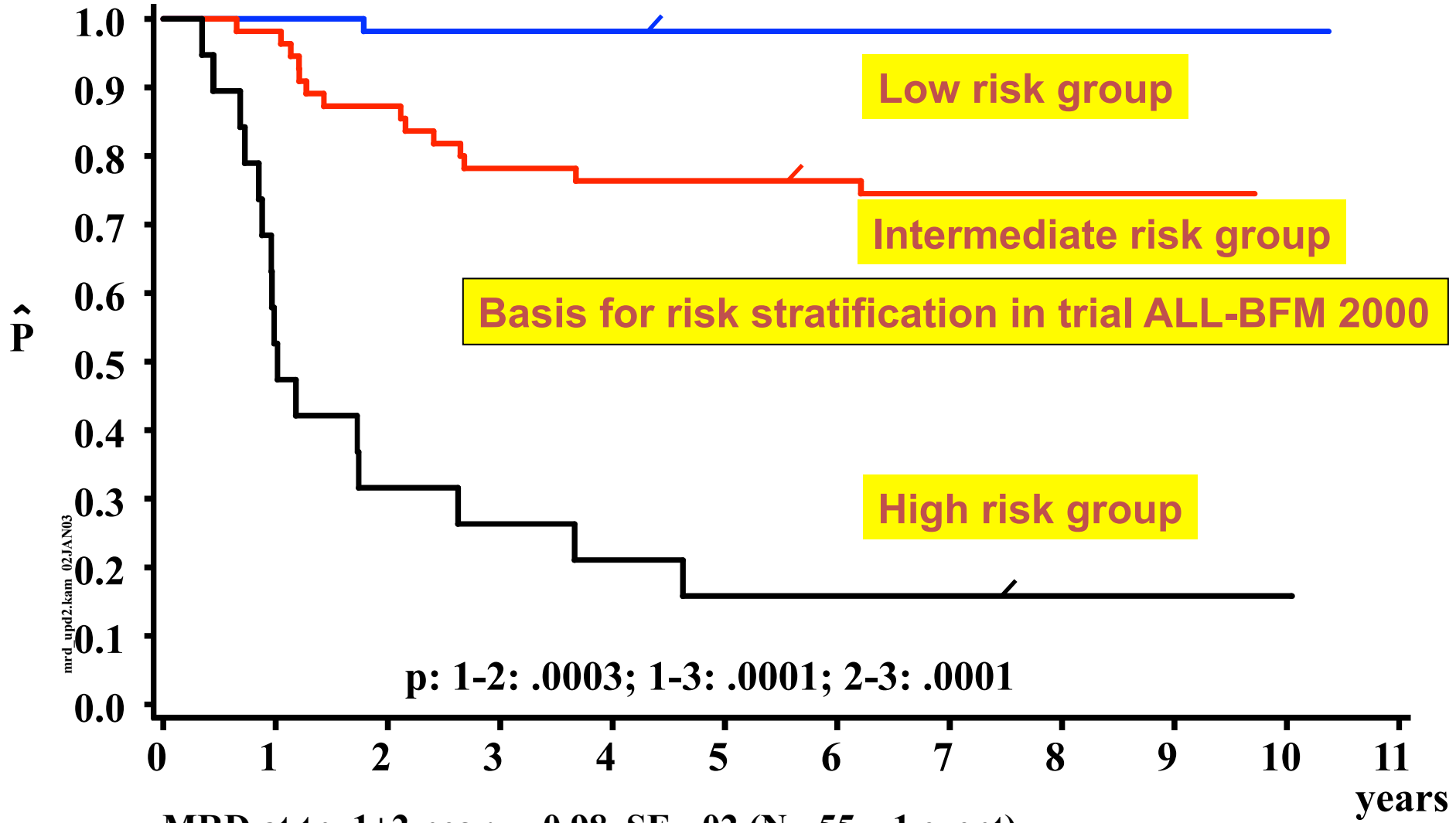
Background

PCR-based MRD measurement has prognostic implications in childhood

I-BFM-SG, Van Donghen JJ,….., Lancet ***1998***

I-BFM-SG ALL-MRD-Study: Update 2002, 5y-pEFS (fromw12)

Outcome by MRD detection levels at w5 (tp 1) and w12 (tp 2)



MRD at tp. 1+2 neg.: 0.98, SE=.02 (N= 55, 1 event)

MRD pos but < 10^{-3} at tp. 2: 0.76, SE=.06 (N= 55, 14 events)

MRD at tp. 1+2 >=10^{-3}: 0.16, SE=.08 (N= 19, 16 events)

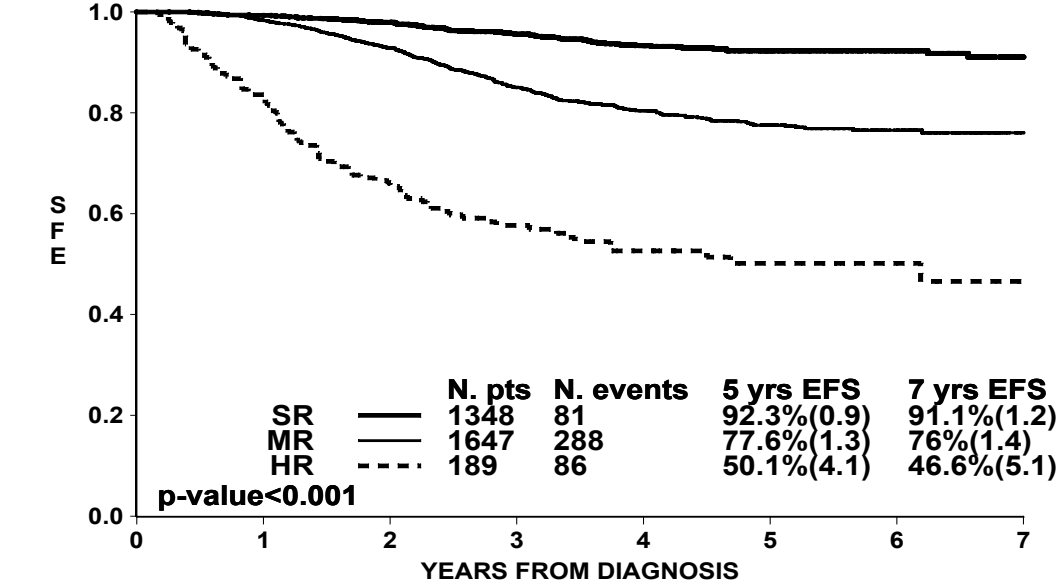
M.S. (ALL-BFM) 13.3.03

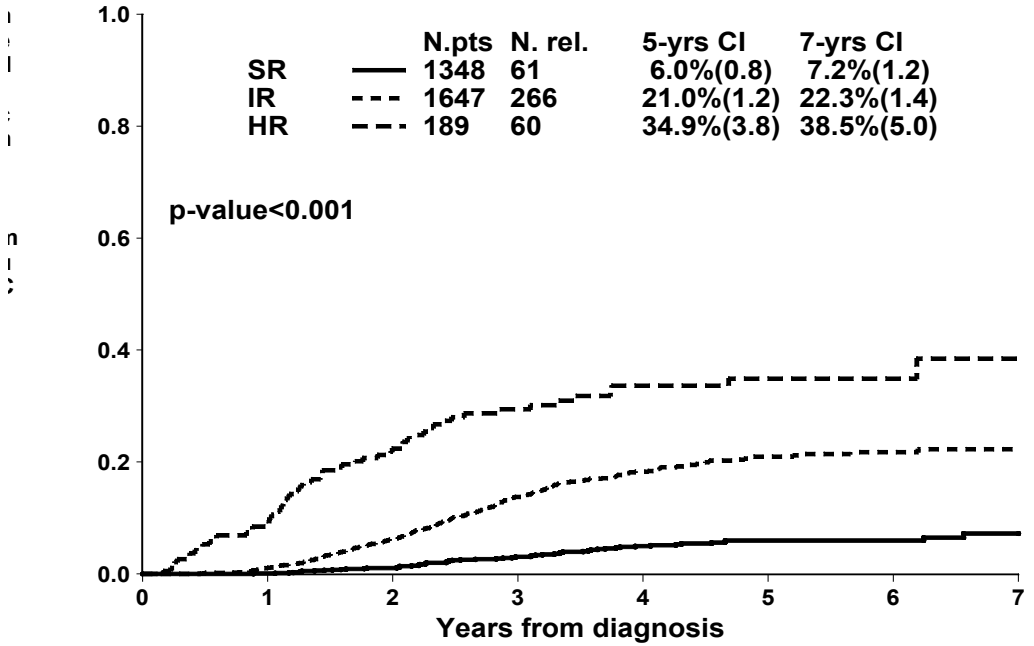
- PCR-based MRD measurement has prognostic implications in childhood ALL *(I-BFM-SG, Lancet 1998)*

- **AIEOP-BFM-ALL 2000 trial demonstrated that PCR-MRD stratification is feasible on a large scale (3184 patients) and allows identification of different patient subgroups.**

IBFM-SG, Conter V, Blood 2010

Figure 1- panel b





Conter V et al, Blood; 2010

ASH Clinical News™

Search

Monitoring minimal residual disease (MRD) has become the standard of care in pediatric patients with acute lymphocytic leukemia (ALL) *based on evidence that it is a strong prognostic factor for patient outcomes – patients who test negative for MRD have better outcomes than those who test positive. However,* ***MRD isn't a panacea. There are still many unanswered questions about what MRD is able to tell us****, and what it isn't –* ***especially in adult patients with ALL****. The use of MRD monitoring in adult patients is much less prevalent due to a lack of* ***clear evidence and inconsistencies among the labs that conduct the tests.***

By Jill Sederstrom; Minimal Residual Disease: ALL It's Cracked Up to Be?

In the United States, the most common method for detecting MRD is flow cytometry: leukemia-associated immunophenotypes…….

In Europe, the common approach is using polymerase chain reaction (PCR) to screen and amplify a DNA in the immuglobulin gene or T-cell receptor to identify a clone associated with leukemia.

Each method has its own set of advantages and disadvantages. Flow cytometry, for instance, is less expensive, can often report quantitative results within a day, and has a larger evidence base (having been used in most U.S.-based trials).

By Jill Sederstrom

The perfect MRD methodology

- High sensitivity and high specificity
- Fast
- Cheap
- Easy in standardization
- Utilized routinely in the majority of patients
- Quantification of positive cells

Practice points

- Immunologic testing of MRD relies on "leukemia-associated" immunophenotypes which can be identified in 95% or more of ALL cases.
- The sensitivity of MRD detection that can be achieved in routine assays by flow cytometry is 0.01%. The development of more powerful flow cytometric methods may extend the sensitivity to 0.001% for most cases.
- Molecular methods for MRD detection rely on PCR amplification of antigen-receptor gene rearrangements (present in approximately 95% of cases) and fusion transcripts (present in approximately 40% of cases). The sensitivity is typically 0.01%–0.001%.
- Both immunologic and molecular MRD assays have relative advantages and disadvantages but yield generally concordant results in samples with MRD 0.01% or higher.
- Results of MRD testing provide unique and clinically important information, and are now used in clinical protocols for risk assignment.

Research agenda

1. The identification of new markers of MRD should increase the sensitivity of MRD testing by flow cytometry.
2. The development of simpler and less expensive flow cytometric and molecular methods is required to widen the applicability of MRD studies.

Coustan-Smith E and Campana D, 2013

Evolution in MRD Technologies

- ***Molecular***

  NGS application increase the ability in multiclones identification and quantification (standardization on going..). False negative.

  Digital PCR and absolute quantification.

- ***FCM***

  The number of antigens simultaneously tested are increasing (sensitivity and specificity are improving);

  quality reagents

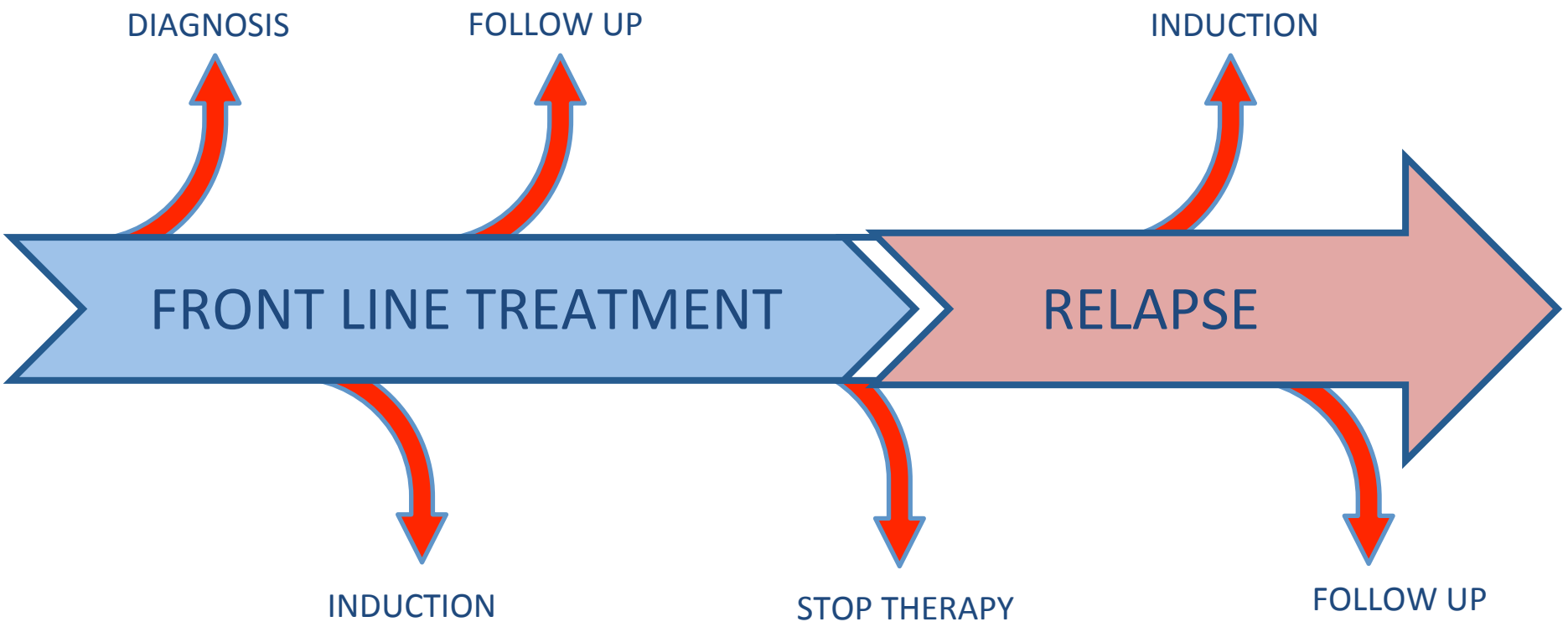
  new generation instrumentations.

Background

- PCR-based MRD measurement has prognostic implications in childhood ALL (I-BFM-SG, Lancet 1998)
- AIEOP-BFM-ALL 2000 trial demonstrated that PCR-MRD stratification is feasible on a large scale and allows identification of different patient subgroups

- **Is it possible to further refine the use of MRD measurements for treatement of childhood ALL?**

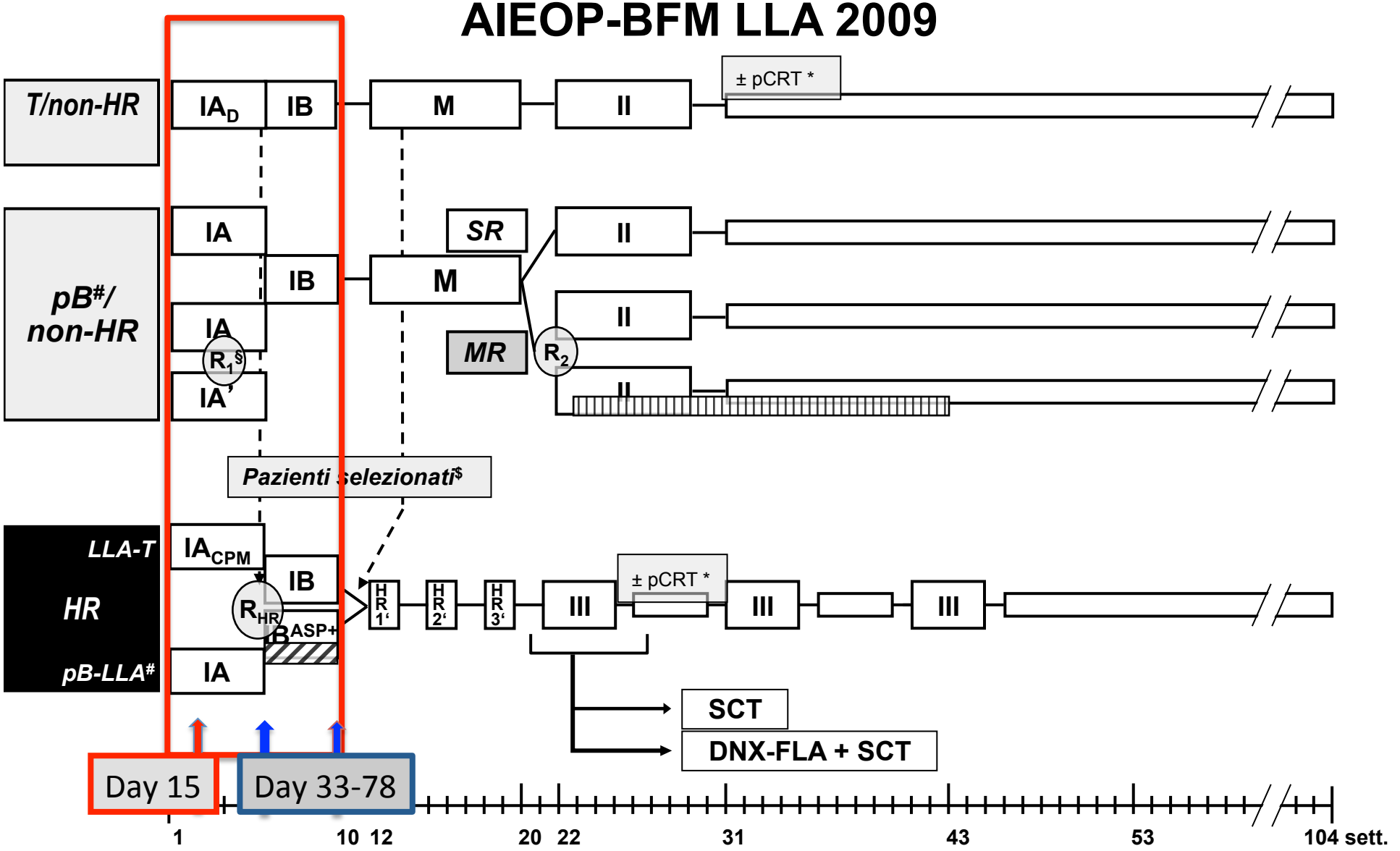
POTENTIAL TREATMENT DECISIONS BASED ON MRD



Clinical relevance of MRD

- Before therapy, as stratification tool
- During therapy, to evaluate effectiveness and to choose the most appropriate therapeutic strategy (stratification)
- ***ALL patients who don't have MRD at earlier points in their therapy tend to have the best prognosis,***
- At stop therapy, to choose the most appropriate therapeutic strategy
- For this reason in the future the MRD technologies will improve there utilization

AIEOP-BFM LLA 2009



IA	Prot. IA (con Pred e 4 dosi DNR nei giorni 8, 15, 22 e 29)
IA'	Prot. IA' (con Pred e 2 dosi DNR nei giorni 8 and 15)
IA_{CPM}	Prot. IA_{CPM} (con Pred, 4 dosi DNR e 1 dose CPM al giorno 10)
IA_D	Prot. IA_D (con Dexa e 4 dosi DNR nei giorni 8, 15, 22 e 29)
IB^{ASP+}	Prot. IB-ASP+ (con 4 x 2500 E PEG-L-ASP
	PEG-ASP data per 20 settimane

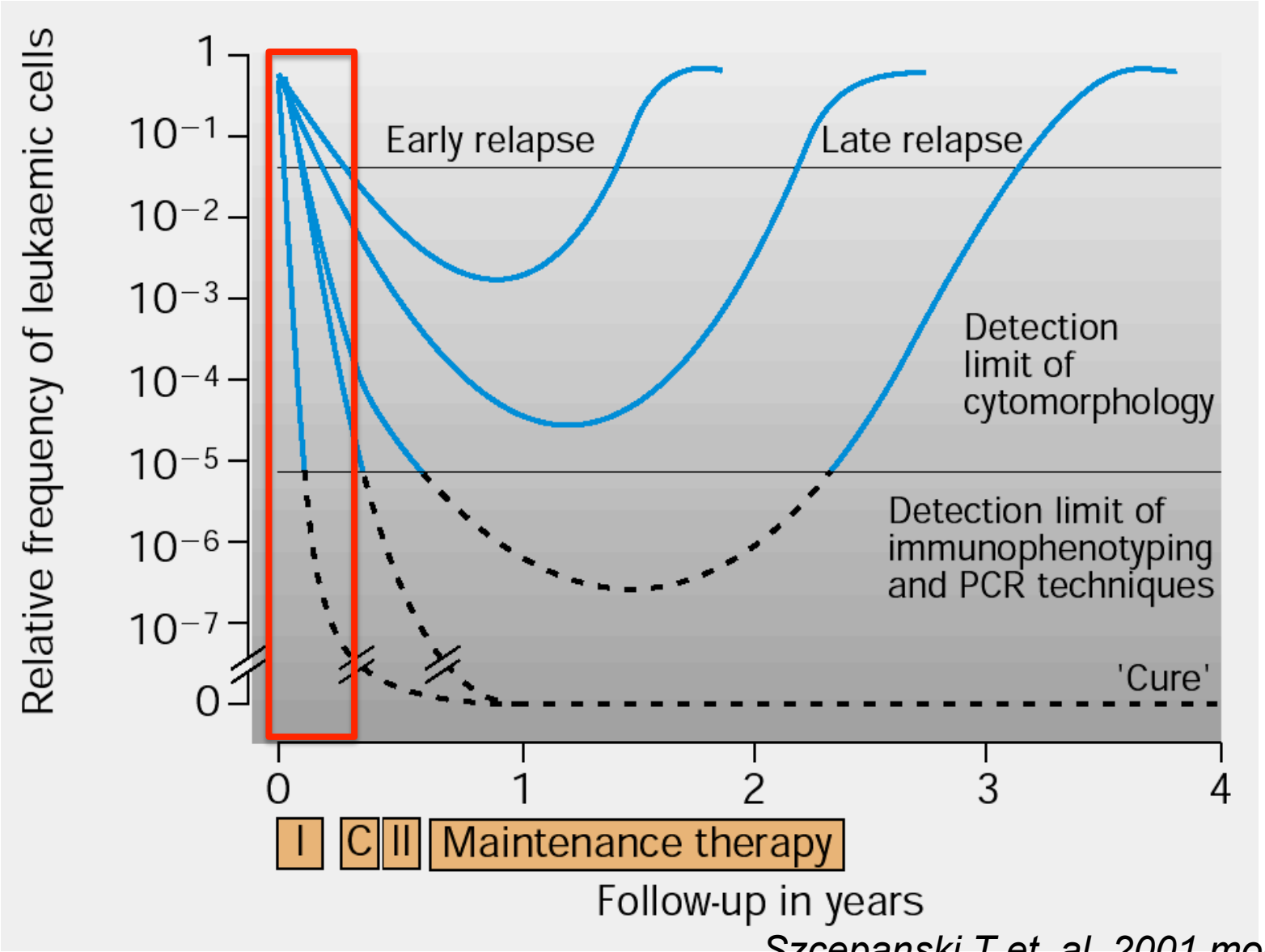
o immunofenotipo non noto

* pCRT 12 Gy se età ≥ 2 aa / in sottogruppi selezionati non pCRT + 6x IT MTX / in pazienti con malattia SNC (SNC 3) tCRT con 12 Gy o 18 Gy (dose età-dipendente)

§ per eleggibilità alla randomizzazione vedi protocollo

$ vedi protocollo

MRD as “surrogate” marker to assess heterogeneity in response to treatment



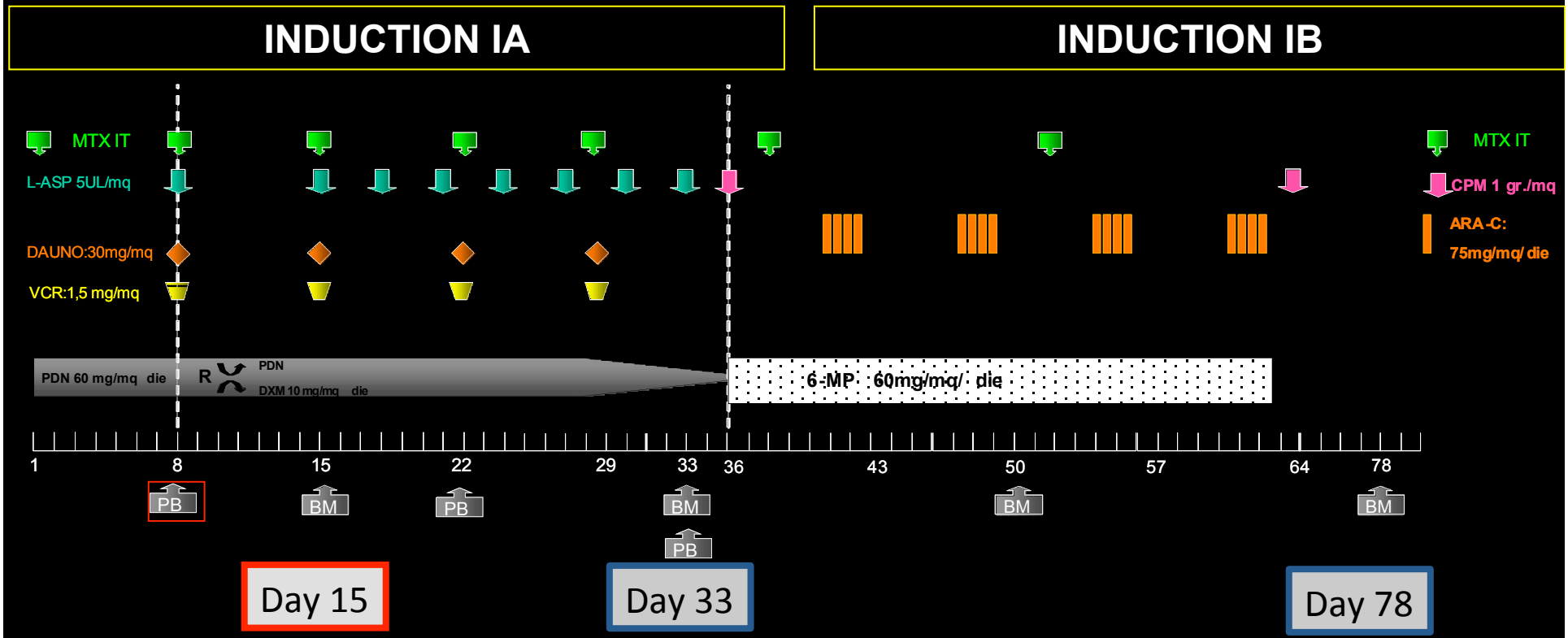
Szczepanski T et al, 2001 modified

Day 15?

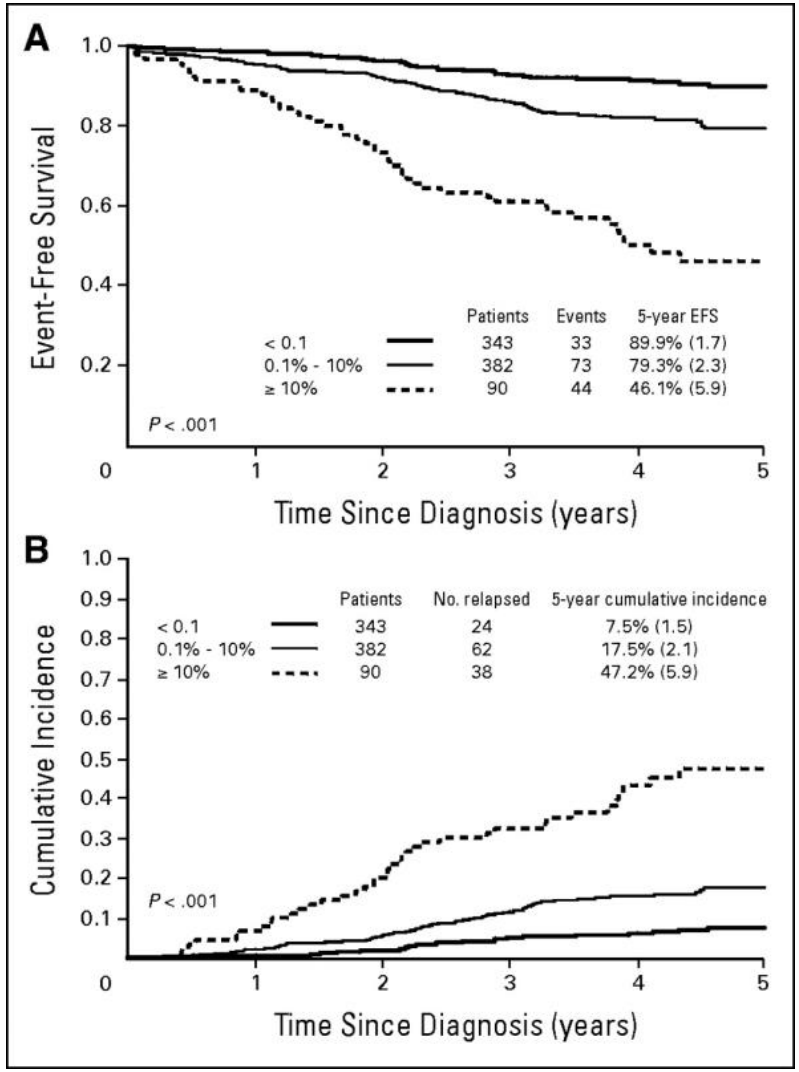
DAY 15 TECHNICAL ADVANTAGES

- Very early *in vivo* response to drugs (PDN, VCR, DN, L-ASP)
- Optimal time point for evaluation of residual disease by flow cytometry:
- - Easy blast cells enumeration
- - No regenerating B cells in BM (avoid bias in dot plots evaluation)
- Easy apoptotic cells enumeration.

AIEOP-BFM ALL 2009



(A) Event-free survival (EFS) and (B) cumulative incidence of relapse in 815 patients treated in the AIEOP-BFM-ALL 2000 trial, stratified into three risk groups according to minimal residual disease on day 15 marrow as detected by flow cytometry.



Basso G et al. JCO 2009;27:5168-5174

JOURNAL OF CLINICAL ONCOLOGY

VOLUME 27 · NUMBER 31 · NOVEMBER 1 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Risk of Relapse of Childhood Acute Lymphoblastic Leukemia Is Predicted By Flow Cytometric Measurement of Residual Disease on Day 15 Bone Marrow

Giuseppe Basso, Marinella Veltroni, Maria Grazia Valsecchi, Michael N. Dworzak, Richard Ratei, Daniela Silvestri, Alessandra Benetello, Barbara Buldini, Oscar Maglia, Giuseppe Masera, Valentino Conter, Maurizio Arico, Andrea Biondi, and Giuseppe Gaipa

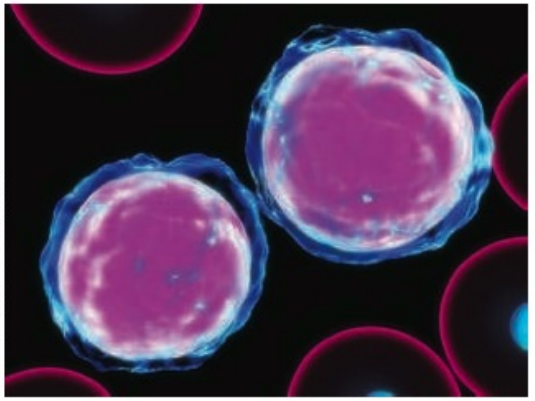
HEMATOLOGY

Early disease predictor for ALL relapse

While most childhood acute lymphoblastic leukemia is cured with modern treatments, relapse is the main cause of treatment failure. Therefore, determining early response to therapy is a primary prognostic goal. Assessment of blast counts in the bone marrow on day 15 is often used to guide therapy. Patients with insufficient response by day 15 have an unfavorable outcome due to leukemia relapse. As part of a clinical trial, investigators have now demonstrated that measurement of flow cytometry minimal residual disease at a single early time point in the bone marrow on day 15 following treatment represents a feasible and appropriate prognostic factor.

Bone marrow samples were assessed for 815 patients who were 18 years or younger with newly diagnosed ALL. The samples were collected on day 15 after 14 days of steroid treatment and single chemotherapy dose. Three groups were identified by flow cytometry: standard (<0.1 blast cells); intermediate (0.1 to <10 blast cells) and high (≥10 blast cells). The 5-year cumulative incidence of relapse was 7.5%, 17.5% and 47.2% for the standard, intermediate and high-risk groups, respectively. Flow cytometry was the most important prognostic factor. Flow cytometry was able to detect relapses in patients according to their bone marrow blast cell counts on day 15. In total, 5.5% of patients with blast counts lower than 0.1% had a relapse compared with 36.8% in patients with blast counts of ≥10.

"Measurement of flow cytometry blast counts on day 15 bone marrow samples was the most powerful early predictor of relapse" conclude the researchers. Patients with a high flow cytometry minimal residual disease score at day 15 had a particularly poor prognosis, independent of all other clinical and biologic features.



While this technology has yet to be standardized, it represents an important additional tool to assess ALL as it is rapid and relatively cheap. The authors plan to use this parameter in all patients in whom PCR may be informative, thus allowing to select patients for treatment tailoring.

Lisa Hutchinson

Original article Basso, G. *et al.* Risk of relapse of childhood acute lymphoblastic leukemia is predicted by flow cytometric measurement of residual disease on day 15 bone marrow. *J. Clin. Oncol.* **27**, 5168–5174 (2009)

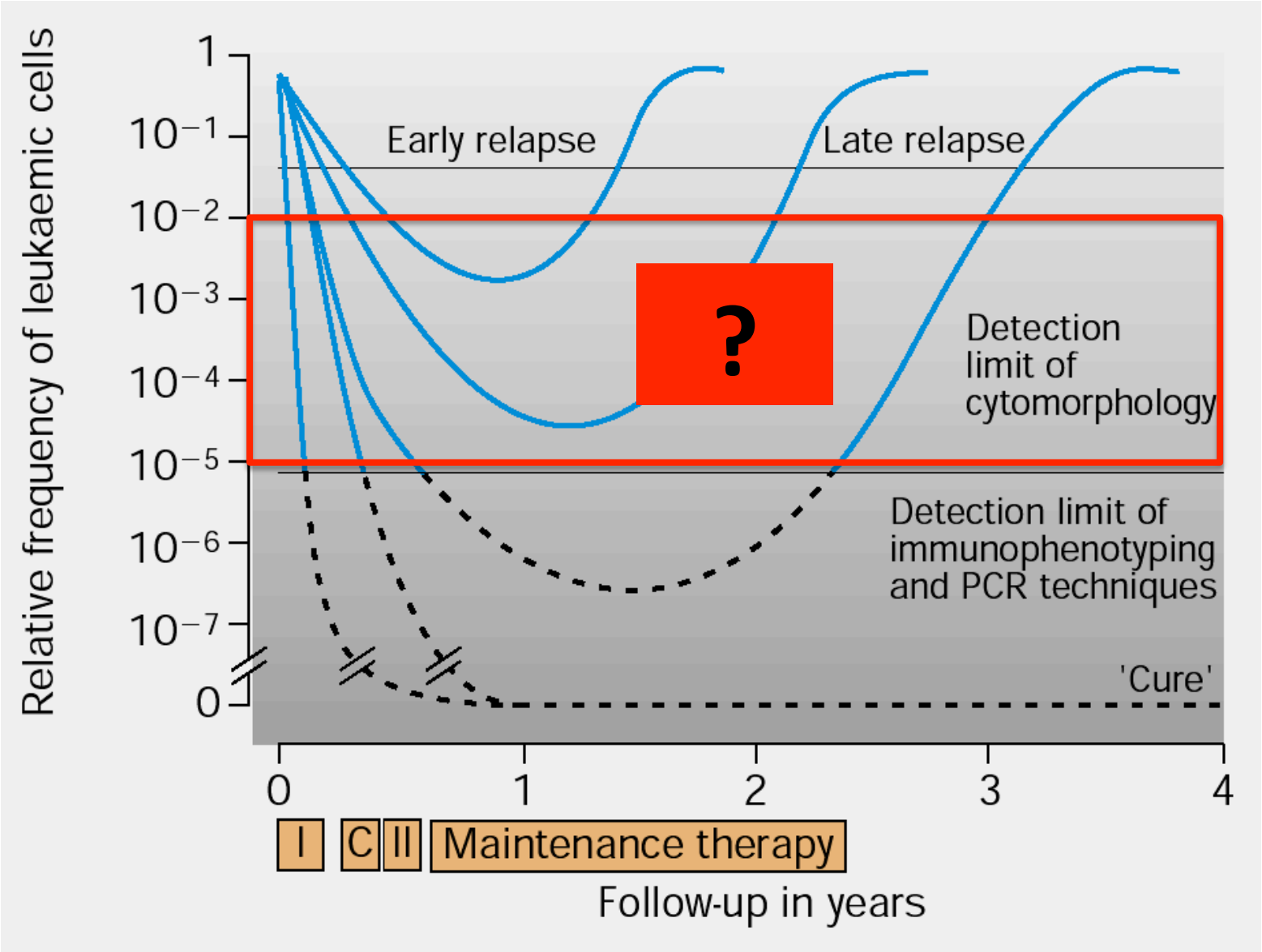
Nature Reviews Clinical Oncology Volume 7, January 2010

Open question

While we do know that ALL patients who don't have MRD at earlier points in their therapy tend to have the best prognosis, ***researchers are still trying to determine the best way to treat patients who appear to be in remission based on morphologic examinations but who still have MRD.*** *Consensus about whether MRD can be used as an endpoint in clinical trials, or whether oncologists should be monitoring MRD to signal relapse, have yet to be reached.*

By Jill Sederstrom

MRD as “surrogate” marker to assess heterogeneity in response to treatment



Clinical relevance of MRD

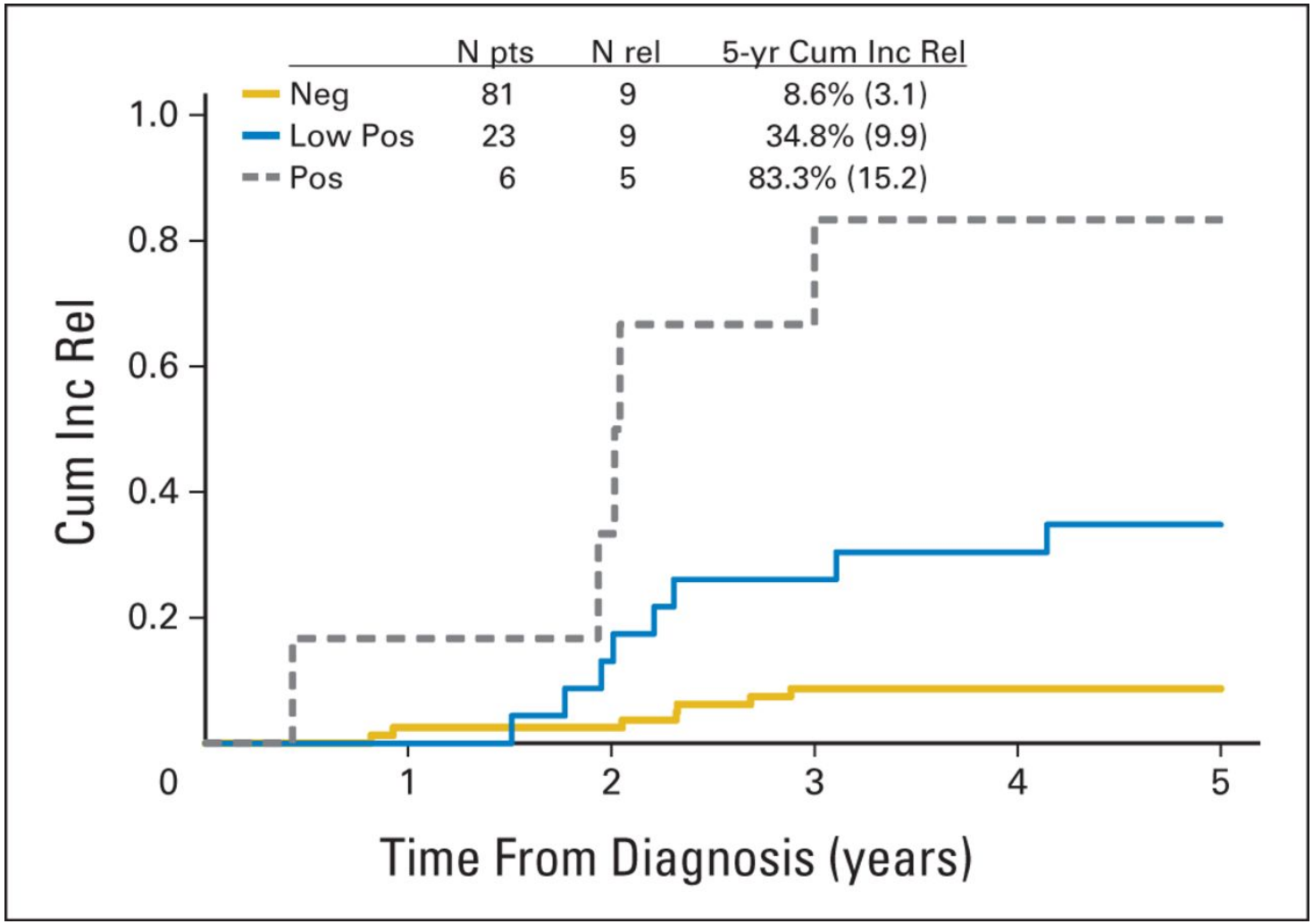
- Before therapy, as stratification tool
- **During therapy, to evaluate effectiveness and to choose the most appropriate therapeutic strategies**
- Clinical utility of sequential MRD measurement in early phase of treatment
- At stop therapy, to choose the most appropriate therapeutic strategy
- For this reason in the future the MRD technologies will improve there utilization

In a retrospective study (AIEOP-BFM 2000), during postinduction treatment 110 patients were categorized as negative, low positive ($< 5 \times 10^{-4}$), or high positive ($\geq 5 \times 10^{-4}$). Patients with at least one low-positive or high-positive result were assigned to the corresponding subgroup.

RESULTS:

Patients who tested during postinduction therapy high positive, low positive, or negative had significantly different cumulative incidences of leukemia relapse: 83.3%, 34.8%, and 8.6%, respectively ($P < .001$).

Cumulative incidence of relapse (Cum Inc Rel) in 110 children with acute lymphoblastic leukemia, according to the results of minimal residual disease during postinduction treatment.



Maddalena Paganin et al. JCO 2014;32:3553-3558

JOURNAL OF CLINICAL ONCOLOGY ASCO

... Two thirds of positive cases were identified within 4 months after induction-consolidation therapy, suggesting that this time frame may be most suitable for cost-effective MRD monitoring, particularly in patients who did not clear their disease at the end of consolidation.

CONCLUSION:

These findings provide further insights into the dynamic of MRD and the ongoing effort to **define mrd relapse** in childhood ALL.

VOLUME 32 · NUMBER 31 · NOVEMBER 1 2014

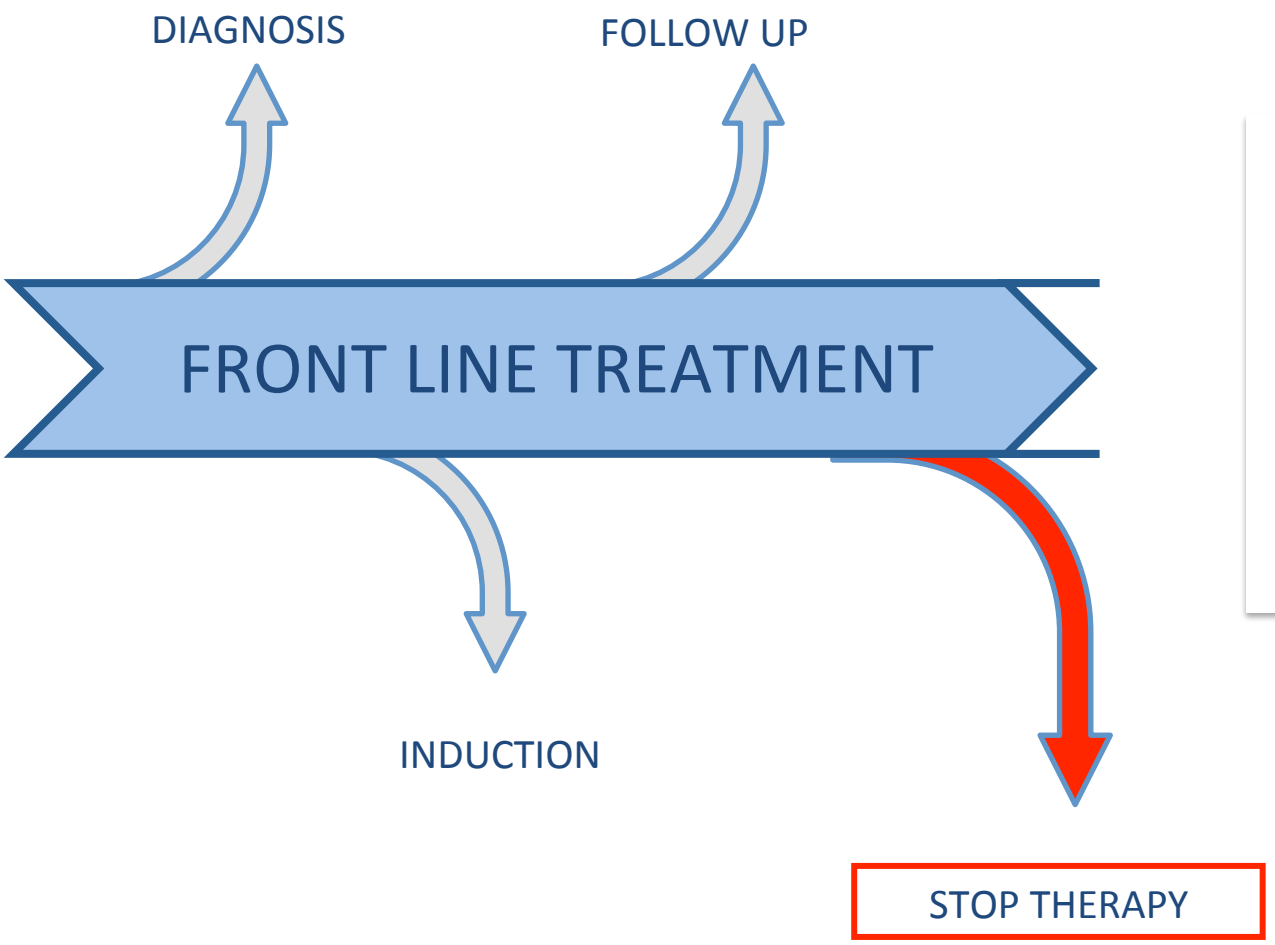
JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Postinduction Minimal Residual Disease Monitoring by Polymerase Chain Reaction in Children With Acute Lymphoblastic Leukemia

Maddalena Paganin, Giulia Fabbri, Valentino Conter, Elena Barisone, Katia Polato, Giovanni Cazzaniga, Eugenia Giraldi, Franca Fagioli, Maurizio Aricò, Maria Grazia Valsecchi, and Giuseppe Basso

POTENTIAL TREATMENT DECISIONS BASED ON MRD



MRD
During the follow up/ Stop Therapy

1. **The suspect cells are blast cells?** identical to those found at diagnosis or have they changed?
2. Is it a relapse or a secondary leukemia?

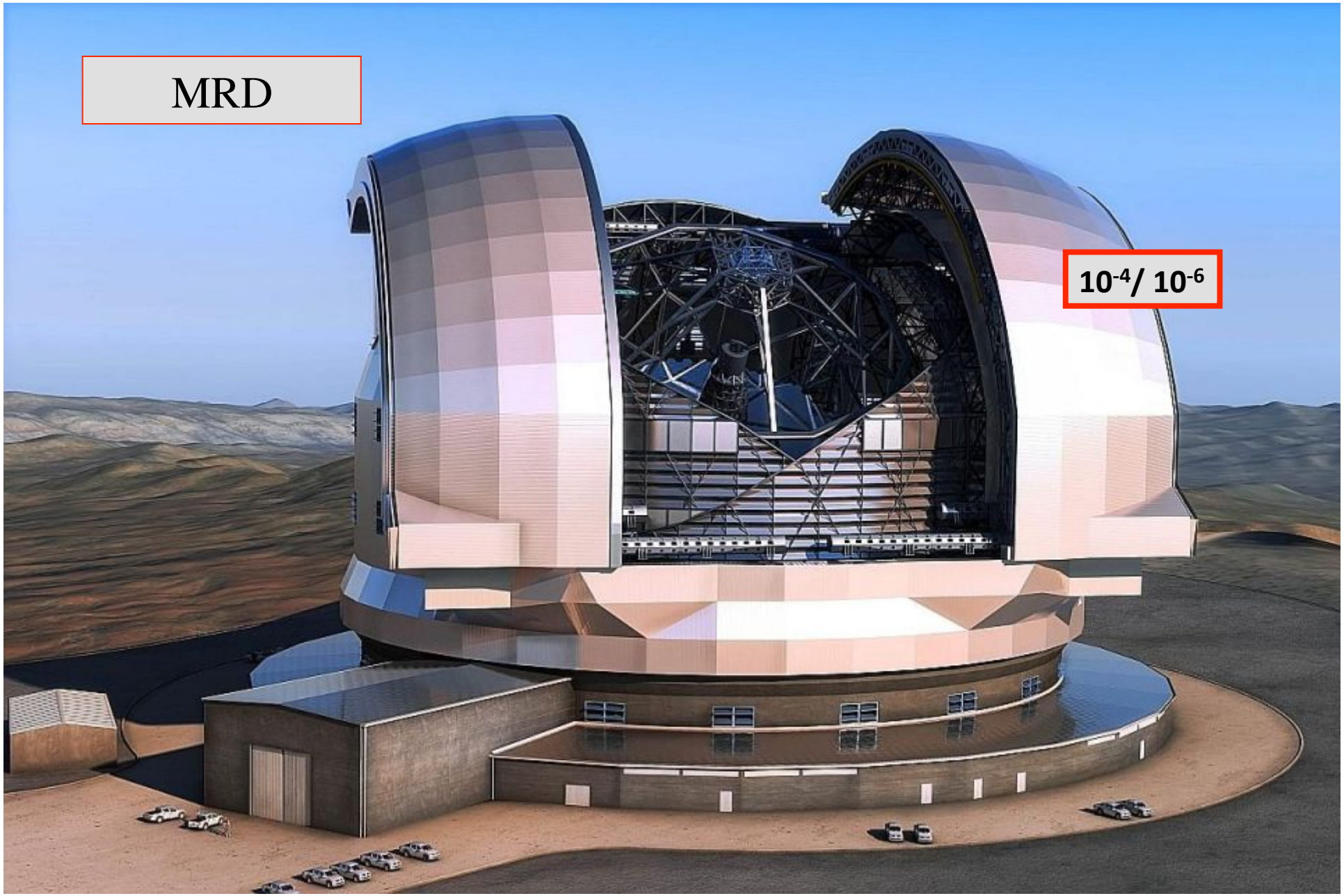
Follow up

*Although he still performs morphologic bone marrow tests**, Dr. Pui no longer depends on them**. MRD monitoring is able to provide greater sensitivity and identify disease not found on bone marrow tests, without adding significant costs.*

By Jill Sederstrom

Morphology

1 - 5%


MRD
10^{-4}/ 10^{-6}

FLOW CYTOMETRIC DETECTION OF MINIMAL RESIDUAL DISEASE

PITFALLS

While the data on the prognostic value of MRD are strong in pediatric ALL, the research in the adult setting is less extensive, and the knowledge of MRD's role in care is more fragmented*. ……*

Compared with pediatrics, the testing process in adult ALL is not as structured or streamlined. With recent comparative analyses of ALL MRD testing laboratories, the National Cancer Institute (NCI) discovered that, although ***there was high concordance between two primary reference laboratories used by the Children's Oncology Group (COG), the adult reference labs that participated in the pilot study were not at all concordant****……"As a result, there was enthusiasm among the adult reference laboratories to participate in a voluntary standardization approach and adopt the* ***COG six-color panel as the starting point for that standardization,"***

By Jill Sederstrom

FLOW CYTOMETRIC DETECTION OF MINIMAL RESIDUAL DISEASE

PITFALLS

- Highly specialized skills and extensive database of reference samples, which only a few laboratories have (*Coustan-Smith E, and Campana D, 2013*)

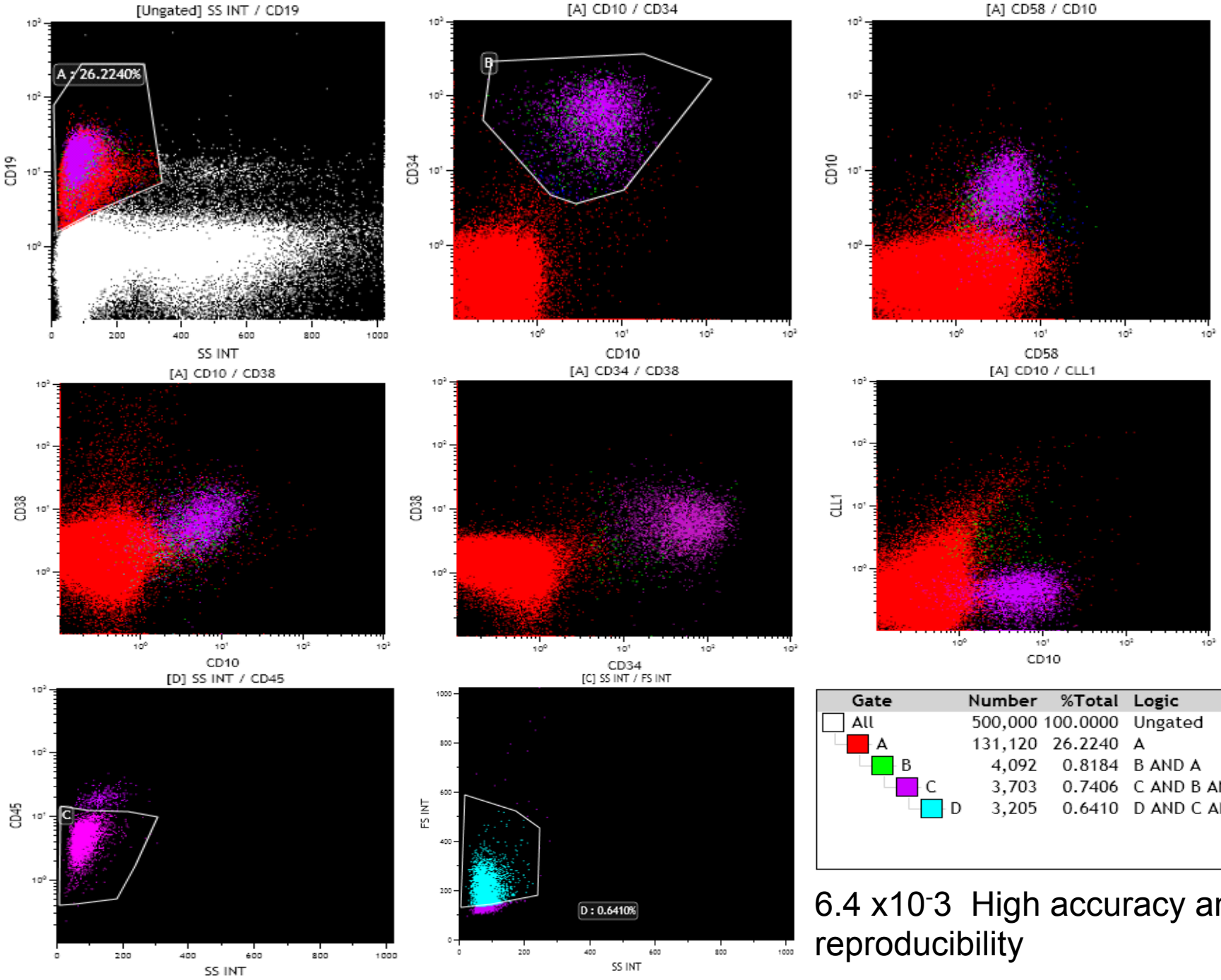
The pitfalls are depending from

- Sample quality …
- Technology; **the iBFM twinning program.....**

Possible Technical Pitfalls in MRD FCM

- Different sample in different sites of BM aspirate (different point of aspiration)
- **Reagents standardization**
- **Antigen modulation**
- Shift immunophenotyping
- Death or apoptotic cell evaluation

ALL DAY +15, "*DURACLONE** TUBE



Gate	Number	%Total	Logic
All	500,000	100.0000	Ungated
A	131,120	26.2240	A
B	4,092	0.8184	B AND A
C	3,703	0.7406	C AND B AND A
D	3,205	0.6410	D AND C AND B AND A

6.4×10^{-3} High accuracy and reproducibility

Possible Technical Pitfalls in MRD FCM

- Different sample in different sites of BM aspirate (different point of aspiration)
- **Reagents standardization**
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FLOW CYTOMETRIC DETECTION OF MINIMAL RESIDUAL DISEASE

PITFALLS

- Highly specialized skills and extensive database of reference samples, which only a few laboratories have (*Coustan-Smith E, and Campana D, 2013*)

The pitfalls are depending from

- **Sample quality …2 or more different aspirations**
- **Reagent quality..”*Duraclone custom tubes*…”**
- **Technology; the twinning program……..**
- **Skill and training; *Flow in Lab*, iBFM….**

While the data on the prognostic value of MRD are strong in pediatric ALL, the research in the adult setting is less extensive, and the knowledge of MRD's role in care is more fragmented*. ……*

Compared with pediatrics, the testing process in adult ALL is not as structured or streamlined. With recent comparative analyses of ALL MRD testing laboratories, the National Cancer Institute (NCI) discovered that, although ***there was high concordance between two primary reference laboratories used by the Children's Oncology Group (COG), the adult reference labs that participated in the pilot study were not at all concordant****……"As a result, there was enthusiasm among the adult reference laboratories to participate in a voluntary standardization approach and adopt the* ***COG six-color panel as the starting point for that standardization,"***

By Jill Sederstrom

WEB SITE https://elearning.unipd.it/flowinlab

INSTITUTION Pediatric Hemato Oncology Laboratory

Department of Woman and Child Health – SDB

University of Padua – Italy

In collaboration with Centro Multimediale E-Learning di Ateneo - CMELA

MOODLE version 2.8.7+ (Build: 20150730)

FLOWINLAB STAFF :

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Tutor	Silvia DISARO'
Tutor	Dr. Chiara FRASSON
Tutor	Dr. Maria GABELLI
Tutor	Barbara MICHIELOTTO
Tutor	Dr. Pamela SCARPARO
External Tutor	Prof. Gianpietro Carlo SEMENZATO
External Tutor	Dr. Renato ZAMBELLO
Admin	Dr. Andrea ZANGRANDO

FlowInLabCategories

Clinical Case Repository

Anonimous Pediatric Hemato Oncology cases analyzed by the Staff

Request a Counseling

Request a counseling to the Staff

Available Courses

Courses or Training periods at Lab or online

Presentations & Documents

Literature and teaching material

Project Requests

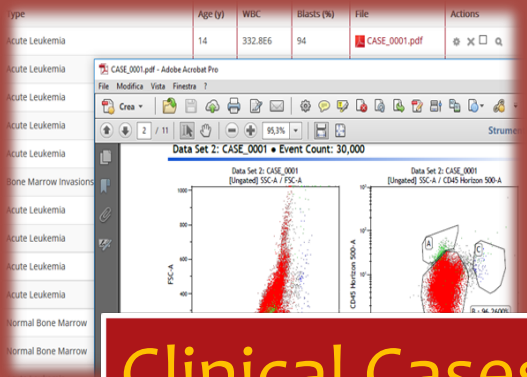
Ongoing projects with the Lab

Previous Courses

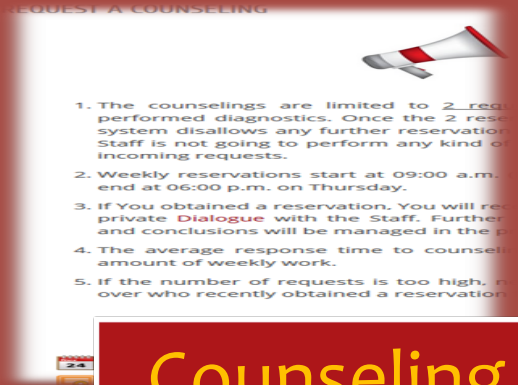
Previous Courses or Training periods at Lab or online

FlowInLab**Features**

Team & Aims



Clinical Cases



Counseling

Literature

Projects

Courses

600/ 700 new diagnosis
3000 Flow MRD per year

Flow Cytometry Facility

NAVIOS

CANTO II

FACSAria III

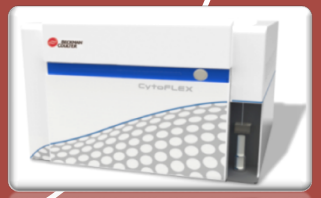
FC500

Core Facility

MoFlo

CANTO II

CytoFLEX



NAVIOS