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FOOD AND DRUG ADMINISTRATION
AMERICAN SOCIETY FOR CLINICAL ONCOLOGY
LEUKEMIA AND LYMPHOMA SOCIETY
AMERICAN SOCIETY OF HEMATOLOGY

Minimal Residual Disease (MRD) as a
Surrogate Endpoint in
Chronic Lymphocytic Leukemia (CLL) Workshop

Wednesday, February 27, 2013

8:00 a.m. to 3:30 p.m.

FDA White Oak Campus
White Oak Conference Center
10903 New Hampshire Avenue
Silver Spring, Maryland

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P R O C E E D I N G S

(8:00 a.m.)

Welcome and Introduction

DR. REAMAN: I'm Greg Reaman from the Office of Hematology and Oncology Products in the Center for Drug Evaluation and Research. I'd like to welcome you all to this workshop on considering minimal residual disease as a potential surrogate endpoint in CLL.

On behalf of my co-chairs, Drs. De Claro and Wierda, I would also like to acknowledge the co-sponsors of this workshop, ASCO, the Leukemia and Lymphoma Society, and the American Society of Hematology.

I also want to acknowledge the planning committee, who has worked fairly laboriously over the past several months, members of the FDA and various divisions, and then external consultants, who were very active participants in today's program.

So our objective here today is to review and discuss available data on the prognostic

1 significance of MRD and its potential as a
2 surrogate to predict clinical benefit in chronic
3 lymphocytic leukemia; to review and discuss
4 advantages and challenges with respect to timing,
5 threshold levels, preferred specimens for analysis,
6 technology platform, and the need for proficiency
7 assessment and standardization; to discuss the
8 potential impact of treatment interventions based
9 on MRD on the analysis of trial endpoints,
10 specifically if this is going to be used as a
11 surrogate; and to discuss the need for centralized
12 or regionalized testing of MRD.

13 So I think I'd also like to have the panel
14 introduce themselves. We can start going around
15 the table. Dr. Montserrat, do you want to just --

16 DR. MONTSERRAT: Emilio Montserrat from the
17 University of Barcelona, Spain.

18 DR. HILLMEN: Peter Hillmen, hematologist
19 from Leeds in the United Kingdom.

20 DR. GHIA: Paolo Ghia, Universita San
21 Raffaele from Milano, Italy.

22 DR. DEISSEROTH: Al Deisseroth, FDA.

1 DR. FARRELL: Ann Farrell, FDA.
2 DR. DE CLARO: Angelo de Claro, FDA.
3 DR. WIERDA: Will Wierda, MD Anderson.
4 DR. KAY: Neil Kay, Mayo Clinic, Rochester,
5 Minnesota.
6 DR. STETLER-STEVENSON: Maryalice Stetler-
7 Stevenson, NCI, NIH.
8 DR. BYRD: John Byrd, Ohio State University.
9 DR. RAWSTRON: Andy Rawstron from Leeds in
10 the U.K.
11 DR. KIPPS: Tom Kipps from the UC San Diego
12 Moores Cancer Center.
13 DR. BECKER: Robert Becker, FDA.
14 DR. BOTTCHEER: Sebastian Bottcher from Kiel
15 in Germany.
16 DR. KAMINSKAS: Edvardes Kaminskas, FDA.
17 DR. WOOD: Brent Wood, University of
18 Washington.
19 DR. LOZANSKI: Gerald Lozanski, OSU.
20 DR. MARTI: Gerald Marti, CDRH, FDA.
21 DR. ROTHMANN: Mark Rothmann, FDA.
22 DR. WIESTNER: Adrian Wiestner, NIH.

1 DR. BROWN: Jennifer Brown, Dana-Farber
2 Cancer Institute.

3 DR. KHOURI: Issa Khouri, MD Anderson Cancer
4 Center.

5 DR. REAMAN: Thanks and welcome.

6 So our agenda is to review some regulatory
7 considerations, just to provide a framework for
8 regulatory, what's required for regulatory
9 approval, and what would constitute acceptable
10 surrogates; to then discuss a single center and
11 international multicenter experiences with the
12 assessment of MRD in CLL; discussion on technical
13 considerations and the need for standardization;
14 and then we'll end with discussion points, and
15 there are questions that have been provided.

16 I just want to point out that this is not
17 like questions to an advisory committee. We're not
18 asking for a vote. There would be no absolute
19 decisions made at the completion of this workshop,
20 but we will use obviously the discussion and
21 consensus, should one be reached, in further
22 consideration of MRD as a potential surrogate.

1 So with that, I'd like to introduce our
2 first speaker, Dr. Al Deisseroth, who will talk
3 about considerations for regulatory approval.

4 **Presentation - Albert Deisseroth**

5 DR. DEISSEROTH: Thank you, Greg.

6 My goal and role this morning is to briefly
7 outline and discuss the basis for approval of
8 marketing applications at the U.S. FDA, and then to
9 make observations on two examples in which
10 measurement of minimal residual disease has evolved
11 into regulatory endpoints for drug approval.

12 So the basis for new drug approval at the
13 FDA is demonstration of efficacy with acceptable
14 safety in adequate, well-controlled studies. And
15 this requirement implies that the clinical data
16 generated enables the definition of the appropriate
17 patient population for safe and effective use of
18 the drug.

19 Now, there are two types of marketing
20 approval, regular approval, in which the
21 demonstration of clinical benefit, prolongation of
22 life, a better life, or an established surrogate

1 thereof, is demonstrated before approval. But
2 because the FDA strives to provide access for life-
3 threatening conditions to new drugs while efficacy
4 data is being collected, a second type of approval
5 was generated called accelerated approval.

6 In this approval paradigm, a surrogate
7 endpoint that's reasonably likely to predict
8 benefit is accepted as evidence of clinical
9 benefit, in contrast to the regular approval, in
10 which clinical benefit is demonstrated before
11 approval. And this is usually for life-threatening
12 conditions in which there is no established
13 therapy, or in which the candidate drug displays an
14 advantage over other available therapy.

15 Now, a third important concept that really
16 is important for our discussions today is that the
17 FDA really is charged with exercising its
18 scientific judgment in determining the kind and
19 quality of data that's required for approval. So
20 this creates an opportunity to explore new types of
21 endpoints for marketing approval.

22 Now, I'll go through two examples in which

1 the identification of assays for minimal residual
2 disease and their study in clinical trials have led
3 to the use of minimal residual disease as a
4 regulatory endpoint for approval.

5 The first is a well-known drama that
6 unfolded in the '90s in which clinical endpoints,
7 like opportunistic infections or CD4 levels, were
8 replaced by measurement of residual or the level of
9 viral RNA after treatment.

10 Now, before 1996, the endpoints that were
11 utilized for approval of new anti-HIV drugs were
12 clinical, decreased CD4 levels, new opportunistic
13 infections, mortality. But the advent of reliable
14 assays for viral load after therapy led to
15 increasing pressure to shift from these clinical
16 endpoints to the more immediate and informative
17 molecular endpoints, so that patients didn't have
18 to wait and watch their viral load go up after
19 therapy until experiencing an unfortunate clinical
20 occurrence.

21 The crux of the development of the use of
22 viral load as a regulatory endpoint was the effort

1 that was expended to standardize these assays for
2 viral load and to demonstrate that the measurement
3 of a reduction in viral load to a predetermined
4 goal was related to clinically important events and
5 could predict long-term outcome of therapy.

6 So industry, academia, and government joined
7 together to test a correlation between measurement
8 of post-treatment viral load in over 5,000 patients
9 in multiple trials, and concluded that the
10 measurement of a short-term decrease in viral load
11 correlated with long-term clinical outcome,
12 duration of response, the increase in progression,
13 and increased survival.

14 So in 1996, a standard was established with
15 this minimal residual disease of 50 copies of HIV
16 RNA per mL in the intravascular space of patients
17 so treated. And in 1997, an antiviral FDA advisory
18 committee approved the use of this endpoint at 24
19 weeks for accelerated approval, 48 weeks for
20 regular approval. And to this day, that standard
21 is used, and it's resulted in approval of over 28
22 new drugs for treatment of AIDS and an acceleration

1 of the regulatory process that leads to approval.

2 Now, the second example is CML and, again,
3 there was an assay available to monitor transcript
4 levels of a fusion gene that was the driver for the
5 clinical events that happened in CML. And the
6 question that was answered through clinical
7 investigation was whether this particular
8 measurement of minimal residual disease after
9 therapy could predict long-term outcome.

10 Again, as in the AIDS example, there was a
11 drive that extended over several years to
12 standardize the measurement of residual transcript
13 levels, and one formulation was based on the
14 establishment of a baseline level that would allow
15 the comparison of results among all laboratories
16 all around the world.

17 This was a ratio of the fusion transcript to
18 the normal transcript among 30 patients and a
19 median ratio establishes a standard baseline, and
20 then the calculation of the same ratio after
21 treatment. The goal in this particular case was a
22 three-log reduction of that ratio, which was

1 designated major molecular response.

2 In Europe, this process of standardization
3 extended from 1999 to 2003. And in 2005, a
4 consensus conference in the United States was held
5 at the NIH to attempt to formalize the
6 standardization of this assay.

7 Now, the major vehicle for understanding
8 whether measurement of minimal residual disease,
9 minimal level of transcripts after therapy, could
10 predict long-term outcome was the IRIS trial.
11 That's the International Randomized Study of
12 Interferon and Imatinib. And this trial entered
13 over 1100 patients newly diagnosed within six
14 months of therapy, that were randomized to receive
15 the new drug, imatinib or interferon and
16 cytarabine.

17 Actually, in the trial, the primary clinical
18 endpoint was progression-free survival. Secondary
19 endpoints included a major shortage in response,
20 hematologic response, but an exploratory endpoint
21 of this trial was to determine the value and
22 relevance of making measurements of BCR-ABL

1 transcripts after therapy.

2 So the first clue that minimal residual
3 disease would be a useful measurement was the
4 analysis at 12 months after treatment in which not
5 only was there a major difference in the percentage
6 of patients who achieved complete cytogenetic
7 response with imatinib or interferon and
8 cytarabine, but the percentage of patients in whom
9 a three-log reduction of transcript levels also
10 tracked with the accepted standard of complete
11 cytogenetic response.

12 In addition, with increasing duration of
13 treatment, the percentage of patients with complete
14 cytogenetic response that also exhibited this
15 three-log reduction increased. And importantly,
16 patients in whom there was a major molecular
17 response had a zero percent incidence of
18 progression to accelerated phase or blast crisis.

19 Later, tracking of major molecular remission
20 showed that early achievement of a major molecular
21 response at 18 months could predict those patients
22 who would either lose or not lose their complete

1 cytogenetic response at seven years.

2 So here is an additional advantage of using
3 MRD. And more recently, it's become clear that the
4 measurement of major molecular response at three
5 months after initiation of therapy can identify and
6 dissect out patients who may have an adverse
7 prognosis in terms of overall survival at eight
8 years.

9 This is particularly interesting because the
10 ability to use transcript reduction levels at three
11 months doesn't seem to be abrogated by succeeding
12 forms of salvage therapy.

13 Now, the first example of the use of minimal
14 residual disease as a primary regulatory endpoint
15 was the submission of the marketing application for
16 a second-generation tyrosine kinase inhibitor for
17 CML, nilotinib, in which the primary endpoint was
18 the percentage or proportion of patients receiving
19 either nilotinib or imatinib at 12 months, who
20 achieved a three-log reduction in their transcript
21 level.

22 This particular trial achieved its

1 prespecified goal of a statistically significant
2 difference and did receive accelerated approval.
3 And again, more recent data suggests that early
4 measurement of reduction of transcript levels at
5 three months can predict longer-term outcome that
6 are clinically important.

7 So what have we learned from these
8 observations? What conclusions or observations can
9 we make about the evolution of MRD as a regulatory
10 endpoint? Well, the first is that both examples
11 indicate that interesting, potentially useful MRD
12 standards were identified in clinical trials.

13 There was an agreement internationally of
14 the importance of standardization of these assays
15 so that results could be compared all over the
16 world. And then, once standardized, these MRD
17 measurements were applied prospectively in clinical
18 trials and shown to be useful.

19 So we thought that this historical
20 perspective might be useful for you to think about
21 as we go through the day. Thank you.

22 (Applause.)

1 DR. REAMAN: Thanks, Al.

2 Our next speaker is Dr. Bob Becker from the
3 Office of In Vitro Diagnostics and Radiologic
4 Health in CDRH. Dr. Becker?

5 **Presentation - Robert Becker**

6 DR. BECKER: Good morning. So as Dr. Reaman
7 indicated, I am from the Center for Devices, and we
8 deal with in vitro diagnostic testing as it is
9 cleared or approved for use in the delivery of
10 essentially retail medicine in the United States.

11 We're talking about a little different
12 setting here, though, than where I actually work
13 day to day. This is in the area of applying
14 markers to clinical trials as endpoints. We do not
15 clear or approve products for that purpose, yet
16 there are some areas of overlap that you can
17 imagine in terms of the interest, especially from
18 an analytical perspective, that are relevant in
19 both places.

20 So I'll touch on some of those aspects.
21 I'll try to help you understand some areas where
22 analytical validation information can be helpful

1 with respect to surrogate endpoints and also
2 keeping clear for you, I hope, the distinction
3 between clearance and approval of devices and the
4 idea of qualification of surrogate endpoints.

5 So the idea of minimal residual disease as a
6 surrogate endpoint is one that does fit into the
7 concept of what a surrogate endpoint is. That is,
8 it's something meant to substitute for a clinical
9 efficacy endpoint. It's meant to have something
10 typically which gives you a forward look, for
11 obvious reasons, in terms of what the clinical
12 effect of the drug might be for patients who will
13 be subjected to use of that drug.

14 This is actually captured in a draft
15 guidance document that's put forward by the Center
16 for Drugs, that's on the Web, in what's called a
17 drug development tool, guidance. And surrogate
18 endpoints are one of four elements of drug
19 development tools that are developed in that
20 guidance.

21 In that guidance is the concept of biomarker
22 qualification. And again, this is something

1 completely separate from the idea of review of a
2 device for delivery of retail medicine of
3 day-to-day clinical practice.

4 From a programmatic perspective, this is a
5 program handled by the Office of Translational
6 Sciences, and it's something which actually has a
7 package that is submitted to the FDA that can
8 establish an interpretable meaning under a specific
9 context of use for the marker that is being put
10 forward as a surrogate.

11 The qualification of a marker is something
12 which is agreed to or declined by the qualification
13 review team. That, as you can tell from today's
14 gathering, surely includes the affected review
15 divisions, as an integral aspect of that
16 evaluation.

17 Once established as a qualified surrogate
18 endpoint, then anyone can use that endpoint within
19 the context that it has been qualified for purposes
20 of use in clinical decisions, in clinical trials,
21 and in regulatory decision-making.

22 I'd point out that the qualification of the

1 endpoint does not have any effect upon the
2 regulatory status of the test for commercial
3 marketing, per se. It only affects the scope of
4 use of the test in the context of the clinical
5 trials.

6 Now, this is a program which has, basically,
7 as its alternative, the one-time use of an endpoint
8 that's simply been agreed to by a trial sponsor,
9 along with the review division for the use of that
10 endpoint in the context of a single trial. The
11 obvious advantage of the qualification is that one
12 is looking at the ability to make the marker
13 essentially transportable between trials that have
14 the same context of use for the marker.

15 So with respect to minimal residual disease
16 as a surrogate endpoint, that is, that MRD does
17 define an endpoint, a clinically significant
18 endpoint, that occurs prior to a standard endpoint,
19 such as PFS or as OS, the idea would be that it's
20 not just something which is correlated with
21 ultimate outcome, but it actually captures in some
22 sense the effect of the drug on that patient in a

1 way that would also project through to the effect
2 of the drug for the definitive endpoints such as
3 overall survival.

4 The alternative, as I mentioned before, is a
5 one-time or a case-by case agreement, which is not
6 the idea of qualification. And we're talking more
7 today about the idea of being able to actually get
8 an agreement about a qualification ultimately.

9 The concept would be that biomarker
10 qualification would allow the use of MRD as a
11 surrogate endpoint under the context of use that is
12 within the qualification in a transportable manner.
13 It is the biomarker that is qualified in the drug
14 development tool process, not a particular test,
15 not a particular test strategy, protocol, or
16 material.

17 One does not even need to have a cleared or
18 approved test for the purpose of applying it in a
19 qualified context. But, as you can imagine, some
20 very strong evidence about the analytical
21 performance of that test or the intended system is
22 an expectation, since you want to know that you're

1 getting good analytical data whether you're talking
2 about a qualified biomarker or whether you're
3 talking about a test that's applied in commercial
4 context.

5 So the idea of an analytical validation is
6 something which is a very strong aspect of
7 assessing the qualification of a biomarker for
8 surrogate endpoint use. And without going into a
9 very long discussion about what can constitute
10 aspects of analytical validation, it basically
11 breaks down into two areas, one of trueness and
12 accuracy versus precision.

13 The idea of trueness and accuracy can be in
14 an average sense, that is, essentially, that the
15 test does not bias across all patients in terms of
16 having given, on average, the correct measurement
17 of the endpoint, of the analyte of interest.

18 Also, though, because things can vary from
19 one patient to another, for example, in terms of
20 interferences that might be existing within one
21 patient to another, the idea that, for each
22 individual patient, you can anticipate that the

1 marker will be expected to be true, expected to be
2 accurate in terms of giving you the correct value
3 for the marker of interest is relevant.

4 Now, also with respect to patient to
5 patient, you can anticipate that issues of
6 precision can creep into our concerns. That is, if
7 the test has a lot of dispersion when repeated
8 measurements are taken, either under the same
9 conditions or across systematically varied
10 conditions, those can be of concern about whether
11 you'll be getting measurements that, at the level
12 of individual patients, are really on target for
13 being able to apply the results of that measurement
14 in assessing the patient's status.

15 In developing analytical qualifications or
16 analytical validations, I should say, there are
17 standards that are published, that are developed in
18 part with industry, part with FDA, part with other
19 governmental bodies to give good approaches to how
20 one can carry out studies that will establish the
21 analytical validity of tests, and I recommend those
22 to your attention.

1 I would say that the biomarker itself is not
2 an in vitro diagnostic test, as I've already
3 alluded to. The biomarker itself is the thing that
4 you measure, an in vitro diagnostic product, which
5 is not the same thing as a qualified biomarker. It
6 is something which is a device, a specific device,
7 that is cleared or approved for use in delivering
8 clinical care day to day.

9 We're talking here about something which is
10 more than the biological marker itself, in terms of
11 having gotten a good validated set of criteria by
12 which you can apply that marker in trials and
13 something less than perhaps a cleared or approved
14 device.

15 So the terminology that separates these two
16 is apparent on the left- and the right-hand side.
17 In the context of a biomarker qualification, the
18 real focus for a drug development tool is on the
19 biomarker itself. There is a test or a variety of
20 tests for which you need to be confident about the
21 analytical performance characteristics of those
22 tests in giving you knowledge of the biomarker

1 status. And this gives you some biological
2 insight, which has a particular context of use for
3 the purpose of applying a surrogate endpoint
4 biomarker in clinical trials. And this is, of
5 course, something which you'd expect to be able to
6 redound beneficially to the more rapid or
7 definitively good assessment of a drug that is in
8 development.

9 With respect to device clearance or
10 approval, it is the test that is the regulatory
11 concern, with respect to a particular analyte and
12 the standards of safety and effectiveness of that
13 test for actual day-to-day clinical use, within the
14 context of an intended use for the purpose of
15 delivering clinical care.

16 So these are two parallel but obviously
17 somewhat analogous kinds of scenarios for
18 considering the status of the device.

19 So what are the effects of using the
20 biomarker via a clinical trial test as a novel
21 endpoint? Well, the hopeful benefit is that you
22 can have shorter, perhaps more informative, perhaps

1 smaller and less expensive clinical trials. The
2 expectation is that the correlation of the
3 surrogate endpoint biomarker to the traditional
4 endpoint will be very strong, if there is a very
5 convincing link of the biomarker to a traditional
6 endpoint across the context of use that has been
7 defined for that biomarker, as it will be used in
8 the surrogate setting.

9 We do know that test versions across sites
10 and trials can affect the read-out, can affect the
11 value that you get from attempts to measure any
12 particular biomarker. So the need for
13 standardization and the validation analytically of
14 those biomarkers is evident. A documentation of
15 that so you can be informed with respect to one
16 approach to measuring the biomarker versus another
17 approach to the biomarker is important.

18 This is something which can in part be
19 captured, at least leveraged, from what might be
20 cleared or approved tests that have been put into
21 clinical practice, but there's no requirement for
22 that to be the case. This is simply one way in

1 which you might be able to get perhaps a jump-start
2 in understanding what the performance
3 characteristics of the tests are as it might be
4 applied in a clinical trial setting as a surrogate
5 endpoint.

6 You want to know that you've got a very well
7 agreed upon testing paradigm as the actual trial
8 itself will be carried forward so that you don't
9 have, essentially, lack of control about the way in
10 which the biomarker will be assessed in the context
11 of the trial. And so this is what's needed in
12 order to be able to be confident you have
13 acceptable performance for purposes of using the
14 outcomes -- using the results of the biomarker as a
15 surrogate for outcome in assessing the drug.

16 So MRD as a surrogate endpoint could be
17 something which is in context of a pharmacodynamic
18 kind of marker or a predictive marker. These are
19 two of the other aspects of drug development tools
20 that are developed in the guidance.

21 The idea, though, here is that you would be
22 extending what is I think in common use clinically

1 already in laboratory-developed tests, the idea of
2 a prognostic usage for minimal residual disease, as
3 it helps to guide patient management in day-to-day
4 use now.

5 To date, we do not have any tests for
6 minimal residual disease that have been cleared or
7 approved for clinical management by FDA. We
8 certainly know that current technologies can vary.
9 They can be cell-based. They can be biochemically-
10 based. An understanding of how those various
11 technologies compare, whether there are advantages
12 of one versus another in terms of ease of use, in
13 terms of the reliability of the output, is of
14 significant importance in understanding the way in
15 which you would carry the MRD measurements into
16 clinical trials as surrogate endpoint biomarkers.
17 And then the question of whether they can be
18 standardized sufficiently for use informatively in
19 the trials is, of course, of relevance.

20 So to summarize really briefly, surrogate
21 endpoints do substitute for clinical efficacy
22 endpoints in the way that Dr. Deisseroth indicated

1 in his talk just before me. These are a category
2 of drug development tool. There are the
3 prognostic, the predictive, the pharmacodynamic,
4 and then the surrogate endpoint biomarker aspects
5 of drug development tools that are recognized for
6 qualification by the agency.

7 The surrogate endpoint biomarker, as it
8 would be used in clinical trials, has to be framed
9 in terms of the specific context of use of the
10 biomarker for the purposes of the trial and the
11 decisions that we made with respect to the drug.

12 There was not a requirement for a cleared or
13 approved test as a surrogate endpoint biomarker,
14 though you can certainly expect that a deep
15 understanding of the analytical performance
16 characteristics of any test that is put into this
17 rule is expected by the agency.

18 Lastly, we do understand that minimal
19 residual disease measurements, as they are carried
20 out currently in the United States, are not widely
21 standardized. There are no cleared or approved
22 tests by which you can easily leverage what's

1 already known by the agency in terms of the
2 analytical performance characteristics that would
3 underscore a surrogate endpoint biomarker use.

4 So part of the discussion that I suspect
5 will be happening today is how one proceeds with
6 respect to these features and being able to apply
7 MRD as a surrogate endpoint. Thank you.

8 (Applause.)

9 DR. REAMAN: Thanks, Bob.

10 Our next presentation is by Dr. Mark
11 Rothmann from the Office of Biometrics in the
12 Office of Translational Science on statistical
13 considerations.

14 **Presentation - Mark Rothmann**

15 DR. ROTHMANN: Thank you. I'm Dr. Mark
16 Rothmann. I'm a statistician at the FDA. I'm
17 going to give a general introduction to surrogate
18 endpoints. I'll briefly describe clinical and
19 surrogate endpoints, how surrogate endpoints are
20 used, and how we evaluate surrogate endpoints,
21 particularly what's of interest to statisticians.
22 I'd also like to acknowledge that some of the

1 slides that I'm using in my presentation come from
2 a presentation by Dr. Thomas Fleming.

3 A clinical benefit endpoint is a direct
4 measure of how a patient functions, feels, or
5 survives. This has been a definition that's been
6 used by Dr. Robert Temple going back to the '80s.
7 The National Institute of Health definition of a
8 surrogate endpoint is a biomarker intended to
9 substitute for clinical endpoint.

10 There are two ways of interest in a
11 regulatory setting related to approvals of how
12 surrogate endpoints are used. They can be used to
13 draw conclusions on effects of a clinical benefit
14 endpoint or, as in accelerated approval, they can
15 be used to reasonably likely predict clinical
16 benefit.

17 For a biomarker to be a surrogate endpoint
18 for a particular clinical benefit endpoint, the
19 effect of an intervention on the clinical benefit
20 endpoint should be reliably predicted by the effect
21 of the intervention on the surrogate endpoint.

22 The analysis on the surrogate endpoint

1 should provide guidance on how the clinical benefit
2 endpoint will compare between treatment arms.
3 Evaluating a marker for a surrogate endpoint
4 involves two important things, one, an
5 understanding of the disease process and an
6 understanding of whether the surrogate or the
7 biomarker is on the clinical pathway of the process
8 towards benefit.

9 As we want to have effects on the surrogate
10 reliably predict effects on the clinical outcome,
11 we need to have results from clinical trials that
12 examine how the effects on the surrogate have
13 related to effects on the clinical outcome. This
14 usually requires a meta-analysis.

15 There are a variety of ways a biomarker may
16 be related to a clinical endpoint. The two
17 endpoints may be correlated, like CD4 counts and
18 the risk of mother-and-child transmission of HIV,
19 but not in a cause-and-effect way. The biomarker
20 might not be on the clinical pathway towards
21 benefit.

22 CD4 counts are correlated with mother-child

1 transmission of HIV. However, CD4 is not on the
2 causal pathway towards benefit. Viral load, which
3 is on the pathway towards benefit, as been
4 discussed earlier, has been a surrogate in many HIV
5 settings.

6 There are other possibilities. The
7 intervention may affect one of multiple pathways
8 towards clinical benefit. Usually, when this
9 happens, and even if there is no off-target
10 effects, large effects on the intervention may
11 correspond to small effects on clinical outcome.

12 An intervention may have a mechanism of
13 action that's independent of the disease process or
14 have what are called off-target effects that may
15 positively or negatively affect clinical outcome.
16 For example, erythropoiesis-stimulating agents, or
17 ESAs, positively affect, increase, hemoglobin. But
18 they also increase the risk of thrombosis, which
19 leads to an increase in the risk of cardiovascular
20 events. So that increase in the risk of thrombosis
21 would be an off-target effect.

22 Prentice's criteria is a criteria that's

1 really set to establish that something is -- what's
2 called a validated surrogate or a surrogate that
3 can be used to draw conclusions on a clinical
4 benefit endpoint.

5 What Prentice's criteria says is that
6 something that is a surrogate in which you're able
7 to draw conclusions of a positive or negative
8 effect on a clinical benefit endpoint, the
9 surrogate must be correlated with the clinical
10 outcome at a patient level, and the surrogate
11 endpoint must fully capture the net effect of the
12 clinical outcome.

13 So if you knew what the value of the
14 surrogate endpoint was, your ability to guess what
15 the clinical outcome is, is not enhanced by then
16 knowing the treatment. That's what's meant by this
17 sub-bullet here that's a little technical about
18 treatment parameter being zero. Now, I'll say it
19 again. After you know the surrogate, your ability
20 to predict the clinical outcome for the patient is
21 not further enhanced by knowing the treatment.

22 Assessing the reliability of a surrogate

1 endpoint, particularly if we wanted to talk about
2 accelerated approval, involves determining the
3 pattern between observed effects on the surrogate
4 endpoint to observed effects on the clinical
5 benefit endpoint, and assessing the amount of
6 deviation from that pattern.

7 For example, this comes from a paper by Dan
8 Sargent in which he did an evaluation of
9 predictability of comparisons on three-year
10 disease-free survival to comparisons on five-year
11 overall survival in adjuvant colorectal cancer for
12 studies that use 5-fluorouracil as base
13 chemotherapy.

14 Plotted here are the disease-free survival
15 and overall survival hazard ratios from 18 such
16 studies. We see here that there is a fairly linear
17 relationship between the hazard ratios, as denoted
18 by this dotted yellow -- we'll get back to the
19 picture here.

20 We see that there is a fairly linear
21 relationship between the disease-free survival
22 hazard ratios and the overall survival hazard

1 ratios, as denoted by this yellow line.

2 We can quantify how accurate this is. The
3 typical amount we're off, by using this line to
4 determine the overall survival hazard ratio from
5 the disease-free survival hazard ratio, is 0.05.
6 So we can quantify how reliable this pattern is in
7 predicting overall survival hazard ratio based on
8 five-year data, from a disease-free survival hazard
9 ratio, based on three years' data.

10 Let me also point out -- I don't want to
11 forget -- we also have here that large effects on
12 disease-free survival correspond to large effects
13 on overall survival, small effects on disease-free
14 survival to small effects on overall survival, and
15 no effects or negative effects on disease-free
16 survival to no effects or negative effects on
17 overall survival. So you have that type of
18 consistency.

19 From such a meta-analysis, the conclusions
20 formerly hold for the types of trials and therapies
21 studied. An endpoint may be an appropriate
22 surrogate endpoint for one class of agents, but not

1 for another class of agents. That concludes my
2 talk. Thanks.

3 (Applause.)

4 **Clarifying Questions**

5 DR. REAMAN: We can open this up to any
6 questions from the panel for any of the presenters
7 from this first session. If there are none --

8 DR. KAY: I do have one.

9 DR. REAMAN: Yes.

10 DR. KAY: So from the standpoint of the
11 assays that will be used for minimal residual
12 disease, I'm not sure exactly who to address this
13 to, but how rigorous does the assay have to be from
14 laboratory to laboratory if there is no clinically
15 acceptable across-the-board assay?

16 Obviously, the question at hand, there are
17 different approaches and many different labs where
18 the laboratories feel they are validated. So I'm
19 guessing from what I heard, that as long as the
20 assay has quality, however we determine it that
21 that assay could be used. But I'm just wondering
22 if that would be a contentious issue as we go

1 forward.

2 DR. REAMAN: Do you want to? No. I was
3 pointing to Dr. Becker, if he'd like to --.

4 DR. BECKER: Different performance
5 characteristics for assays across laboratories can
6 be a potentially confounding factor in the way that
7 you would apply the marker in a clinical trial, so
8 that it's of course for the review division to
9 decide what is sufficiently consistent resulting
10 from the assays to meet the purposes of the trial
11 itself.

12 But at a minimum, I guess I would expect
13 that you'd want to be sure that you didn't have
14 some kind of systematic division between assays
15 that give you one kind of result versus another
16 kind of result, say, across treatment arms. There
17 are many ways in which assays that vary in their
18 analytical performance might in some sense confound
19 or complicate the interpretation of trial results.

20 DR. REAMAN: I suspect we'll have much more
21 discussion about that later on today, as we talk
22 about assays and performance characteristics.

1 Dr. Byrd?

2 DR. BYRD: Thank you. And I guess an even
3 more complex issue is the assay as it relates to
4 the evolving -- the change in therapy. So if we
5 looked at combined cytotoxic chemotherapy, in many
6 of the studies that we're going to hear about,
7 where you obtain MRD-negative status using a
8 reproducible assay, it's very, very predictable.
9 Peter and others have shown this, and even Andy
10 from the blood. And you, say, add a monoclonal
11 antibody to it that's very good at clearing out
12 blood disease, and all of a sudden, you shift where
13 that may not be as important because the patients
14 may have big lymph nodes that you're not seeing.

15 I think -- and my talk will challenge people
16 relative to the BCR antagonists as well, how do you
17 incorporate the change of therapy into guidelines
18 for surrogate endpoints. Progression-free survival
19 is probably not going to change relative to being
20 somewhat predictive of overall survival, but
21 surrogate endpoints like this, where the therapy
22 changes the biology of the disease, very well

1 could.

2 I think that's a point that we have to
3 consider in our discussions today.

4 DR. HILLMEN: I agree that's the case, but
5 it's likely that MRD will remain -- if you become
6 negative, it will remain a predictive marker, and
7 maybe some therapies don't need that improvement in
8 the biomarker to result in a prolonged remission.
9 But the addition of those therapies that you
10 mentioned to chemo might actually increase the
11 negative direction, so I think it's a valid assay.

12 DR. REAMAN: I think there was a comment
13 earlier about context of use. So I think any
14 decision about its applicability as a surrogate is
15 really going to depend on the specific drug being
16 evaluated and the clinical situation. But you're
17 absolutely right, different drugs impacting or
18 affecting the biology of the disease.

19 So I don't think we're looking for a single
20 solution for every new therapeutic approach for
21 this disease or any other disease. So I think
22 surrogate endpoint considerations will be drug-,

1 maybe not even class-dependent.

2 Dr. Kay?

3 DR. KAY: I think we will be talking a lot
4 more about this as the day goes on, but we're
5 preempting some things. But I think the assays
6 that we all use do vary from academic center to
7 academic center. And that's one of the reasons I
8 ask that question. But I think it's also
9 complicated by where you sample the MRD from.

10 I agree with John that if you're looking at
11 blood, that's obviously going to be maybe different
12 than if you're looking at bone marrow or if you're
13 assessing lymph nodes. So I think it does get to
14 be more complicated than just the assay itself.

15 DR. REAMAN: I think we'll move on to the
16 second part of today's session. Dr. De Claro, you
17 take over.

18 DR. DE CLARO: Good morning. I am Angelo De
19 Claro. I'll be chairing the second session. The
20 title of the second session is Current Status of
21 MRD Assessment in CLL. Our first speaker is Dr.
22 Paolo Ghia from Milan, Italy. The title of his

1 talk is minimal residual disease in CLL, coming
2 from far away.

3 **Presentation - Paola Ghia**

4 DR. GHIA: Thank you. Good morning to
5 everyone. I want first to thank FDA, ASCO, ASH,
6 and the Leukemia Lymphoma Foundation for organizing
7 this interesting meeting, workshop that was kind of
8 unbelievable only a few years ago. But we have to
9 keep in mind that, indeed, the MRD studies in CLL
10 are not really young and it's at least 10, 15 years
11 that investigators are using it in clinical trials
12 and for experimental studies.

13 So what we learned in these 10 or 15 years
14 is that, indeed, even also in chronic
15 lymphocytic -- when treating chronic lymphocytic
16 leukemia, the quality of response is the best
17 predictive marker for clinical outcome. So this is
18 quite obviously many other hematologic disorders,
19 but also in CLL, so the better there is a clinical
20 response, the better is the probability of survival
21 of a single patient. And this became real and
22 visible thanks also to the improvement in new

1 therapies and combination of chemotherapeutics.

2 So when we had, 30 years ago, only
3 chlorambucil in our armamentarium, then it was
4 difficult to see how the quality of response could
5 really improve the clinical outcome. But then it
6 became quite obvious when we started having better
7 therapies, like monoclonal antibodies or a combined
8 chemotherapy, like a safe darabine,
9 cyclophosphamide, and better overall FCR, when we
10 combine FC with CD20 antibodies.

11 As we were able to increase and improve the
12 number of completed remissions, clinical-complete
13 remissions in CLL, then it became also quite pushy
14 to go and analyze MRD, so minimal residual disease
15 status in our patients. And indeed, while we
16 increase the complete remission rate, we also were
17 able to improve on the quality of the response at
18 the biological level, meaning that we were able to
19 get rid of most of the cells in reaching minimal
20 residual disease status.

21 Just to make a summary of the last 10 or
22 15 years, several studies indeed used the MRD

1 assessment when using different combinations of
2 therapies, even in monotherapies like with
3 alemtuzumab. And as you can see here, while we
4 improve the percentage and proportion of a complete
5 remission, we were also able to improve on the
6 number of cases reaching the minimal residual
7 disease status.

8 As you can see here from the bottom line, in
9 many cases, in many studies, correlated to it,
10 indeed, the clinical outcome, meaning that it
11 correlated with progression-free survival, duration
12 of response, or even overall survival.

13 This was not clear in every study. But this
14 is indeed the problem of these 10, 15 years of
15 work, because what we have really to understand is
16 what we mean for minimal residual disease in CLL
17 and how we detect it, because, of course, in 10 or
18 15 years of time, the technology improved, changed,
19 and indeed also the quality of the assessment of
20 MRD definitely changed and improved.

21 So I go here just for simplicity and just to
22 set the ground for the following discussion during

1 today. I am using the new guidance for CLL
2 management by the IWCLL NCI. And the first thing
3 that we learn from here is that when we define MRD,
4 minimal residual disease status in CLL, we do not
5 mean that we eradicate every single cell, every
6 single leukemic cell. It just means that we are
7 able to reach a certain level of eradication of the
8 disease, which is indeed one cell out of 10,000.
9 We change 10 to the 10^{-4} . And this is a number that
10 you have to keep in mind because this will be
11 repeated throughout all other talks after me.

12 So that means that, nowadays, in 2013, we
13 are able to reliably detect 1 out of 10,000
14 leukemic cells. And that is the limit of our
15 technology at the moment and that is considered to
16 be minimal residual disease. That doesn't mean
17 that this level cannot be overcome, and in the
18 future, this probably will be the case.

19 So how do we detect reliably 1 cell out of
20 10,000 in CLL? Again according to the guidelines,
21 that can be done with two methodologies. One is
22 the PCR, quantity PCR. We are going to go a little

1 bit more in detail in the next slides, and the
2 other one flow cytometry, multicolor flow
3 cytometry. These are the two techniques, as we are
4 going to see, that has been standardized and used
5 throughout the multicenter studies, and so they
6 have been validated in international studies.

7 As I said, this is not the end of it. It's
8 just the beginning of it. So maybe, in the future,
9 we can definitely improve on both the level of
10 detection as well as the quality of our assay. So
11 one I just mentioned, because this may come up
12 later in the discussion, is NGS, next-generation
13 sequencing approach, which indeed can apparently
14 reach lower levels of detection, like 10 to the -5.
15 And this correlates very well with both PCR and
16 flow cytometry.

17 It also has a great advantage that it is
18 universally applicable, so it can be used to any
19 patient without really knowing in advance the
20 biological features of the patient. But still,
21 this is a technique that is in its infancy. So
22 first of all, it costs still a lot, but that will

1 decrease in the future, but it has not been
2 validated in clinical trials. It has not been used
3 in many cases; besides, it has also a technical
4 limitation because, so far, it's not reliable and
5 reproducible. And this, of course, cannot be used
6 throughout international studies.

7 But this is just to mention that the
8 technological landscape is a moving target, so it's
9 something that will change in the future. So I
10 think that we don't have really to focus too much
11 on what specific assay we need to use, but we have
12 only to define the quality of an assay so that the
13 reproducibility and reliability of an assay -- in
14 order to get down to 10 to the -4 level of
15 detection.

16 So I'll just quickly go through the two
17 established methodologies, PCR and flow cytometry.
18 And, again, just to make you understand the
19 progress that we made in 10, 15 years. And the
20 difference is also between different studies, so we
21 have really to go and read the materials and
22 methods before judging the quality of the MRD

1 studies and the reproducibility of MRD studies.

2 The first way to detect MRD -- so first of
3 all, using PCR, we use the immunoglobulin chain
4 rearrangement as the target to detect the presence
5 of leukemic cells. And at the beginning, this was
6 done by consensus PCR, so let's say generic primers
7 for every patient. And the level of detection was
8 only 10 to the -2, 10 to the -3. Then of course,
9 the situation became definitely better with
10 allele-specific primer, so we need to know the
11 sequence of the immunoglobulin of each patient,
12 design specific primers. And this gets down to
13 10 to the -4, even probably 10 to the -5 in expert
14 hands.

15 Nowadays, the technology is quantitative
16 PCR, RQPCR, when we use, indeed, probes,
17 fluorescent probes, to detect the presence of MRDs.
18 And this is what has been standardized. You will
19 hear much more later by Sebastian Bottcher in the
20 German studies and many other colleagues.

21 The other technique is the flow cytometry.
22 Again, also in the path, the flow cytometry has

1 been used widely with only two colors, like the
2 classical CD5, CD19 staining or kappa lambda
3 ratios. And these again were not able to get down
4 to 10 to the -4, so we were able just to detect 1
5 out of 100 cells, 1 out of 300 cells, which is not
6 enough, again. And instead, in most recent
7 periods, the flow cytometry protocol has become
8 standardized and validated in international
9 studies. And it needs to include at least the four
10 colors, but now it is improving. It is increasing
11 to six and even eight colors. And you will hear
12 more from Andy Rawstron and this is able to get
13 down to a level of detection of 10 to the -4.

14 Here, I show you very quickly, we see much
15 more with Andy Rawstron. The established protocol,
16 four-color protocol, four-color panels, are used in
17 several international studies. And this has been
18 evaluated thanks to the ERIC, the European Research
19 Initiative on CLL. But this was part of an
20 international study, including also colleagues from
21 the U.S. So this is good, something on which a
22 consensus has been reached.

1 Now, we know how to detect MRD in CLL
2 reliably, and where should we detect it, as
3 Professor Kay discussed earlier. So again,
4 according to the guidelines and based on the
5 literature and the data provided in the literature,
6 we can do that definitely on blood, with the only
7 exception of the use, when using the monoclonal
8 antibodies, they of course tend to clean the
9 leukemic cells much earlier from the peripheral
10 blood than from the bone marrow. So in the first
11 three months after using monoclonal antibodies, one
12 should avoid testing MRD in peripheral blood, but
13 after that, it is a valuable predictor of the
14 status of MRD also in bone marrow.

15 Again, here, you will see from Andy, again,
16 so there is a very nice correlation between the MRD
17 status in the blood versus the MRD, with the
18 exception of the use of monoclonal antibodies in
19 this case, alemtuzumab, but the same thing occurs
20 when using C20 antibodies.

21 So what is the best technology? I don't
22 think that this is the aim of this workshop, and

1 generally speaking, there is never a best. I think
2 both technologies have advantages and
3 disadvantages. Both are able to get down to 10 to
4 the -4, which is now the level that we want to
5 reach, but that doesn't mean that, in the future,
6 we can go lower than that.

7 Maybe flow cytometry has some advantages
8 because you don't need to have a sample testing,
9 the diagnosis. And of course the complexity of the
10 procedure is much lower as compared to PCR. And,
11 indeed, even to improve the simplicity of the
12 technique, as Andy Rawstron will show you, now we
13 are moving from a four-color panel to a six-color
14 panel to an eight-color panel. But nevertheless,
15 the point is that with both technologies, RQPCR and
16 flow cytometry, we are able to detect the level
17 that we need in order to assess MRD status in CLL.

18 So is it meaning when we consider this -- so
19 when we use a standardized protocol and validated
20 protocol, is still MRD negativity meaningful in
21 CLL? Does it really improve our management of
22 patients? And indeed, the answer is yes. In these

1 most recent works, you can see that, indeed,
2 reaching MRD negativity status, it correlates with
3 a better treatment-free survival or even overall
4 survival. And this is independent of the therapy
5 used. So it doesn't matter how you get there. The
6 important thing is to get to MRD negativity stages.

7 The best confirmation of these data are
8 actually shown by the data of Sebastian Bottcher,
9 who will present later, from the CLL8 study, the
10 German CLL study group, where the FCR was compared
11 to FC. It showed indeed a higher efficacy of FCR
12 versus FC.

13 But the data, when MRD status was analyzed,
14 was very interesting. Of course, with FCR, you are
15 able to get to more MRD-negative status as compared
16 to the use of FC. But the important thing is
17 regardless of how you get, again, to MRD negativity
18 status, so using FC or FCR, if you get down to less
19 than a 10 to the -4 level, then you're probably to
20 survival or it's definitely equal.

21 The other important thing is that MRD status
22 is predictive of the clinical outcome, regardless

1 of the clinical stages. So it's not only
2 independent of the type of therapy that you use,
3 but also independent of the clinical response that
4 you have. So if you consider only people who
5 achieved a partial response, still those who
6 reached MRD status in the peripheral blood had the
7 better outcome as compared to those who did not
8 reach it.

9 Having said that, I want just to remind you
10 that MRD may be in its infancy in CLL, but I think
11 that the future is quite bright. And I hope that,
12 really, we can definitely reach a consensus on
13 using the MRD assessment as a surrogate clinical
14 endpoint in clinical trials.

15 Thank you all for your attention. Here are
16 all the people in my lab, in my group, who worked
17 for MRD. And I want also to thank the ERIC, the
18 European Research Initiative on CLL, and all the
19 people who participated actively in the MRD
20 studies, and in particular Andy Rawstron and
21 Sebastian Bottcher. Thank you very much for your
22 attention.

1 (Applause.)

2 DR. DE CLARO: Thank you, Dr. Ghia. Our
3 next speaker will be Dr. Sebastian Bottcher from
4 Kiel, Germany. The title of his talk is German
5 Multicenter Experience with MRD Flow Principles of
6 EuroFlow CLL MRD Assessments.

7 **Presentation - Sebastian Bottcher**

8 DR. BOTTCHER: Thank you. Good morning.
9 I'd like to thank the organizers for allowing me to
10 address actually two topics. I will share German
11 multicenter experience with MRD flow, and then will
12 talk about EuroFlow and EuroFlow principles for
13 doing MRD testing in CLL.

14 What I'd like to do to start off with the
15 clinical part and talk about how we can use MRD to
16 predict progression-free and overall survival. And
17 then I would like to discuss a couple of variables
18 that might impact on the prognostic significance of
19 MRD. I will be discussing the levels, the
20 treatment, the relationship to clinical staging,
21 the relationship to additional risk features,
22 biological risk features in CLL, and will also talk

1 about the time point, when we should best assess
2 MRD.

3 I will not cover as the use of material and
4 the regrowth kinetics after treatment, but I've
5 been providing some slides in the backup. And then
6 I will be discussing technical aspects that will
7 show a comparison between MRD flow and PCR, and
8 also talk about principles of EuroFlow CLL MRD
9 detection.

10 Most of the data I'm going to present today
11 is from the CLL8 trials that showed the efficacy of
12 adding rituximab to a standard FC chemotherapy.
13 Within that trial, we were able to analyze 5,000 or
14 so samples from the trial. And we were able to
15 analyze after three cycles of therapy, after an
16 additional three cycles of therapy, and then during
17 follow-up.

18 Now, if you turn to the FC, to the chemo-
19 only part of the trial, you can see that, during
20 therapy, after three cycles of therapy, you see a
21 dramatic decrease in median MRD levels, and you see
22 even more decrease in MRD levels after an

1 additional three cycles of treatment. And the
2 medians are symbolized with the red bars. And if
3 you don't sit back and wait, MRD levels will
4 increase over time.

5 In principle, you see the same thing if you
6 turn to the FCR arm. There's a notable difference
7 that for each and any individual point, you see
8 lower MRD levels for FCR-treated patients.

9 So how can we use it to predict progression-
10 free and overall survival? I will first discuss at
11 this time what we call final restaging. That's
12 three months after initiation of the last treatment
13 cycle.

14 If you combine both treatment arms from that
15 trial and simply cut at 10 to the -4, according to
16 the IWCLL guidelines, you get two almost equal
17 groups of patients. One was above, one below 10 to
18 the -4. That's measured in peripheral blood. And
19 you can obviously see that the prognosis for those
20 with MRD negativity is much better compared to the
21 prognosis in patients who have higher MRD levels.
22 However, we found that we can do much better by

1 additionally subdividing the group of MRD-positive
2 patients at a threshold of 10 to the -2.

3 You can see that the red line shows patients
4 with very high MRD levels, above 10 to the -2, and
5 they have the poorest outcome. And then we have a
6 group of intermediate-risk patients with
7 intermediate MRD levels.

8 Now, that is important because we observed
9 that, actually, the poorer responders, the very
10 poor responders, are actually those patients who
11 have inferior overall survival. In this current
12 follow-up, it is now like six to seven years from
13 that trial. We don't see a very pronounced
14 difference in overall survival between
15 intermediate-level and low-level subgroups. So it
16 is not statistically significant at the moment.

17 Now, how does treatment affect our ability
18 to use MRD as a surrogate endpoint? And Paolo has
19 already shown this data. I just want to go briefly
20 again about that because I think that is really
21 important for using MRD as a surrogate endpoint.
22 You see, the Kaplan-Meier curves are virtually

1 superimposable once you divide patients according
2 to MRD status. And the superiority is the added
3 efficacy rituximab FC. It's fully captured by a
4 higher frequency of patients who become MRD
5 negative, as seen in the histogram on the far
6 right.

7 So if you want to go into more formal
8 Prentice statistically analyses, then you can do as
9 done by Paul Delmar here. You can simply subgroup
10 according to the threshold of 10 to the -4. Then
11 you see again that low-level MRD has been official
12 for the patient. You see that FCR versus FC is
13 associated with a hazard rate of .6, but once it
14 adjusts for MRD response, you don't see that effect
15 any more. So that means that the treatment effect,
16 at least for this, is fully captured by MRD
17 assessments. That makes a point for using it as a
18 surrogate marker.

19 Now, if you want to use it as a surrogate
20 endpoint, you have to be reproducible, obviously.
21 And so we try to find data to confirm this. And we
22 first compare it to our own measurements using a

1 different trial. This is a single-arm trial using
2 B or bendamustine rixutimab. It's called CLL2M.
3 On the right-hand side, you see the results from
4 the CLL2M trial. And you again see these three
5 groups of MRD that you have defined, according to
6 the levels 10 to the -4, 10 to the -2. And on the
7 left-hand side, you see the two arms of CLL8 with
8 FCR and FC.

9 Then you see the median progression-free
10 survival. And you can estimate that for both
11 situations, usually for the poorest patients, you
12 have medium progression-free survival of about one
13 year and about three years for the intermediate MRD
14 level subgroup.

15 Now, that is done in our own lab in Kiel.
16 How does it compare to other labs? And I leave it
17 to your judgment, whether you will call this a good
18 correlation or a bad correlation. At least it
19 shows you a little bit of the variability that is
20 in this measurement.

21 All these three trials looked into FCR
22 first-line treatment, and one is our own and the

1 other one are two French studies. They recently
2 published. And you always find something like one
3 sort being MRD positive after FCR treatment in
4 peripheral blood.

5 The lipid trial had 17p-deleted patients in
6 it, and a retrospective study published by Bouvet
7 had an issue with dose intensities that might
8 account for some of the differences, but you will
9 never know for sure.

10 When it comes to predictability, also the
11 Bouvet data seemed to show that if you have MRD
12 above 10 to the -4, you usually end up with a
13 medium progression-free survival of something like
14 two years in first-line patients treated, probably
15 with FC, FCR, and BR.

16 Now, what about the time point of sampling?
17 Here, I will present data from CLL8 comparing MRD
18 measured during therapy and after therapy. On the
19 left-hand side, you'll see Kaplan-Meier plots for
20 measuring during therapy, and on the right-hand
21 side, you'll see the final restaging data that is
22 after completion of therapy.

1 As you can see here, you have a proportion
2 of patients who becomes MRD negative already after
3 three cycles of therapy. And those seem to have a
4 very good prognosis. And it does not seem to be
5 much better for patients after six cycles of
6 therapy. However, as shown with the pie charts on
7 the bottom left, the proportion of patients who
8 become MRD negative obviously increases, meaning
9 that you save a fair proportion of patients by
10 adding another three cycles of therapy.

11 If you now look into the previous groups,
12 symbolized with red and green lines, you can see
13 that their outcome is worse if they still have high
14 levels of MRD after completion of treatment. And
15 that probably translates to the general principle
16 that you have higher hazard rates after completion
17 of therapy because then you have captured all the
18 treatment effect in your measurement. That
19 probably is the most preferable time point for
20 measuring it. And that also holds true, from my
21 perspective, for the new drugs. As long as you
22 have an induction sort of treatment, you should

1 measure at a time point when you're sure that the
2 complete treatment effect is captured in your MRD
3 measurement.

4 Now, how does clinical staging influence
5 that? This is the hardest part usually in the U.S.
6 I'll try anyways. As already shown by Paolo,
7 clinical PR patients, if they happen to become MRD
8 negative, have a very comparable outcome compared
9 to clinical CR patients. Having said that,
10 obviously there is a correlation between being CR
11 and having a high likelihood of becoming MRD
12 negative.

13 Now, what about biological parameters? And
14 out of many biological parameters we have analyzed,
15 I will show you mutational status. So if we
16 subdivide it from the FCR arm of our trial, we
17 subdivided patients according to MRD status and
18 mutational status of the immunoglobulin gene.

19 As you can see, patients who have an
20 intermediate or low level of MRD result after
21 treatment and happen to carry an unmutated IGVH
22 gene are those patients who have a shorter

1 progression-free survival. And that probably
2 relates to a faster speed of regrowth. I can't
3 show you the data, but that probably is the
4 explanation for that.

5 So in the German CLL study group, based on
6 those data, we combined patients who have either
7 very high levels of MRD or intermediate levels of
8 MRD plus an unmutated immunoglobulin gene status,
9 and considered those candidates for maintenance
10 therapies.

11 Now, if you put everything into multivariate
12 analyzers, it does not come as a surprise that MRD
13 really remains and is still a very important
14 prognostic parameter. And the other things that
15 really remain prognostically significant is 17p
16 deletions and mutational status.

17 So now turning to methods, if you compare in
18 Germany again from CLL8, MRD flow to RQPCR. We did
19 this within CLL8 and separated the true arms of the
20 CLL8 trial. First, focusing on FC-treated
21 patients, we found a very high correlation, a very
22 high quantitative correlation, between the two

1 methods. And luckily, that worked out for
2 FCR-treated patients as well.

3 Then we had some patients that gave us
4 quantitative results, was one of the methods and
5 very positive by the other method. And for those
6 samples, out of a total of 530 or, we found there
7 are very few in total, about 1 percent having this
8 situation. And then there were a couple of samples
9 where we had positive but not a quantifiable result
10 by PCR. And about one-third of those are positive
11 by flow, and about two-thirds of those are negative
12 by flow cytometry.

13 Now, if you apply the IWCLL standard
14 threshold of 10 to the -4, you again see a very
15 high correlation between the two methods with a
16 concordance rate of about 95 percent within the FC
17 arm and similar concordance rate in the FCR arm.
18 So I would say they are the best validated methods
19 at the moment one could use.

20 Now turning to the last part of my talk,
21 that is EuroFlow. It colors CLL MRD. You might
22 know that EuroFlow is an international or more or

1 less European consortium that actually tries to
2 standardize flow cytometry for diagnosis and
3 follow-up.

4 Here, we have a subproject where we actually
5 tried to develop standardized MRD assays, more or
6 less on our experience with flow cytometry, based
7 on our standardization, our technical
8 standardization. This is also influenced by a
9 really in-depth look at normal maturation, and
10 finally on the availability of large databases of
11 human immunophenotypic information.

12 The standardization has been an effort for
13 like six years just in this consortium. And we
14 tried to make the measurements as comparable
15 between the different labs as we could. And we did
16 so by really standardizing everything, samples,
17 preparation, instrument setup, data analyzers, and
18 as I said, we tried to reach really reproducible,
19 through a sense, intensities between the
20 participating lab.

21 Then we did quality control rounds after
22 implementation of all this and tried to measure how

1 much would we differ just by technical variation
2 between the labs. And usually, this is the last
3 three consecutive years of standardization rounds.
4 Usually, you cannot end up with a CV below
5 50 percent. So for the first time, you can
6 actually measure how much variation is introduced
7 into the whole process by the simple fact that
8 different people do the same thing.

9 Now, here is a panel that's actually being
10 made to diagnose the various kinds of -- Hodgkin's
11 lymphomas. We measured 100 CLL patients and a
12 couple of normal peripheral blood and bone marrow
13 samples as well.

14 Then we combined the whole immunophenotypic
15 information and compared this to peripheral blood.
16 And then we came up with a list of markers that
17 contribute to this separation on the one-hand side,
18 and you can see them on the gray box on the right.
19 If you also calculate, it's a fast positive rate
20 that gave us background.

21 As you can see, the fast positive rate, with
22 all its markers currently is like 8 times 10 to the

1 -5. And we did not consider this sufficient
2 because we were aiming at 10 to the -5.

3 So we developed it for additional rounds of
4 testing using panels like this. And then we
5 analyzed which markers would contribute the most to
6 the separation between CLL and peripheral blood and
7 bone marrow samples. And as you can see here,
8 marker slide 22, 280, 81, probably should not be
9 dropped from such panels.

10 We also really analyzed the gating strategy,
11 and compared different gating strategies, and found
12 that, first, focusing on a 27-5 combination would
13 give us the best results in terms of the lowest
14 background.

15 We also tried to achieve an even higher
16 resolution by adding new markers to this concept.
17 And that is the total, standardized gating strategy
18 here, so you actually superimpose CLL samples over
19 the normal peripheral blood. And then you apply
20 the principle component analyzers to the whole
21 immunophenotype, and you simply calculate how much
22 of the normals would fall into the area of the CLL,

1 really using the standardized phase, standardized
2 to standard deviation of the CLL cells.

3 With that, we were able to approach
4 something like 2 times 10 to the -6 in terms of
5 false positivity range. And we are currently still
6 working a little bit on technical aspects of this,
7 but we hoped we could actually reach 10 to the -5
8 using a flow assay. We also looked if we were able
9 to would recover, really, all the cells from that
10 initial testing set and were, indeed, able to do
11 so.

12 So to summarize, I think I have convinced
13 you, and I hope I have convinced you, that the
14 prognostic significance of MRD is independent from
15 the type of induction therapy. This forms the
16 basis to use MRD as an endpoint in randomized
17 clinical trials.

18 After induction therapy, you cannot and we
19 don't have data for maintenance sort of therapies,
20 and we cannot use it as an endpoint in such a
21 clinical setting. I would really propose to use
22 the level of 10 to the -4, according to guidelines,

1 as a primary endpoint and would propose to also
2 check for 10 to the -2. I hope that I could
3 convince you a little bit that even in PR patients,
4 it makes sense to look for MRD.

5 I did not show you data on the comparison
6 between peripheral blood and bone marrow. It's put
7 in the backup. What we found is that you find more
8 patients being positive in bone marrow. However,
9 the clinical significance is comparable between
10 peripheral blood and bone marrow.

11 MRD is prognostic at all time points, but I
12 would propose to use a time point when the full
13 treatment effect has been captured by the
14 measurement. And I showed you that MRD is
15 influenced or the prognostic significance of MRD
16 will depend on the actual cohort you study.
17 Considering cohort, having all patients with
18 mutated IGVH status would give you much longer
19 progression-free survival. That's why, if you want
20 to use it in an endpoint, you should use it in a
21 randomized trial that you have control for all
22 known and unknown data that might influence the

1 prognostic significance of that test.

2 For the technical aspects, I would really
3 say you have to have a quantitative measurement,
4 down to the -4. That's really necessary. I know
5 that we see that MRD flow and PCR is a prime -- IGH
6 PCR, I mean -- is really suitable standardizing
7 cross-validated enlarged set of samples; 550 should
8 be something that you could really rely on. And I
9 would say that all available data has been done, at
10 least with a good sensitivity, down to the -4, has
11 been from a centralized lab.

12 So we cannot really know what will happen if
13 we use labs from everybody, so I would currently
14 call for centralized diagnostics. And for optimal
15 results, I would propose to use the best
16 standardization that is available in a particular
17 field, just to be sure that the variation we
18 observe is not a technical variation but really
19 comes from biological differences.

20 Quality control rounds are essential and
21 what happens with novel technologies, nobody knows
22 until we've studied a really large prospective

1 series of samples. And within EuroFlow, we are
2 currently developing a fully-standardized
3 eight-color MRD flow tube.

4 With that, I would like to thank you for
5 your attention, all the people who contributed to
6 this data. Many I did not mention on this slide.
7 In particular, I would like to thank the German CLL
8 study group, the EuroFlow consortium, and people
9 who made this and supported this, including the
10 European Commission and Hoffman-La Roche. Thank
11 you.

12 (Applause.)

13 DR. DE CLARO: Thank you, Doctor.

14 DR. LOZANSKI: Did you observe a skewed
15 segregation of patients with adverse cytogenetics
16 to an MRD-positive group versus patients which have
17 good, for example, 13q- to MRD negative group? You
18 show some data on the impact of the mutational
19 status, but this is very complex. We know that, in
20 CLL, it's a very heterogeneous disease.

21 So could it be that people with MRD-negative
22 status do so well because they represent lower-risk

1 patients without 17p, without 11q, and with 13q
2 deletion?

3 DR. BOTTCHEER: Yes. We actually studied
4 this in large extent, and it's impossible to
5 predict MRD status with the notable exception of
6 17p. All the other risk features did not correlate
7 with statistical significance to MRD status.

8 That's probably because many things
9 influence MRD status. It's not only the
10 cytogenetics, but also let's say dose intensity,
11 whether it's Christmas and somebody relates the
12 next treatment cycle, things like that. Everything
13 is really captured within MRD. For instance,
14 pharmacogenetics, renal function, all this might
15 influence actual dose intensity in a particular
16 patient and it's reflected probably because it
17 integrates everything, being a response parameter.
18 That's why only 17p came out as a marker to predict
19 for MRD positivity, obviously.

20 DR. DE CLARO: I think we're running out of
21 time, so I think I'll allow one more clarifying
22 question for the speaker.

1 DR. KHOURI: I just want to, again -- (off
2 mic)

3 DR. DE CLARO: We would ask to preserve the
4 data for the transcript, please identify yourself
5 before asking the question.

6 DR. KHOURI: Issa Khouri, MD Anderson. Just
7 again to bring up what has been brought up by the
8 other members of the panel regarding different
9 centers, different lab techniques, and other things
10 like lympho size at the time of treatment, if you
11 look at your first slide, for instance, and you
12 look at the MRD from the lab from MD Anderson, and
13 if you look at the patient where they are CR and
14 MRD negative, the curve is a flat line. And that
15 is a longer follow-up than the German study. If
16 you look at the German study, the curve continues
17 dwindling down. And you look progression-free
18 survival, and there is little hope for a cure, even
19 with MRD negative, it seems like.

20 What do you think?

21 DR. BOTTCHE: I mean, MD Anderson, what's
22 been published have been used different, different

1 assays, so it's really hard to -- with different
2 sensitivities, at least the published data from
3 Kuntz (ph), Tom. So it's really hard to judge how
4 that will affect it.

5 All I can say, we tried our best to really
6 look into CR and PR rates. And there's been lots
7 of monitoring because it's been a study where,
8 actually, they applied for submission with this
9 study, so there's been lots of monitoring going on.
10 And I can only hope that the quality of the
11 clinical staging was good. And I did not really
12 look into the data. I don't have access. It's
13 been blindly analyzed. I can't say more as to the
14 differences between your results and the German
15 results.

16 DR. DE CLARO: Thank you, Dr. Bottcher.

17 We'll now move on to the third speaker. We
18 will probably have time for more clarifying
19 questions after Dr. Wierda's talk. My next speaker
20 is Dr. Wierda from MD Anderson Cancer Center from
21 Houston, Texas. The title of his talk is MRD
22 Status and Clinical Outcomes in CLL, from the CLL

1 Research Consortium and MD Anderson Cancer Center.
2 Dr. Wierda.

3 **Presentation - William Wierda**

4 DR. WIERDA: Thank you.

5 Good morning. I'm going to summarize for
6 you results of three different studies, two of
7 which are studies that are CLL Research Consortium
8 studies, and the third one is a snapshot of an
9 ongoing prospective analysis of MRD status in
10 patients getting FCR at a single institution at
11 Anderson.

12 So we have done two trials in the consortium
13 with alemtuzumab, looking at the ability of
14 alemtuzumab to consolidate responses after
15 chemotherapy or chemoimmunotherapy. The first one
16 was a trial with 58 patients using I VCAM path.
17 And the intent here was to take patients who had
18 had some response to chemo or chemoimmunotherapy,
19 PR, nodular PR, or CR with residual disease by
20 flow, and treat them with an I VCAM path for four
21 to eight weeks, and then to evaluate their
22 improvement in response.

1 So we were looking for improvement from PR
2 to CR, or nodular PR to CR, or elimination of
3 minimal residual disease in the CR patients.

4 In this trial, John Gribben evaluated
5 minimal residual disease in the bone marrow
6 following end of treatment by an allele-specific
7 PCR assay. This is just the trial design. The
8 trial was modified early on because patients had
9 initially been treated with a 10-milligram dose,
10 and then we bumped the dose up to 30 milligrams.

11 So the overall response by the criteria that
12 we initially proposed, which was improvement in PRs
13 to CR, et cetera, the response rate was 53 percent.
14 We didn't see a significant difference in terms of
15 the doses. And in terms of the outcomes by PCR,
16 minimal residual disease status in the bone marrow
17 at the end of treatment are shown here, where you
18 can see improvement in outcomes. And this is time
19 to treatment failure for those patients who are MRD
20 negative in the yellow curve. And this is overall
21 survival again, improvement in overall survival for
22 those patients who are MRD negative by this method.

1 Now, as was mentioned earlier, the sensitivity of
2 this method is 10 to the -4.

3 Then we did a second trial evaluating self-
4 administration of subcutaneous CAM path for
5 patients who had a similar entry criteria. They
6 were patients who had residual disease at the end
7 of chemo or chemoimmunotherapy, and the same
8 response criteria were applied. Patients did self-
9 administration of subcutaneous CAM path three times
10 a week at 30 milligrams for four to eight weeks.

11 Now, we looked retrospectively at MRD in
12 this trial as well as evaluated drug levels and
13 made correlations between outcomes with drug level
14 and PK analysis in the limited sampling that we
15 had. MRD was evaluated in these patients by a
16 four-color flow assay, which was done on archive
17 tissue, and it was sent to Genzyme. So they did a
18 four-color flow, and the sensitivity of that assay
19 was reportedly .01 percent or 10 to the -4.

20 Now, the problem with this study is that it
21 was a limited number of patients enrolled. There
22 were only 31 patients. And we didn't see a large

1 proportion of the patients becoming MRD negative.
2 And so our follow-up and curves, I didn't put them
3 here, but because of the limited numbers of
4 patients, the outcomes were not significant.

5 So that's the CAM path and consortium data.
6 Michael Keating developed the FCR regimen many
7 years ago. The initial study started in '98.
8 We've done a number of clinical trials subsequent
9 to improve on the outcomes of the FCR regimen, and
10 we haven't identified a regimen that had superior
11 outcomes compared with our standard FCR regimen.

12 So what we did most recently, starting in
13 2008, was to do a prospective analysis of
14 prognostic factors, pretreatment characterization
15 of prognostic factors, evaluation of minimal
16 residual disease. And the intent is to make
17 correlations with outcome in this trial with MRD
18 and again evaluate prospectively the prognostic
19 factors.

20 The trial is intended to enroll 300
21 patients. We have about 250 patients enrolled, and
22 I'm going to show you data here for about 225

1 patients. Patients are evaluated for response both
2 by the standard criteria we used when we wrote the
3 protocol. It was the '96 NCI working group
4 criteria. They were evaluated before cycle
5 number 4 and at end of treatment.

6 I'll refer to end of treatment. It's
7 essentially the same as Sebastian was saying for
8 their final response analysis, so it's two months
9 after the end of the last cycle.

10 These patients were all evaluated for
11 minimal residual disease in the bone marrow, and
12 the methodology is the four-color flow cytometry
13 assay that was adapted from the ERIC experience and
14 Jeff Jorgensen, who I thought was going to be
15 here -- oh, he's here -- has been working with Andy
16 in terms of validating and developing this assay.

17 We're following patients for progression-
18 free and overall survival. One of the issues with
19 the data that I'm going to show you is the limited
20 follow-up for these patients. And I think it
21 limits our ability so far to appreciate the full
22 impact and importance of MRD, although, as you will

1 see, it is a significant endpoint.

2 This is just a treatment regimen, again, 237
3 patients who have completed treatment, and have
4 their response evaluation and some follow-up time.
5 The intended treatment is six cycles. As you'll
6 see, not all patients receive all six cycles. So
7 about 25 percent of the patients receive three
8 cycles or fewer; 75 percent received more than
9 three cycles, and you'll see the importance of
10 that.

11 I'm just going to quickly show you the
12 features that correlate with the standard criteria
13 used for response, particularly complete remission
14 and overall remission. In our original trial, we
15 reported a complete remission rate of about
16 70 percent. In this analysis of this group, the
17 complete remission rate is 65 percent, which is
18 consistent with what we've seen. Overall response
19 rate is 97 percent.

20 You can identify high-risk patients at risk
21 for lower complete remission by virtue of having
22 the 17p deletion. And interestingly, the

1 trisomy 12 patients tend to have a higher complete
2 remission rate.

3 Other clinical and laboratory features that
4 correlate with remission, age has a lower complete
5 remission rate, advanced stage, and high beta-2
6 microglobulin. Interestingly, the newer prognostic
7 factors didn't significantly correlate with either
8 complete remission or overall response.

9 Now, this shows you that the 17p-deleted
10 patients are the high-risk group, having the
11 shortest progression-free survival in the blue
12 curve, and also a mutation status correlated with
13 progression-free survival, showing those patients
14 who have an unmutated V gene in the red curve.

15 Now, this gives you an idea about MRD status
16 at the end of treatment by NCI working-group
17 response, so 75 percent of the complete responders
18 were MRD negative. And this is at two months after
19 end of treatment. The overall MRD negativity in
20 this group is 59 percent.

21 What are those features that correlated with
22 MRD negativity at the end of treatment? Those are

1 shown here in this multivariate model, so there
2 were things that we could identify that correlated
3 with MRD negativity.

4 Surprisingly, mutation status correlated, so
5 the unmutated group had a lower MRD-negative
6 frequency than the mutated V gene patients,
7 advanced stage. Interestingly, again, the
8 trisomy 12 patients did better, so they had a
9 higher incidence of MRD negativity in that group
10 than the others. And 17p again was an unfavorable
11 group.

12 So this on the left is progression-free
13 survival, and on the right showing you that the MRD
14 at end of treatment, final response assessment does
15 correlate with both outcomes. So the progression-
16 free survival, red curve, is those patients who are
17 MRD positive as well as, again, overall survival.

18 The NCI working-group criteria for complete
19 partial remission, also, as we've seen in many of
20 our trials in the past, correlates with outcome,
21 both progression-free survival. And it does
22 correlate with overall survival, although, again,

1 this is an untreated patient population and the
2 importance of achieving complete remission will
3 probably be demonstrated more so in a longer
4 follow-up.

5 So one question is, how does MRD status
6 figure in, in considering the NCI working-group
7 response? And I think the important point here is
8 that you can see -- significantly in the left panel
9 for progression-free survival, you can see a
10 significant difference in terms of progression-free
11 survival for patients who achieve a partial
12 remission by virtue of their MRD status. So the
13 pink curve are MRD-positive PRs and the green curve
14 are MRD-negative PRs.

15 If you'll pay attention to the red and blue
16 curves, those are all complete responders. And so
17 far, we don't see a statistically significant
18 difference in those curves, although I think with
19 further follow-up, we will.

20 So as I mentioned, the intended treatment is
21 six cycles. About 25 percent of the patients
22 stopped treatment by three cycles, and 75 percent

1 received more than three cycles. So again, we
2 looked at MRD status after cycle 3, before cycle 4,
3 and then at the final response assessment. So you
4 can see here the distribution of patients, based on
5 the amount of treatment that they get, their MRD
6 status.

7 So about half the patients who got only
8 three cycles were MRD negative at the end of
9 treatment; 14 percent of the other half that only
10 received three or fewer cycles were MRD positive.
11 If you look at those patients who were negative at
12 3 and negative at 6, there were 18 of the total
13 group positive, converted to negative. That's
14 30 percent of the total group. In the patients who
15 remained positive, that again is about 25, 26
16 percent of the group.

17 These are the curves for those groups. So
18 paying attention to the red and the blue curve, the
19 red curve being those patients who only got three
20 or four fewer cycles and who were MRD positive
21 having the shortest progression-free survival; in
22 the blue curve are those patients who were MRD

1 negative at three cycles and didn't receive any
2 additional therapy.

3 If you look at the green curve, those are
4 patients who were MRD negative at three cycles and
5 continued on treatment. That curve is very similar
6 to the blue curve. And the pink curve, which are
7 those patients who converted from MRD positive to
8 negative, is similar to the other blue and green
9 curves. A shorter progression-free survival was
10 seen for the black curve. Those are patients who
11 completed more than three cycles of treatment and
12 were MRD positive at the end of treatment.

13 Pretreatment factors that correlated with
14 progression-free survival are shown here, so
15 17p deletion is an unfavorable feature, as is an
16 unmutated V gene.

17 If you look at the end-of-treatment factors
18 and do a multivariate model, MRD status comes into
19 this model, as well as the NCI working-group
20 criteria for response. And this is a model
21 combining for progression-free survival, both
22 pretreatment characteristics and end of treatment.

1 And again, we're seeing for progression-free
2 survival, end of treatment, MRD status is
3 independently associated with outcome.

4 This is the model for overall survival so
5 far, so 17p deletion, again identifying a high-risk
6 group as is MRD status at the end of treatment.
7 And I think that's all I have to show you. So
8 thank you for your attention.

9 (Applause.)

10 DR. DE CLARO: I think I will open the floor
11 for questions for any of the first three presenters
12 we had for this session. Any questions for
13 Dr. Ghia, Dr. Bottcher, Dr. Wierda?

14 DR. KAY: I have a quick question for Bill.
15 You must have been surprised, if I had read that
16 slide right or heard the slide right, that the
17 unmutated had lower -- correlating better with MRD
18 negativity?

19 DR. WIERDA: No. Positivity.

20 DR. KAY: With positivity. I'm sorry.
21 Okay.

22 DR. DE CLARO: Dr. Byrd?

1 DR. BYRD: So this is a question for all
2 three of the panelists, but the last two, Bill in
3 particular. So the follow-up on all the studies
4 are relatively short, particularly for the
5 IGVH-mutated patients that do quite well, how do
6 you separate this being a good surrogate endpoint
7 for survival if your follow-up doesn't take into
8 account competing issues like secondary
9 malignancies and other things?

10 As we consider these things, in addition to
11 the valid surrogate endpoint, should we consider
12 this relative to the higher-risk genetic groups?
13 Because, really, most of the MRD studies that have
14 been published have focused on higher-risk
15 patients, not the patients whose disease is often
16 indolent when it's treated and they have long
17 remissions with or without MRD.

18 DR. WIERDA: So as I mentioned, this is an
19 early analysis of our data. Certainly, though,
20 they are confounding issues in these patients,
21 because most of them will receive multiple courses
22 of treatment, and it's not only their first

1 treatment that impacts on their overall survival.

2 So I think we need to continue to collect
3 the data and do these analyses. It does appear,
4 though, in this early analysis that MRD status at
5 the end of treatment is an important endpoint in
6 terms of those patients who do well or those
7 patients who do achieve MRD negativity. And the
8 MRD negative cases are enriched for that
9 population, as enriched for the favorable features
10 that we see in the patients who have a mutated V
11 gene, et cetera.

12 DR. DEISSEROTH: For patients who achieve a
13 CR after therapy, what additional advantage is
14 there for achieving MRD 4-logs reduction above and
15 beyond CR, as far as you can tell from your current
16 data? And the second question is, is there any
17 value in trying to consider the rate at which MRD,
18 objective of 4-logs reduction is reached versus
19 just the final percentage after four to six cycles?

20 DR. WIERDA: So I'll start with the second
21 question. So there were patients who did convert
22 from MRD positivity to negativity, from MRD

1 positivity at the end of three courses to MRD
2 negative at the end of six courses. Their
3 progression-free survival in this early analysis is
4 similar to those patients who are MRD negative at
5 the end of three or at the end of treatment. So in
6 our analysis so far, we don't see a difference.

7 Now, again, this trial was initiated in
8 2008.

9 DR. DEISSEROTH: It's early.

10 DR. WIERDA: And it's early. And we need to
11 continue with the follow-up and continue with
12 collecting the data.

13 If you look at the complete responders,
14 patients who achieve complete remission with FCR,
15 in this analysis, you don't see a significant
16 difference in the curves if you split them by MRD-
17 negative versus MRD-positive patients. But again,
18 I think we need to continue to do the follow-up.

19 Probably, Sebastian can comment on that
20 aspect better because they have a longer follow-up
21 and more events in their group.

22 DR. BOTTCHE: Yes. Our data, virtually, in

1 keeping with your data, considering the follow-up,
2 we see now -- and we didn't even see when we
3 started analyzing -- an additional benefit of
4 looking at MRD in CR patients. But now, first
5 analyzed, like, three years ago, we did not see
6 that. So I expect that we will see this in the MDN
7 data with longer follow-up. And otherwise, I feel
8 almost a complete overlap in the key messages
9 between the two studies using the same regimen,
10 same analysis time points.

11 DR. DE CLARO: Dr. Rothmann?

12 DR. ROTHMANN: Yes. I have two comments, if
13 I may.

14 DR. DE CLARO: Yes. Please go ahead.

15 DR. ROTHMANN: I guess, in Dr. Bottcher's
16 talk, the interpretation of that covariate
17 analysis, you don't test for similarity or no
18 difference between arms by testing for a difference
19 and failing to show a difference. Lack of evidence
20 of a difference is not evidence of lack of
21 difference. There's a confidence interval around
22 that 1.12. A hazard ratio of less than 1 for the

1 treatment parameter in that analysis would mean
2 that there are additional positive effects of
3 rituximab on PFS not captured by the effect of
4 rituximab on MRD in the patient-level relationship
5 between MRD and PFS.

6 A hazard ratio of greater than 1, which is
7 1.12, for the treatment of that analysis would mean
8 that the effect of MRD overstates the effect on
9 PFS, or that there is an additional negative effect
10 of rituximab on PFS not captured by the effect of
11 rituximab on MRD and the relationship between MRD
12 and PFS.

13 My second comment. And this comes from a
14 paper Bob Temple wrote in '99 on cardiovascular
15 surrogate endpoints. One quality that is more
16 favorable for a surrogate as far as biological
17 plausibility is that the surrogate is relatively
18 late on the biologic pathway. The timing of the
19 evaluation surrogate is important. A surrogate
20 endpoint that is evaluated very early, long before
21 treatment ends, likely will not capture all the
22 positive and negative effects on the therapy on

1 clinical outcomes.

2 Also, it does not distinguish short-term use
3 of a therapy from long-term use. So if you just
4 used it, then got the MRD reading, and stopped
5 therapy, it wouldn't distinguish between the
6 stopping of therapy or the continuation of therapy.

7 Thanks.

8 DR. DE CLARO: Thank you.

9 Dr. Bottcher, do you want to respond?

10 DR. BOTTCHEER: Yes. Even the thing about
11 the Prentice criteria -- I'm only a doctor, so I'm
12 not a statistician. But to me, it seems, if it's
13 not statistically significant anymore, it does not
14 really tell you much about the effect. I mean, if
15 the hazard rate after adjustment for MRD is not
16 statistically significant anymore, I will consider
17 that it can't be really shown. At least, that's
18 what I would think about it.

19 DR. ROTHMANN: But there is a difference
20 between not statistically significant being
21 different from a hazard ratio of 1 and having a
22 confidence interval that's entirely close to 1.

1 Your example will have a confidence interval
2 that's very wide. It will contain 1, but it's also
3 going to contain some rather large hazard ratios,
4 which would correspond to negative effects or
5 large, negative effects of rituximab on PFS not
6 captured by the effect of rituximab on MRD and the
7 relationship at a patient level between MRD and
8 PFS.

9 DR. BOTTCHER: With respect to the
10 maintenance thing, then, it's obvious -- I mean, if
11 you intervene after measuring -- that was my
12 point -- then you cannot expect to predict any
13 more. I mean, we are talking about ongoing or a
14 second intervention, and this would not be covered
15 by MRD, at least not from my understanding.

16 DR. DE CLARO: Dr. Hillmen?

17 DR. HILLMEN: Just a comment about the
18 duration of follow-up and the significance of MRD
19 in that context and referring to Bill's experience.
20 And we have now had to show the experience of up to
21 15 years' follow-up of MRD with different
22 treatments. And the benefit is seen early with

1 bad-risk disease because of not falling
2 (indiscernible - technical interference) -- you see
3 the benefit later on with good-risk disease because
4 the MRD-negative remissions, it takes 10 years
5 before the survival is falling off in that group,
6 and you see the difference in MRD.

7 So I don't think it's, a lack of evidence;
8 it's not a lack of effect. And we'll show that the
9 effect actually is true for all groups of CLL. It
10 just takes longer to show it for the better-risk
11 patients.

12 DR. DE CLARO: Thank you. Dr. Khouri?

13 DR. KHOURI: Yes. I would like to go back
14 to the question that was raised by Dr. Deisseroth.
15 If a patient achieves a CR, is it really needed to
16 do MRD? And Dr. Wierda answered that there is no
17 difference really with the CR patients. This
18 depends on the point also of how CR is defined
19 because there is a study by Rosen from Chicago in
20 the CAM path study when he used the IWCLL criteria
21 for defining CR, yet, at the same time, when he
22 used the CT scans on the same patients, the CR rate

1 dropped from 26 percent to 6 percent.

2 So the question is, if we stage patients and
3 define CR rate by CAT scans in addition to bone
4 marrow, et cetera, do really still we need MRD.

5 DR. DE CLARO: Dr. Kipps?

6 DR. KIPPS: Yes. I think there are two very
7 important questions that I hope that we address and
8 come to some consensus on. The first question is,
9 is it reasonable to use MRD as a surrogate marker?
10 And that's what we're discussing.

11 The second question is, what is an
12 acceptable threshold? This is something we
13 grappled with when trying to draft the IWCLL
14 guidelines. And as far as the patient that was
15 referred to over there, the patient would not have
16 been CR because they had an adenopathy on CT scan.
17 That's not a CR.

18 So that's the guidelines for response
19 assessment. But getting to MRD, this is something
20 that Andy and I were debating even late last night,
21 is that you can easily say that the more sensitive
22 the test for MRD, the better, of course. And you

1 may be approaching levels down to a -5, perhaps,
2 with more sensitive flow methods and deep
3 sequencing, as Dr. Ghia mentioned. But when is
4 enough, enough?

5 I'm intrigued by, of course, the data that
6 was presented with the frontline study for FCR, the
7 CLL8 study, where a difference between patients
8 with MRD assessed at between 10 to -2 versus 10 to
9 -4 was not significantly different than patients
10 who had less than 10 to -4.

11 Now, obviously, over time, that difference
12 may bear itself out with more patients and a longer
13 follow-up. And we all intuitively feel that's
14 correct. However, when we try to push the envelope
15 to higher and higher resolutions, we start to
16 introduce some ambiguity into what we define as
17 this standard for MRD, and that can create
18 regulatory confusion.

19 Also, there may be a difficulty in trying to
20 introduce this into clinical labs throughout the
21 country or abroad. Obviously, I think that's
22 something we have to grapple with.

1 Perhaps I could address this with a question
2 to Sebastian about the push with EuroFlow to reach
3 a threshold of 10 to the -5. What is the
4 compelling argument for that?

5 I do think we need to try and define a
6 threshold that we can live by. And obviously, with
7 improvements in technology, that threshold may be
8 moved further down afield. But to come to the
9 conclusion that, yes, it's reasonable to assess for
10 MRD, but we're not yet there with regard to
11 defining an acceptable threshold, I think, would be
12 a mistake.

13 DR. DEISSEROTH: Could I just introduce
14 another possible opportunity here? In the CML
15 data, in addition to the 3-log reduction, whether
16 or not a patient was above or below a 10 percent
17 reduction very early was useful in dissecting out
18 the poor-prognosis patients.

19 So I'm just mentioning this because driving
20 down the threshold goal may not be the only way to
21 use MRD. Maybe, if you looked early at patients
22 who fail to reach a 10 percent reduction as an

1 example, early, that might unveil a very poor
2 prognosis group of patients. So you don't
3 necessarily have to go to greater and greater
4 depths of remission.

5 DR. KIPPS: I think that's correct, although
6 one might have to deal with different therapies
7 having different kinetics of response outcome.
8 That makes it a little bit more complicated.

9 DR. DE CLARO: Dr. Bottcher, if you would
10 like to, comment?

11 DR. BOTTCHEER: Yes. Thank you. I would
12 really like to state that we should actually use 10
13 to the -4. And it's only that we try and push the
14 sensitivity lower because we think that, in the
15 future, after we have good experience with 10 to
16 the -4, it might be that in 10 years, we're going
17 to change that level we want to achieve. But for
18 now, we would really like it to be this 10 to the
19 -4, to be applied all over the world, basically.

20 DR. DE CLARO: With that, I'm going to close
21 this first part of the session. We will now go
22 have a break for 15 minutes. Please help

1 yourselves to complimentary water and coffee in the
2 back of the room, thanks to the American Society of
3 Hematology.

4 A little housekeeping, the restrooms are on
5 the outside, on the right, and we will come back at
6 about 10:20. Thank you.

7 (Whereupon, a brief recess was taken.)

8 DR. DE CLARO: Welcome back. We're
9 continuing the second session on current status of
10 MRD assessment in CLL. Our next speaker is
11 Dr. Montserrat. The title of his talk is the
12 Impact of MRD Status on the Outcome of Patients
13 with CLL, the University of Barcelona Hospital
14 Clinic Experience. Dr. Montserrat.

15 **Presentation - Emilio Montserrat**

16 DR. MONTSERRAT: Thank you, and good
17 morning. First of all, I would like to join the
18 other speakers by thanking the organizers for
19 giving me the opportunity to attend this very
20 interesting meeting. Secondly, I would like to
21 apologize because, in the handouts that you have
22 received, my presentation is not in, so this is my

1 only mistake. So if after hearing my presentation
2 do you still consider that my slides are worth it
3 to be seen and to be kept, feel free to ask me or
4 to ask the staff for them. We are going to be very
5 glad to distribute these slides to you.

6 When preparing this meeting, I was asked to
7 provide some kind of daily life information of the
8 MRD value on the outcome of patients with CLL
9 treated at the CLL institution. And this is what I
10 am going to do. I will state it's an approach that
11 have some positive aspects and some downsides also.

12 So this is a retrospective study of 255
13 patients with CLL, treated at our institution. The
14 medium age was relatively young. Patients were
15 under the age of 60 and treated during a very long
16 period of time.

17 MRD was assessed after frontline therapy, so
18 I am not going to present data on my patients
19 treated with second-line, third-line therapy, but
20 just frontline therapy. I would like also to
21 emphasize that in no case has MRD been used to
22 guide therapy in these patients and that the

1 objectives of these analyses have been to correlate
2 MRD achievement with the type of therapy and also
3 to investigate the relationship between MRD on
4 treatment outcome in terms of treatment-free
5 survival and overall survival.

6 So MRD has been assessed in bone marrow
7 after three months of therapy and, and subsequently
8 every three and six months either in bone marrow or
9 in blood. I mean, we have really been following
10 ERIC and Rawstron's recommendation. And in the
11 recent years, we have moved to peripheral blood
12 much more than marrow.

13 As you can see, we have been using a
14 different panel of monoclonal antibodies, again in
15 agreement with Andy's work and with ERIC
16 recommendations. In no cases was the sensitivity
17 of the technique or the lower level of detection of
18 the technique, as Andy likes to say, is below 10^{-4} .
19

20 So this is the first slide, which
21 recapitulates a little bit what has already been
22 shown by Paolo and others, which is the

1 relationship between different treatment modalities
2 and the type of response. And as you can see, from
3 the left to the right, either in patients treated
4 with chlorambucil only or with CHOP-like regimens,
5 or even with purine analogs, that's a single
6 agent -- that means either cladribine that was used
7 in our institution for a while or fludarabine.

8 The proportion of complete responses was
9 really very, very small, and there are virtually no
10 patients achieving MRD-negative complete response.
11 So when we use the combination of fluda/cyclo and
12 mitoxantrone, I mean, we have been combining, on a
13 relative basis, fluda and cyclophosphamide with
14 mitoxantrone.

15 We obtained a much larger number of complete
16 responses, and a substantial portion of these
17 responses were with no detectible disease, or in
18 other words, MRD negative. And the results are
19 quite impressive. The reasons, maybe we have to
20 resuscitate and revisit whether RFCM should be used
21 instead of FCR.

22 As you can see here, the proportion of

1 MRD-negative compared responses achieved with RFCM
2 is really quite impressive. Still, the number of
3 patients is small, and this is a measure of
4 limitation to make or to further elaborate on this
5 point.

6 So what are the characteristics of diagnosis
7 which correlate with MRD achievement? So would you
8 please note -- and this is very important, this is
9 the important caveat -- that in some of these
10 patients, biomarkers were obtained after initial
11 diagnosis. So biomarkers, as you know, can change
12 over time. But in all cases, all these biomarkers
13 were obtained before therapy, actually in start
14 samples obtained either at the time of diagnosis or
15 before starting therapy.

16 So as you can see here, actually, there are
17 very few parameters analyzed in that way which
18 correlate with the possibility of achieving
19 MRD-negative status, which is namely age, no
20 clinical stage, no beta-2 microglobulin, no ZAP
21 expression, no CD38 expression, no FISH imitations.
22 Again, FISH is completely tricky because p53

1 abnormalities are required at the time.

2 IGVH mutations. NOTCH-1, for reasons that I
3 really don't know, was correlated with achievement
4 of MRD-negative status, and other novel genes, such
5 as the splicing gene factor, this FvD1, and also
6 the MYD88 gene, that mutation was not correlated.
7 So not surprising, the kind of therapy was highly
8 correlated with the possibility of achieving
9 MRD-negative status.

10 So what about prognostic factors? And I'm
11 going to deal first with prognostic factors related
12 to treatment-free survival, and after that,
13 overall survival.

14 I mean, for treatment-free survival, you can
15 see in this table this is a summary of the
16 univariate and multivariate analyses. And the
17 important parameters, again, for treatment-free
18 survival, were beta-2 microglobulin, IGHV
19 mutational status, NOTCH-1 mutated or unmutated,
20 and MRD.

21 Significantly early, univariate analogies
22 were age, clinical stage, the ZAP, SF3B1 mutations,

1 and treatment modality. And that data, which were
2 not significant at univariate or multivariate
3 analogies, were FISH and MITT (ph).

4 For overall survival, which I think is the
5 most important information, only four parameters
6 were retained as important in predicting survival
7 of patients with CLL; not surprisingly, age, beta-2
8 microglobulin levels, IGHV mutational status, and
9 achievement of MRD negativity.

10 You can see here in these plots exactly the
11 same kind of curves that have already been shown by
12 many others. In panel A, you can see the response
13 in terms of MRD negativity. Let me tell you that
14 we have a small fraction of patients that were a
15 partial response by NCI criteria or IWCLL criteria
16 and MRD-negative, a tiny fraction of patients. And
17 these patients have been analyzed together with
18 complete responses.

19 In the panel B, you can see the treatment-
20 free survival based on beta 2. Panel C shows
21 exactly the same thing for IGHV mutational status,
22 and panel D for NOTCH-1 mutations. And then of

1 overall survival, again, here's response, H, beta-2
2 microglobulin, and IGHV gene status.

3 I think that this is fitting very much what
4 has been presented. So it's not any kind of
5 surprise that if you combine any of those, you can
6 really sort out different groups of patients with a
7 much better prognosis comparing, as Sebastian has
8 suggested or has shown, MRD status with IGHV
9 mutational status.

10 So let me talk about limitations of the
11 study, also some strengths among limitations, but
12 this is the name of the game. I was asked to
13 analyze MRD in daily life. And the study is
14 prospective -- not (indiscernible) prognostic
15 markers were obtained at diagnosis or immediately
16 before therapy. This is an important caveat if we
17 are trying to make any attempt of correlating MRD
18 with biomarkers.

19 The study actually was not intended to
20 investigate variables correlated with the
21 achievement of MRD negativity. And obviously,
22 remember the FCR, FCRM story, the study is not

1 powered enough for subgroup analysis.

2 Among the strengths, I think it's the fact,
3 which is a large signature of patients or a
4 relatively large signature of patients treated at a
5 single institution and selected patients with a
6 variety of treatments on reflecting MRD assessment
7 and use in, to say so, daily life.

8 In conclusion, I would like to finish by
9 saying that MRD negativity in this study was more
10 likely to be achieved with chemoimmunotherapy than
11 with other agents and was associated, as shown by
12 many others this morning, with a longer treatment-
13 free survival and overall survival.

14 The only factors in the multivariate
15 analysis correlated with overall survival were age,
16 beta-2, mutational status of the IGHV gene, and MRD
17 negativity. And finally, the correlation of MRD
18 status with both different types of therapy on
19 overall survival favors, in my opinion, considering
20 MRD as a surrogate endpoint to the level of
21 efficacy of novel therapies in CLL. That's all.
22 Thank you very much.

1 (Applause.)

2 DR. DE CLARO: Thank you, Dr. Montserrat.
3 We will move onto our next speaker, Dr. Peter
4 Hillmen from the U.K. The title of his talk is MRD
5 in U.K. CLL Clinical Trials.

6 **Presentation - Peter Hillmen**

7 DR. HILLMEN: Thank you. Thank you very
8 much for the invitation to present our data. And
9 also, I'd like to reiterate the comments that one
10 or two other people have made about the importance
11 of this process and also for those of us who have
12 been looking at MRD for quite some time with CLL,
13 the importance of it becoming a surrogate endpoint
14 in CLL more generally.

15 So I'm going to review data from our own
16 center, where we've been looking at MRD for 15 or
17 more years, and then how we're using that data in
18 the U.K. CLL trials, and that's increasing, as I'll
19 show you.

20 So we initially started looking at MRD in
21 the mid-1990s, late 1990s, with many because of
22 alemtuzumab CAM path, because we were seeing very

1 deep remissions without the drug. And that led to
2 the development of the flow assay, which Andy
3 Rawstron, who will talk about it later on, was key
4 in the lab. And then parallel to our use of MRD in
5 clinical trials has been the validation of the
6 assay, or assays, for MRD.

7 So the first trial that we applied MRD to,
8 as really as an exploratory endpoint rather than a
9 primary endpoint, was within patients with very
10 bad-risk disease. So this first phase 2 trial,
11 which started recruiting in 2001, was for patients
12 who were refractory to fludarabine by conventional
13 criteria. So expected survival about a year on
14 average with conventional therapy at the time,
15 treated with CAM path subcutaneously for up to 24
16 weeks, some patients with the addition of
17 fludarabine.

18 What we've shown with this trial was that,
19 as we've seen with all the studies and, actually,
20 of all the studies, depth of response is the most
21 important predictor of outcome for patients. And
22 in this context, MRD negativity was the strongest

1 predictor. So if you achieve MRD-negative
2 remission, it's a small number of patients, you
3 have a better outcome than the patients who did not
4 achieve that remission. And this is an update of
5 the data from two or three years ago that was
6 initially presented several years before that.

7 The follow-up study to that, which was led
8 by Andy Pettitt from Liverpool, just took patients
9 who were 17p deleted, so the worst group of
10 patients, both frontline and relapsed patients, who
11 were treated with CAM path plus hydrosteroids as an
12 attempt to overcome 17p.

13 Within this study, we looked at MRD as well.
14 And, again, as you can see on the left, if you
15 attained a CR, you had a better outcome. But MRD,
16 again, was the best predictor for outcome; although
17 in porous disease, it doesn't overcome the porous
18 disease. It just helps with differentiating the
19 patients.

20 So since the mid-1990s, we've been applying
21 MRD assessment to all of our marrows and
22 assessments of patients both within clinical trials

1 and without clinical trials in our lab, not changed
2 the treatments on the basis of it, but looking at
3 it.

4 So we presented two or three years ago now,
5 and this is an update of data from this year of
6 that analysis, of all the patients in Leeds who had
7 been treated for CLL between '96 and 2007, who had
8 achieved at least a PR and had a marrow to assess
9 response in which we had MRD assessment. This is
10 across all treatment types apart from allogeneic
11 transplant, which is almost certainly different in
12 terms of MRD.

13 We used a threshold of 1 in 10 to the -4 for
14 all of these studies over time and generally used
15 marrows at the end of treatment as an assessment
16 point, although we do have some good assessment.

17 The advantage of this data set is that
18 there's a very long follow-up, up to 15 years, and
19 we have multivariate analysis across multiple
20 different treatments. And I'll show you the
21 multivariate analysis for PFS and overall survival.

22 So there were 133 patients, as I mentioned,

1 over a 10-year period; started younger than the
2 average CLL patient. Of those patients, 41 percent
3 were having their first line of therapy and
4 assessment at that point; 59 percent had prior
5 therapies and relapsed.

6 Listed are the various therapies that were
7 used. And, of course, they evolved over the
8 10 years of the study. So most of the patients
9 were treated in the FC before the rituximab era.
10 There were quite a few CAM path patients.
11 Autologous transplant is no longer done, I think,
12 certainly not in the U.K. And then there was a
13 small proportion of patients with chlorambucil
14 therapies, who actually achieved good remission.
15 So there's a mixture of all patients who managed.

16 This is just to show the data we've seen
17 from Sabotren (ph), that the depth of remission is
18 a continuous variable. So if you achieve a CR with
19 less than 1 in 10 to the 4 cells, shown in the top
20 curve, you have a better outcome than the patients
21 who have over 1 in 100 cells, which is the worst
22 curve, and then the intermediate patients are

1 intermediate. So it's continuous variable, but the
2 10 to the -4 cutoff is a robust cutoff for an
3 endpoint of response.

4 If you look at PFS now by prior therapy in
5 MRD, this is now, as you can see, up to 15 years'
6 follow-up. Patients who are on the blue curve, on
7 the yellow curve are the no-prior-therapy patients,
8 separated by MRD positivity or negativity at the
9 end of treatment. The green and red curve of the
10 prior therapy patients is separated by MRD
11 negativity and positivity. So it shows in either
12 group, MRD negativity predicts for a better
13 progression-free survival.

14 This is overall survival now. It's up to
15 15 years. And, again, we see a better overall
16 survival of the blue curve compared to the yellow
17 curve by MRD response, and then the green and red
18 for the prior therapy patients. So it's consistent
19 for both progression-free and overall survival.

20 This is the multivariate and univariate
21 analysis of the various things I showed you on the
22 first slide. So in univariate analysis, marrow

1 function, stage, prior therapy, whether you've had
2 prior therapy or not, treatment type, FISH,
3 17p deletion, and response in MRD are significant
4 for PFS, but for multivariate analysis, the only
5 things that stand out are adverse FISH by 17p with
6 a hazard ratio of .24 and MRD, which is using a
7 continuous variable, but down to less than -1 to 10
8 to the 4, and this beats response rates.

9 So the question was asked before about CR
10 and whether you can use CR instead of MRD.
11 Clearly, the MRD is a better outcome in long term
12 for patients than response rates in the
13 multivariate analysis, from our experience.

14 Then this is the same data for overall
15 survival, so again, most of the factors are
16 significant as a univariate analysis. The ones in
17 red are significant by multivariate analysis, so
18 obviously age and whether the patients had a prior
19 therapy or not, but the MRD and 17p stand out as
20 biological markers. And again, this is a
21 continuous variable for MRD. So MRD is the
22 strongest of the outcomes with 17p.

1 So our data from every patient treated in a
2 10-year period in Leeds with MRD assessment is
3 supportive of MRD as an endpoint.

4 The question that's been raised, to some
5 extent, and I think will be discussed a bit more,
6 is about the timing on the tissue to look out for
7 MRD. And the trial I'm going to show you, which we
8 tried to change MRD-positive patients to
9 MRD-negative patients, which I think we can learn a
10 lot about, used CAM path as the therapy to do that.

11 Now, CAM path is probably the therapy which
12 has the biggest compartment effect. It clears the
13 blood clearly better than it clears the marrow, and
14 it clears the marrow clearly better than it clears
15 the lymph nodes. And our experience is that if you
16 use chemotherapy in this area, most of it was not
17 with antibodies, shown on the left of the curve.
18 The bone marrow assessments against the peripheral
19 blood is very similar, and there's no systematic
20 error, whereas, if you look at CAM path
21 alemtuzumab, during therapy or up to three months
22 after therapy, there are many more patients with

1 clear blood who don't have clear bone marrow.

2 So in this context, you probably can't use
3 peripheral blood. And I will show you an update in
4 a moment or two, that probably six-month peripheral
5 blood for CAM path is the best time frame to use.
6 This may well be different with other therapies, as
7 we have heard already.

8 So the CLL207 study was a phase 2 trial
9 leading to a phase 3, where we took patients who
10 were MRD positive and tried to change them to being
11 MRD negative with CAM path consolidation. We
12 screened, I think it was, 61 patients in this trial
13 at the end of treatment so they could have first-
14 or third-line therapy for MRD.

15 If the patient was MRD negative -- and there
16 were 15 patients who were MRD negative -- they were
17 followed. They were not consolidated. Only the
18 MRD-positive patients were consolidated. And I'll
19 come back to the 15 MRD-negative patients in a
20 moment, because it allows us to look at whether we
21 change people to MRD negative, whether that is the
22 same as if you're MRD negative after chemotherapy.

1 The joint primary endpoints were eradication of MRD
2 and the safety of CAM path in this context.

3 So this is the marrow at the end of
4 treatment. Now, we didn't have a marrow at three
5 months because we were using a marrow to define
6 therapy. And I'm not going to talk in detail about
7 marrow because marrow, at the end of therapy, is
8 not a good endpoint for CAM path-type treatment.
9 What we achieved is an MRD-negative marrow in over
10 80 percent of the patients who were consolidated in
11 this trial; 39 out of 47 patients.

12 If you look at the data -- and this is
13 peripheral blood -- at end of treatment, three
14 months, and six months -- so the green curve, this
15 is progression-free survival, is the patients who
16 are MRD negative in the peripheral blood six months
17 after consolidation. The blue curve are the
18 patients who are MRD positive at three months. And
19 the red curve are the ones that convert to MRD
20 positivity between three and six months.

21 So it shows that the patients who are
22 converting to positivity by six months are doing

1 the same as the MRD-positive patients. And I think
2 this is a redistribution of the disease with CAM
3 path, so that disease that's possibly in the nodes
4 or in the marrow is distributing into the
5 peripheral blood.

6 So using this analysis, MRD at six months in
7 the peripheral blood is a robust endpoint. And the
8 same is as seen in overall survival, just to show
9 you the data. The blue is the positive. The red
10 is going negative to positive at six months, and
11 the MRD-negative are in the green.

12 So now, five years, this is looking at
13 peripheral blood in the 207 study for patients who
14 are consolidated -- at six months, post-
15 consolidation. And you see that the MRD-negative
16 patients do a lot better than the MRD-positive
17 patients out at five years, and this is
18 significant.

19 Then if you look at overall survival, you
20 see the same thing, that if you achieve an
21 MRD-negative remission six months post-CAM path
22 consolidation, you have a better overall survival

1 in the same patients who don't achieve an
2 MRD-negative remission with therapy.

3 This slide shows those first 15 patients who
4 were MRD negative in the red curve at the end of
5 therapy. So these patients were not consolidated;
6 they were just followed at an MRD level. The blue
7 curve are the patients who were MRD positive and
8 became negative. And then this is a relapse of
9 MRD.

10 So what we see is, the patients who achieve
11 an MRD-negative remission with consolidation seem
12 to have the same duration of MRD negativity as
13 patients who are MRD negative after chemotherapy,
14 suggesting there actually was a change in the
15 outcome for that group of patients back up to where
16 the MRD-negative patients were originally.

17 Then this is a mixture of the two data sets,
18 so we're taking the MRD-positive patients and we
19 expect a 40 percent PFS, from data from our group,
20 and then the MRD-negative patients certainly seem
21 to be doing better than the MRD-positive patients.

22 So this was a trial leading to a phase 3

1 trial, which we had planned, and I'll mention in
2 the next slide.

3 So we have more data that we're collecting
4 and will be presenting later on this year from two
5 large randomized phase 2 trials with FCR-based
6 treatment, 415 patients. Just to show you what
7 data we're collecting now. So we're now collecting
8 marrow and peripheral blood at three months, and
9 then continuing MRD assessment in the peripheral
10 blood over time for the negative patients and for
11 the ones that become positive to look at the
12 dynamic of relapse.

13 So that data, these trials, are now fully
14 recruited, and we're seeing MRD-negative rates
15 similar to the sub-data from the German 8 trial in
16 both of these studies.

17 Going forward, we have two trials planned
18 which use MRD as part of the studies. CLL8 was a
19 follow-on to the C207, the consolidation trial. As
20 you will I'm sure be aware, alemtuzumab or CAM
21 path, the license has now been withdrawn for CLL in
22 Europe and is not being sold. So there are issues

1 over availability if we do a phase 3 trial and the
2 toxicity of that drug. However, there's a third-
3 generation antibody, C20 antibody, GA101 or
4 obinutuzumab, which seems to result in
5 (indiscernible) antibody -- remission. So we have
6 a planned randomized trial where we're using GA101
7 as consolidation in MRD-positive patients post-
8 treatment.

9 The other question which I think we need to
10 address is with the novel therapies. I know John
11 is going to talk about this later on. We have a
12 planned trial, a phase 3 trial, for FCR FITs
13 patients, comparing FCR with ibrutinib. And we're
14 MRD assessment within this trial to define the
15 duration of ibrutinib therapy. And I think it may
16 be well an important use of MRD in this context.

17 As we've seen in CML, the problem is what to
18 do with your negative patients. CLL is a different
19 disease, of course, because we think we can control
20 the disease, as they're opposites in MRD level.

21 So my conclusion really, first of all,
22 testing. I think we're getting increasingly

1 confident that peripheral blood is a good tissue to
2 look for MRD and CLL, given the caveats of the
3 different therapies affecting the different
4 compartments differently. I think we may well get
5 away from doing marrows at all, which probably
6 aren't a very reliable way of assessing CLL
7 generally, because it's a patchy disease. We don't
8 get good quality samples. It's important that we,
9 especially the bigger trial groups, collect the
10 data so that we can prove that peripheral blood is
11 as effective as marrow or more effective than
12 marrow.

13 Why is MRD important as a primary endpoint
14 for frontline trials? Well, our big problem with
15 CLL are the patients who are good-risk patients or
16 standard-risk patients who would conventionally be
17 treated with FCR. The median progression-free
18 survival for FCR is probably in the region of six
19 years and the time to next treatment is longer than
20 that.

21 So in reality, using PFS as an endpoint,
22 we're not going to be developing new drugs for that

1 group of patients, and obviously, that's why we're
2 here. In addition, increasingly we believe as a
3 community that MRD positivity is a bad thing, so
4 our trials are designed to eradicate MRD in a
5 randomized way to prove that the changing of
6 MRD-positive FCR patients to a negative patient
7 improves outcome, and the other data suggests
8 that's true.

9 So actually, if we use PFS as an endpoint
10 for approval, we'll never approve a drug because
11 we're changing it after the initial treatment. So
12 if we don't change to an appropriate surrogate
13 endpoint, we end up with studies that we have to
14 look across from all the trials, all the great
15 groups of patients, to treat our younger, fitter
16 patients.

17 The two groups of patients where we're going
18 to approve drugs are the elderly, which clearly is
19 not a comparative group compared to the patients
20 we're talking about, who have sometimes 10-year
21 remissions without current therapy, and secondly,
22 the 17p deleted fludarabine refractory, which is

1 biologically a very different disease.

2 So in my opinion, it's much better to use an
3 appropriate surrogate endpoint -- and MRD is the,
4 I'm sure, appropriate surrogate endpoint -- than
5 trying to infer across from all the disease areas
6 of CLL with inappropriate comparisons.

7 So that's I'm sure why we're all here. And
8 obviously, I represent the U.K. group, and I'm sure
9 I've missed out lots of people from this slide
10 because every time I present it in the U.K.,
11 someone goes up and comments on it. But it's a
12 very large collaborative group, shown here. Thanks
13 for your attention.

14 (Applause.)

15 DR. DE CLARO: Thank you, Dr. Hillmen. Our
16 next speaker is going to be Dr. John Byrd from Ohio
17 State University. The title of his talk is
18 Targeting B-Cell Receptor Signaling: A Pause for
19 MRD as a Required Endpoint.

20 **Presentation - John Byrd**

21 DR. BYRD: The title of my paper or my talk
22 is focused on B-cell receptor signaling. But I

1 think what we've heard from this point are the
2 studies that have been done with minimal residual
3 disease of chemotherapy and adding something to
4 chemotherapy or chemotherapy alone. And I think as
5 we look forward, many of us are looking toward the
6 chronic maintenance drugs.

7 So when I prepared this, I could have
8 substituted immune-modulating agents, such as
9 lenalidomide, BCL-2 antagonizing agents, ABT19,
10 ABT263, and a variety of other drugs that patients
11 go on and they take for an extended period of time,
12 a maintenance -- an induction, consolidation,
13 maintenance-type strategy as opposed to just taking
14 a set period of therapy and then assessing after.

15 So my talk is more to get us to thinking
16 about the future and how we can use a very powerful
17 technique, MRD, as Peter sort of alluded to, to
18 drive our trials, but at the same not paralyze new
19 drugs coming forward by saying, "If you hit a
20 surrogate endpoint that we know is valid,
21 progression-free survival, but you're not getting
22 MRD; well, that's not good enough," because I think

1 the patients in general like non-toxic therapies,
2 and we're being paternalistic to say, "You have to
3 take chemotherapy versus something that's better
4 tolerated."

5 So that's the context of my talk. And I'm
6 going to focus on B-cell receptor signaling. There
7 are a variety of therapeutic agents that are in the
8 clinic. I'm going to focus on the two that are
9 furthest along, ibrutinib and GS1101, and describe
10 some of the data and what very, very little data is
11 available on MRD. And I'll share some anecdotal
12 cases from our center with ibrutinib, and then try
13 to put this into context. Then Neil Kay is going
14 to follow me and talk about the U.S. intergroup
15 studies, where we're going to be examining some of
16 these MRD questions.

17 So fostamatinib and dasatinib were both
18 early BCR antagonists. They both showed activity,
19 but really not enough to move forward. It was
20 really the first truly successful BCR antagonist to
21 enter the clinic that now is in phase 3
22 development. That's CAL-101, now GS-1101, which is

1 a selective PI3-kinase delta inhibitor. It was
2 very, very well-tolerated, enough that a healthy
3 volunteers study could be done to identify a dose,
4 where activity was seen in the first dose cohort.
5 It was a strong preclinical rationale for looking
6 at this. And the phase 1 study included multiple
7 types of lymphoma in CLL.

8 The dose -- it was an extended phase 1
9 study, very large, that included 55 patients with
10 CLL, where the dose was defined based upon PK, PK
11 toxicity, and also efficacy. And the patients in
12 the study were very heavily pretreated with median
13 therapies of five.

14 What you're called to here, we always think
15 of response as a surrogate endpoint. And what you
16 see here is that the response to this agent is
17 relatively low using standard criteria, 24 percent.
18 And it's mainly driven by this asymptomatic
19 lymphocytosis, which is typical of these agents.

20 But the toxicity of this agent is quite
21 favorable. And when you look at the progression-
22 free survival for all patients in this very

1 refractory group, it's quite respectable. And
2 these data will be updated, but the progression-
3 free survival for these patients is 18 months. The
4 correlation that we see with CR, PR, no response is
5 not as clear with this agent. So patients benefit.

6 Where we would have expected maybe the same
7 finding that we've seen with antibodies is adding
8 this to cytotoxic or biologic therapies. And the
9 company, Calistoga and Gilead went on and looked at
10 combining it with chemoimmunotherapy, bendamustine
11 or rituximab. And you clearly increase the overall
12 response, and you get a few CRs. And our follow-up
13 of the progression-free survival and overall
14 survival, again, is quite respectful. But in this
15 study, there are very, very few CRs. So the data
16 are very early.

17 I've written of the second agent. It
18 targets BTK irreversibly. There's strong
19 preclinical work to support it. And this prompted
20 moving forward with a phase 1 study initially in
21 lymphoma, where, again, through multiple cohorts of
22 lymphoma in CLL patients, a dose was established

1 based on inhibition of the target.

2 The response, this was well-tolerated in the
3 56-patient cohort. No MTD was obtained. And the
4 response in this group again was very respectable,
5 but there were very few CRs again with this
6 monotherapy, yet a great number of these
7 patients -- this study started in 1999 -- are still
8 on treatment.

9 This prompted a phase 2 study that was
10 presented at ASH this past year in several
11 different groups of patients, again with treatment-
12 naive, and relapsed, and refractory patients. And
13 in the treatment-naive group, this is a really,
14 really active agent. We might have expected a lot
15 of CRs.

16 So again, this just looks at the
17 demographics of the patients, again 31 treatment
18 naive over the age of 65 and 85 relapsed and
19 refractory patients. Again, looking at our
20 response in the relapsed and refractory patients,
21 while the response is 70 percent, the CR rate is
22 only 2 percent using classic criteria. In the

1 previously untreated patient group, the CR rate is
2 only 10 percent.

3 When we look at the progression-free
4 survival with follow-up, this is quite respectable.
5 In the treatment-naive group, again, where we had
6 CRs in only 10 percent of the patients, with
7 patients continuing to take therapy, the estimated
8 progression-free survival at 26 months was
9 96 percent.

10 So historically, comparing apples to
11 oranges, this is better than what we see with most
12 of our other clinical agents. And similarly, in
13 the relapsed and refractory patient population, the
14 26-month progression-free survival was 75 percent.

15 This just looks at the response by
16 mutational status in cytogenetics. The 17ps are
17 not doing quite as well, but are still doing quite
18 respectfully.

19 This looks at survival and, again, nobody is
20 becoming MRD negative with this. We're seeing very
21 extended progression-free survival and overall
22 survival is shown here. So in combination, this

1 has also been combined with ofatumumab with the
2 first cohort of giving ibrutinib for a month and
3 then adding ofatumumab was presented at ASCO.
4 Again, just like with GS-1101, you increase the
5 response rate to 100 percent with this, but you
6 only see a 4 percent CR rate. These are heavily
7 pretreated.

8 What I'll say here is as we followed these
9 patients out, we checked bone marrows at one year.
10 And four patients have gotten under the 1 percent
11 number, so not the .01 percent, but the 1 percent
12 number, 1 relapse five months later, who had 17p,
13 two in remission and one died of an unrelated
14 cause. So again, that's the data that we have.

15 The same follows with bendamustine,
16 rituximab. Adding this, you see a 13 percent CR
17 rate and a 93 percent overall response rate. But
18 what we don't have is sufficient data with MRD yet.

19 So I think the summary that I would make
20 with the MRD data with GS-1101, with ibrutinib and
21 NGS-1101, is that we have very few CRs thus far
22 with monotherapy or with combination therapy in the

1 relapsed setting and with monotherapy in the
2 upfront setting with ibrutinib. Despite that, we
3 see very extended progression-free survival.

4 So the question becomes, with extended
5 follow-up, will these differences come out? Will
6 that 10 percent of patients that become truly MRD
7 negative do better or not? I think the question is
8 still open and we need much longer follow-up.

9 Dr. Kay is going to talk about the new
10 trials that the U.S. intergroup is doing and how
11 MRD studies are going to be incorporated in this.
12 But I think MRD assessment should really not be a
13 mandatory expectation for new targeted drugs coming
14 forward. So if the patient is doing well, the
15 tolerability of the therapy is well, this should be
16 a demand moving forward. It should augment
17 approving good drugs. Thank you.

18 (Applause.)

19 DR. DE CLARO: Thank you. Our last speaker
20 for this session is going to be Dr. Neil Kay from
21 the Mayo Clinic. The title of his talk is MRD in
22 Clinical Trials, North American Intergroup.

1 phase 3 trials where we're going to address MRD.

2 So that is unique. And I am going to
3 present this talk on behalf of John, Jennifer, and
4 I should have put Tait Shanafelt down here, as well
5 as Curt Hanson, who's our flow cytometry hemepath
6 person at Mayo Clinic.

7 So I just want to begin then with the first
8 trial. This is the so-called E1912, which is a
9 randomized phase 3, and it's dramatically what it
10 sounds like Peter Hillmen's group is doing, where
11 we're comparing ibrutinib plus rituximab to
12 standard FCR for untreated younger patients with
13 CLL.

14 This trial will be led by Tait Shanafelt,
15 but will be North America intergroup. Just to take
16 you through the trial design, the patients
17 obviously need treatment. They're younger than 65.
18 They cannot have 17p. There's a 2 to 1
19 randomization, where an expected 346 patients will
20 get the ibrutinib or/and rituximab for three
21 months, and then, for responders, continue on PCI
22 until prog. Then at the investigator's option,

1 they can go onto FCR if there's progression during
2 a certain period of time.

3 With respect to FCR, this is the standard
4 approach, and there will be 173 patients. And
5 patients who have prog'd will have access to PCI,
6 to the ibrutinib. And we're doing this because we
7 think this will really help attract patients into
8 this protocol.

9 Now, the primary endpoint is PFS for reasons
10 that John has just told you. And we think this is
11 a very rational way to go. I won't take you
12 through the agony of how we got to actually get
13 this trial finally to its, hopefully, last steps.
14 God forbid sequestration.

15 But in any case, it is CTEP approved. It's
16 been revised many times. The leukemia steering
17 committee has approved a revised phase 3, and there
18 is a revised protocol that is resubmitted to CTEP
19 very recently. We're hoping to activate this in
20 the spring of 2013. And I take you through this
21 agony because I want you to understand that we will
22 be doing these studies once the trial is activated,

1 but we're hoping it will happen in 2013.

2 Now, there are a lot of correlative studies
3 that are embedded in an ROR1 that was submitted to
4 the NCI. But one of the critical objectives is
5 minimal residual disease. But I just want to
6 emphasize, the primary objective for this trial is
7 to look at PFS in this comparison between the two
8 approaches for younger patients. One of the
9 secondary objectives is eradication of MRD
10 following chemotherapy and CIT, and to see if it's
11 an independent predictor of PFS and OS.

12 Now, that's a gross oversimplification, and
13 I'm going to go into a hypothesis related to this
14 in a moment. But, basically, we will be looking at
15 MRD using flow cytometry, and I'll explain this
16 assay to you in a moment. These we looked at, at
17 different time points during and after therapy to
18 determine if it's an effective surrogate marker for
19 prolonged PFS and OS.

20 Now, with respect to our own trial, we will
21 be looking at this, as I said, in CIT and non-CIT.
22 Our hypothesis is that MRD status either

1 immediately after CIT, or some reasonable period
2 after CIT, or study over time in the non-
3 CIT -- that is the ibrutinib rixutimab -- will be
4 associated with both PFS and OS.

5 We're really trying, then, if you will -- I
6 think this is one of the quests of this meeting
7 today -- to identify a robust and timely surrogate
8 endpoint for those clinical parameters. And there
9 will be multiple time points. And you see in the
10 yellow the time points that we each have chosen to
11 use for sampling of the MRD status over the time of
12 this trial.

13 So what are expected outcomes? So these are
14 kind of a hypothesis, if you will. We anticipate
15 that MRD status at the time of the 12-month
16 response will correlate with PFS and ultimately OS
17 in patients treated with FCR. We also anticipate
18 the MRD status will correlate with PFS and OS for
19 patients treated with a non-CIT regimen. However,
20 the optimal timing of the MRD assessment will
21 probably need to occur at a later time point.
22 We're projecting 24 or 36 months. And that

1 obviously is different than that used for a
2 conventional chemotherapy.

3 So again, this is really maybe just an
4 overlaboring of our hypothesis. But for
5 conventional CIT, the hypothesis is that the CIT
6 arm will have the highest proportion of
7 MRD-negative patients at the 12-month response,
8 whereas MRD status at the time of the 12-month
9 response -- and it will be an accurate predictor
10 for the conventional CIT arm at the 12-month, but
11 will be less useful for the non-CIT.

12 If you will, if the converse for the non-
13 CIT, the proportion of patients in the non-CIT arm
14 who achieve an MRD-negative disease status will be
15 higher at the later time points compared to the
16 12-month time point, and that these later time
17 points will be a better predictor of PFS and
18 ultimately OS for that approach than earlier time
19 points.

20 So having taken you through that, let me
21 also mention the other, if you will, twin trial,
22 companion trial. This is chaired by Jennifer

1 Woyach with John as the correlative study co-chair.
2 And this is being led by the Alliance, but is a
3 North America intergroup trial. And, again, a
4 randomized phase 3 comparing bendamustine plus
5 rituximab versus ibrutinib plus rixutimab, versus
6 ibrutinib alone in older patients. In this case,
7 65 years is the cut point.

8 Here, you see the schema where patients get
9 bendamustine rixutimab and can, if they prog on to
10 ibrutinib, and then comparison to ibrutinib and
11 rixutimab at the doses and cycles you see there.

12 So the primary objective here is to
13 determine whether progression-free is superior
14 after therapy with bendamustine in combination with
15 rixutimab or ibrutinib alone, or the combination of
16 ibrutinib and rixutimab in older, previously
17 untreated CLL patients.

18 The secondary objective, just to mirror then
19 what we are doing in the younger CLL trial, is to
20 determine the impact of MRD-negative disease at the
21 time of CR documentation, added at two years, on
22 PFS and OS in each of the treatment arms. And I

1 have been told by Jennifer and John that MRD will
2 at least be evaluated at the nine-month and two-
3 year time point. Hopefully, this trial will also
4 be activated in three to four months.

5 So let me just end with just a brief
6 description of the MRD assay we will be using in
7 E1912. And to do this, just initially with a bit
8 of an apology, this is Curt Hanson's work, our
9 hemepath person at Mayo Clinic. And I'm really
10 presenting this approach on his behalf. This was
11 presented at IWCLL a couple of years ago and has
12 been published in this reference cited here for
13 you.

14 So basically, what he has done is develop a
15 single-tube six-color with the antibodies shown
16 there. And this has been used repetitively over
17 the last several years to assess MRD in our
18 patients at Mayo Clinic.

19 So the single tube contains six-color
20 antibody panels, 45, 19, 20, CD5, and kappa and
21 lambda. The assay was validated by comparing it to
22 a previous standard four-color assay in 562

1 specimens.

2 Just to take you briefly through what's
3 done, 500,000 events were collected in all cases,
4 analyzed on a Canto. And positive events were
5 based on this flow, if you will, identification of
6 lymphoid cells by light scatter, gating on the
7 CD19, positive B cells, then dual expression of
8 low-expressing CD20 and CD5, and then evaluation
9 for kappa and lambda clonality.

10 In this case, the level of MRD is assessed
11 by calculating the percent of MRD as CLL events
12 divided by the total live and non-aggregated white
13 cells times 100. And the outcome of this basically
14 is summarized in this last line, that there's a
15 high level of sensitivity with the six-color and
16 the ability to consistently detect MRD events at
17 the .005 percent level. And it is our impression
18 that is below that of the historic standard using
19 three- or four-color approaches.

20 I think that's my last slide, and I thank
21 you for your attention.

22 (Applause.)

1 actually, for the clinical benefit outcomes, it's
2 after treatment is cleared, so usually about three
3 months.

4 DR. REAMAN: Could I just follow up? Have
5 there been any studies of the kinetics of MRD
6 clearance? And does that have any prognostic
7 significance or does it correlate at all with the
8 number of patients who are negative at the end of
9 therapy and then ultimate outcome?

10 DR. KIPPS: Unfortunately, this is a moving
11 target, and I think it's going to be
12 therapy-dependent, in that some therapies may be
13 more effective at certain compartments, which make
14 it problematic to assess a compartment for MRD, and
15 in the studies that were published in the British
16 group, I would imagine that some of the patients
17 treated with alemtuzumab who are MRD negative in
18 the blood and marrow probably had adenopathy at the
19 end of therapy by CT scan.

20 Do you have any idea about that, Peter? But
21 I think, if you have a reservoir for the tumor
22 that's still present, then you assess a compartment

1 for MRD negativity, then that may be a false read
2 early on. When the disease redistributes over
3 time, then you get MRD-positive disease and
4 relapse.

5 So I think the complexity here is not only
6 getting a time organized, but looking at the
7 activity of each of these different therapies, what
8 compartments is it affecting. And we may have to
9 try and develop some algorithms for each type of
10 therapy.

11 I know that's not a very satisfactory
12 response.

13 DR. HILLMEN: Could I just address Tom's
14 question or two questions there? One is that we
15 did CT scans in the consolidation trials in the
16 early patients with less than 2-centimeter nodes
17 consolidated, so we didn't include patients with
18 bulky lymphadenopathy in those trials.

19 To address the question in comparison to
20 ALL, I guess the outcome for sera, as you've seen
21 from the survival curves, is a long-term outcome.
22 The PFS for our clinical trials, if we use that,

1 you're waiting six years for the control arm. And
2 a time point at the end of treatment, three months
3 after the end of treatment, which is what has been
4 in the guidelines for quite some time, predicts for
5 outcome; whereas in ALL, you're trying to avoid the
6 early relapses, and there's a different use of the
7 surrogate, if you like.

8 We can talk a lot about different time
9 points, different tissues, but what we know is that
10 at three months post-treatment, at the assessment
11 point, if you're negative, you do better. And I
12 think as an endpoint for trials, that's what we
13 will be doing, and it's probably the best surrogate
14 for the early approval of some drugs.

15 I'll take the point that John makes. Just
16 because you don't achieve negative remission
17 doesn't mean the drug doesn't work, but if you do,
18 it means it does work. And we would then test the
19 other timings for relapse, and dynamics relapse, we
20 do that anyway. But I guess we don't want to
21 confuse the issue.

22 DR. DE CLARO: Dr. Kay?

1 DR. KAY: Yes. I just want to also make the
2 obvious point that we've not tested MRD in the
3 signal inhibitors in great detail. And I like what
4 Tom said a lot. I do think that it might be
5 fascinating to observe that patients who have rapid
6 drops in their MRD but do not get to be MRD
7 negative actually do quite well. And I think also
8 the possibility that blood could be a surrogate
9 even for CT scans may still be out there.

10 In other words, if you have a blood
11 compartment that's completely cleared at three
12 months or six months with the inhibitors that we're
13 proposing to use, that that may be -- it's possible
14 that that might end up being associated with an
15 excellent clinical outcome. We just don't know.

16 So I think it's an exciting time to be
17 looking at MRD. There's all kinds of possibilities
18 about how this may play out.

19 DR. DE CLARO: Dr. Byrd, first. Or do you
20 want to respond to Dr. Kay?

21 DR. MONTSERRAT: I mean, he is connected to
22 me -- no, no, John.

1 DR. DE CLARO: Okay. Dr. Montserrat?

2 DR. MONTSERRAT: I mean, it is related
3 to -- well, maybe a naive approach, but my
4 perception is that the necessary condition to cure
5 any kind of cancer is first to get rid of the
6 cancer. And all these new agents, these kinase
7 inhibitors that you offer us, you have emphasized
8 that two reviews were very good. I mean, they
9 offer promise, but it's still a long way to see
10 whether the concept changes. We can't reform this
11 disease in a kind of chronic disease.

12 So I am not completely clear probably
13 because of my lack of -- I mean, it's my lack of
14 really going in deep in these papers. But can any
15 of you mention what is the -- you have shown, which
16 makes sense, data based on progression-free
17 survival. And so, in terms of classical complete
18 responses by either NCI or IWCLL criteria, do you
19 have -- my understanding is that the complete
20 response rate with any of those new agents is
21 really quite small.

22 Is that correct? I mean, in 10 -- because

1 it's a kind of different treatment approach. I
2 think that my personal view is if we are
3 considering what has been classical therapy for
4 CLL, which is nowadays chemoimmunotherapy, maybe I
5 am biased. But I think that the data that we have,
6 indicating that achieving MRD-negative status is an
7 important endpoint for these patients -- I mean,
8 all these data think they are quite solid.

9 I fully agree with that. It's a moving
10 target. So the techniques would improve over time,
11 that's for sure. But I think that now we have a
12 kind of general agreement about the sensitivity
13 that the technique should have and maybe the time
14 point, as you have indicated.

15 DR. DE CLARO: Dr. Byrd?

16 DR. BYRD: Having been the conservative one
17 relative to MRD the whole meeting thus far, I'll
18 say that we've not seen a single ounce of data that
19 when you become MRD negative with chemotherapy or
20 chemoimmunotherapy, that that doesn't serve as a
21 surrogate outcome for favorable outcome long term.

22 So with these new agents, whether it's

1 ABT-199, it's all of the agents, I'm not sure we
2 need a new paradigm if we're adding a new agent to
3 standard therapy, because the surrogate endpoint of
4 the minimal residual disease negative in patients
5 that are getting chemotherapy plus something has
6 held strong for everything.

7 I think that's an important point to
8 distinguish because, again, if the new drugs coming
9 forward greatly enhance the effect of chemotherapy,
10 we don't want to delay development of drugs based
11 upon what we know.

12 DR. KIPPS: It may help in terms of sorting
13 out treatment-free survival versus progression-free
14 survival. So there are two different things.
15 Patients undergoing continuous treatment may do
16 quite well with these kinase inhibitors and MRD may
17 become less relevant. MRD may become very relevant
18 to look at treatment-free survival after therapy.

19 DR. STETLER-STEVENSON: I would like to
20 state something that I think is obvious. You're
21 talking about MRD positive and negative. If you're
22 talking about a sort of chronic therapy approach,

1 if you don't want to think just in terms of
2 positive and negative, but monitoring, and if you
3 have a very sensitive and precise assay, if you can
4 demonstrate that you're having maintenance at a
5 very low level, this is very useful.

6 DR. DE CLARO: I think, regarding the newer
7 therapies that are emerging, previously our
8 therapies were limited by the number of cycles you
9 could give. With the newer therapies, typically
10 the duration of therapies until progression, we're
11 no longer being limited by this -- for FCR at six
12 cycles, because patients cannot tolerate it
13 furthermore and cytotoxins because of hematologic
14 toxicities.

15 So perhaps, given those -- probably we're
16 going to see a shift with regards to how long we're
17 treating patients, and it's a good idea that we're
18 continuing to assess what's the best time for
19 assessment of MRD.

20 Dr. Brown?

21 DR. BROWN: I just want to echo some of the
22 comments about kinase inhibitors, which are showing

1 a lot of promise, really. People still have
2 macroscopic disease, and we're not sure what the
3 impact of MRD will be, even at 24 and 36 months
4 when people are doing very well, progression free,
5 but still have clinically evident disease.

6 I would say that, for younger patients, who
7 are currently treated with FCR and have long
8 remissions, there's some concern that having
9 persistent disease over many years may lead to
10 outgrowth of subclones and resistance.

11 We just don't have the long follow-up yet
12 with the kinase inhibitors. And there's been some
13 inhibition of study of combinations with FCR for
14 upfront therapy in younger patients because of the
15 lack of a potential surrogate endpoint.

16 We have MRD-negative remissions in
17 chemoimmunotherapy plus kinase inhibitor patients
18 in second-line, but moving that up to first-line is
19 something that has not happened so much because of
20 the potential difficulties of setting progression-
21 free survival in the absence of an MRD-negative
22 endpoint.

1 So for those younger patients, the potential
2 for novel therapy development, as Pete said, has
3 been somewhat inhibited. And we're not limited to
4 chemoimmunotherapy over time. As we look forward,
5 we may be combining kinase inhibitors with other
6 novel agents that do clear MRD. And that may be
7 where we're really going eventually, but again, MRD
8 negativity would --

9 DR. DE CLARO: Dr. Hillmen?

10 DR. HILLMEN: I agree. Just the points
11 about the novel therapies. First of all, John's
12 really shown the E2071 and I believe some
13 data -- the data for other therapies, better
14 antibodies perhaps, 199, do suggest that we might
15 get some MRD-negative remissions with some of the
16 novel therapies as well. Virtually all the data we
17 have for the novel therapies is in
18 relapsed/refractory disease. We don't have
19 any -- the tiny amount of data with one drug for
20 two years in a previously untreated group of
21 patients.

22 So we're comparing apples with oranges,

1 really. We are really looking at the patients we
2 can achieve MRD-negative remissions, and it may
3 well be, "Now, do we want to have people on kinase
4 inhibitors for 10 years as Jennifer alludes to?"
5 We don't know what the long-term effects of these
6 drugs will be and what the long-term outcomes will
7 be. So it may be that I'll suspect we'll be adding
8 those drugs to our conventional chemotherapies.
9 And we will then want to see improved MRD-negative
10 remission rates, which will predict for a better
11 outcome because it doesn't have any of the context.

12 DR. BROWN: I have treated only three
13 patients treated with FCR, and it wouldn't
14 (indiscernible) have been a second-line setting.
15 And two of them are MRD negative two years out and
16 the other one is having reducing MRD levels on
17 persistent ibrutinib. And this is just a very
18 limited experience, but that's because there's
19 limited interest for studying that combination,
20 which eventually hopefully will get rid of
21 chemoimmunotherapy.

22 But right now, these potential combinations

1 have --

2 DR. BYRD: I agree with Jennifer that -- I
3 mean, there are two paradigms. And one, we don't
4 know the long-term outcome of these, but we do know
5 the long-term outcome of chemoimmunotherapy, that a
6 proportion, 3 to 10 percent, with FCR combinations
7 get secondary leukemia, and virtually all of those
8 patients die of a complication of therapy. And
9 then there are other things as well.

10 But MRD clearly -- MRD assessment allows us
11 to get more novel drugs into patients, available to
12 patients quicker, and so it's clearly validated for
13 that.

14 DR. GHIA: I am sorry if I interrupt. Maybe
15 I am getting confused. I think that, now, somehow
16 the discussion shifted on what's the future of
17 treatment in CLL, which I think is not the subject
18 and the topic of this workshop.

19 So none of these things that have been said
20 by all our colleagues are in contrast. I mean,
21 maybe in the future it will turn out that we don't
22 need to eradicate the disease. Maybe, as Professor

1 Montserrat said, you don't want to leave it for
2 40 years with a cancer that can't somehow even
3 transform or anything. But, I mean, this is just a
4 philosophy.

5 I think that we are here just to define can
6 we use MRD assessment as a surrogate endpoint, not
7 for all clinical trials, but just for those where
8 it's needed to get the drug approved much earlier
9 than before.

10 DR. KAY: Can I just add quickly something?
11 I think we're getting a little lost, too. And they
12 don't have to become MRD negative necessarily.
13 It's just that, as you brought up, Greg, it could
14 be that even just the fact that they're getting a
15 drop, that there's some rate of drop of the MRD
16 that is associated with enhanced PFS and OS may be
17 sufficient. And I think we're really trying to get
18 clinical trials done as efficiently as we can to
19 get those drugs out. So I think we shouldn't lose
20 sight of that in this discussion.

21 DR. REAMAN: I would just sort of echo
22 Dr. Ghia's point. I don't think we're here to

1 consider the situation where MRD will be a
2 potential surrogate for every single drug that has
3 to come in for potential approval. So we're really
4 trying to be as flexible as possible and to
5 accelerate the process.

6 I think your point about potentially
7 inhibiting or slowing the process for newer novel
8 therapies is right on target. And I really don't
9 think this is something we have to use all of the
10 time. But in the situations where it can be used
11 and where it would facilitate, I think that's where
12 we're looking to see if we can do that, and if we
13 can do that, how best to do that.

14 DR. DE CLARO: Dr. Hillmen?

15 DR. HILLMEN: Just to add to that, the point
16 I made at the end, we are not able to develop
17 therapies for patients who have frontline fit for
18 FCR without a surrogate endpoint. We just can't do
19 it with PFS as an endpoint. So whatever drugs or
20 therapies we approve, unless we use a surrogate
21 like MRD, we're inferring across from other patient
22 groups that are not comparative.

1 So without this endpoint, we're going to be
2 approving drugs in the wrong group of patients that
3 we want to use the drug in; other therapies rather
4 than drugs. And I'm not talking regulatory. I'm
5 talking for operative groups internationally.

6 DR. REAMAN: For fear of getting off the
7 topic again, just to talk about the future of CLL
8 therapy, do you see these new signaling agents
9 replacing conventional chemoimmunotherapy in total
10 or possibly being integrated or used in addition?
11 And I know we're not supposed to be talking about
12 the future of CLL therapy, but I think it does have
13 some impact on the rest of our discussions here.

14 (Laughter.)

15 DR. REAMAN: Are there two competing camps
16 here?

17 DR. HILLMEN: This feels like a hospital
18 pass, but I think there's probably three groups. I
19 think for the patients, we don't have effective
20 therapies for; so 17p-deleted to frontline patients
21 we do not have effective therapies for. This will
22 replace those, and we're already getting there for

1 those.

2 For the patients who are younger, fitter
3 patients who are having very good remissions with
4 FCR, I think we all believe that these therapies
5 will answer to the treatment. And in some way will
6 be part of the paradigm of our treatments in the
7 future.

8 Whether it's continuous use of a single
9 agent over many years or whether it's a
10 combination -- we have several different targets,
11 new targets that probably are complementary or even
12 with chemotherapy. There's also the issue of long-
13 term effects of chemotherapy. So I think we will
14 be used, but not probably as a single agent forever
15 in a patient with good-risk CLL.

16 DR. DE CARO: Dr. Montserrat?

17 DR. MONTSERRAT: Yes, I think that -- I
18 mean, that took for us something like 10 years or
19 so to get the concept of chemoimmunotherapy as a
20 related concept of the new paradigm for treating
21 patients with CLL. I mean, we are talking about a
22 number of agents which are extremely promising, but

1 the data are completely immature.

2 We shouldn't refrain from using what we know
3 that works and is useful and important for our
4 patients, which is all we have learned in the past
5 20 years or so about chemoimmunotherapy, the
6 importance of MRD, I mean, to expedite the process
7 of getting better and novel drugs for
8 conventionally-treated patients with CLL.

9 So I don't have a crystal ball, but I would
10 say that maybe these new agents will have certainly
11 maybe for patients not completely fit for standard
12 chemoimmunotherapy or a combination. I mean, it's
13 single therapy. I don't believe in single therapy.
14 And I am very glad seeing that there are studies
15 combining monoclonal antibodies with kinase
16 inhibitors and so on. But, I mean, we are far away
17 from just knowing.

18 If you allow me to say so, I think that
19 wishful thinking shouldn't refrain us from really
20 consolidating and making step-by-step progress in
21 daily life, in real life.

22 DR. DE CLARO: Yes. Dr. Kipps?

1 DR. KIPPS: It's clear that there are a lot
2 of exciting new agents out there, and we're all
3 very excited about paradigm shifts in the therapy
4 of CLL. However, it's clear that, also, the use of
5 the kinase inhibitors and perhaps other maintenance
6 drugs might not eradicate MRD. But the supposition
7 is that we will have to continue therapy.

8 So again, I go back to the point of whether
9 MRD can be used as a surrogate marker for
10 treatment-free survival, in which case, patients
11 who are being treated with these various agents,
12 with the addition of another agent, who achieve
13 MRD-negative status might be able to have a holiday
14 off drug altogether.

15 I think many patients would agree that to
16 not take therapy would be better than taking
17 therapy continuously, particularly if you're a
18 younger patient. This is a paradigm that has been
19 established with regard to CML, where the ability
20 to achieve MRD-negative status has allowed for a
21 trial to discontinue the kinase inhibitors with
22 some success. And I think that's an area that

1 needs to be further explored.

2 DR. DE CLARO: Dr. Byrd?

3 DR. BYRD: I think your point of doing
4 well-controlled trials and not throwing things out,
5 I think just the advances that we've made over the
6 past several decades have not been with
7 chemotherapy, no chemotherapies. So adding one
8 chemotherapy to another hasn't improved survival in
9 any CLL patients. It's really adding a targeted
10 therapy to chemotherapy that modestly improves
11 survival, which is the endpoint that we're shooting
12 for.

13 At least the patients that we see -- and I
14 think we would all agree going around the
15 table -- they don't want chemotherapy. And we know
16 the long-term risk of chemotherapy. Right? We
17 don't know the long-term risk of targeted therapy,
18 but we know the long-term risk of chemotherapy.

19 So the concept of testing in well-controlled
20 studies, non-chemotherapy approaches, and trying to
21 throw out something that we know in the past,
22 through decades of studies, hasn't improved

1 survival, I think, is a worthwhile thing.

2 DR. MONTSERRAT: I mean, although we have
3 been prevented from talking about the future of
4 therapy with CLL, I couldn't agree more with you.
5 But no matter how disappointing are the data, this
6 is what we have. And then the best shouldn't work
7 against the better or what is really possible.

8 I agree with you that the major advance has
9 been the introduction of monoclonal antibodies
10 along with chemo (ph) agents. And maybe in the
11 future, hopefully -- I mean, kinase inhibitors to
12 this or to other biologicals will even provide much
13 better with those.

14 As Paolo said, I think that -- we have here
15 three questions. Is MRD a meaningful surrogate for
16 agents, novel agents in CLL? That's probably the
17 first question, and that's why we are here. I
18 think that would be yes.

19 So the question is, do we have additional
20 techniques to assess MRD? Again, my answer would
21 be yes. I know the rest. It's a matter just to
22 introduce them in clinical trials. And so the

1 point that MRD should not be actually the endpoint
2 or the surrogate for all kinds of therapy in CLL, I
3 think is very good. And hopefully, MRD will be
4 replaced by some other kind of surrogate in the
5 future.

6 But again, I mean, the only point that I was
7 trying to make, John, is that the future, no matter
8 how exciting it is, shouldn't refrain us to make
9 progress step by step in their lives.

10 DR. KAY: Can I just say one quick comment?
11 Thank you for asking that question. Not. You have
12 to understand that the North America intergroup
13 trials are not just relying on MRD. That's first
14 of all.

15 Secondly, with the attempts that we are
16 going through and comparing ibrutinib with the
17 novel therapies to the standard, the gold standard
18 FCR, is not necessarily a replacement. It is of
19 equivalence or of providing patients with options.
20 For example, it is possible that novel therapy will
21 be equivalent to FCR. However, the toxicity of FCR
22 is very different than would be the case, as far as

1 we know for IR, for the novel therapy.

2 To me, quite apart from the MRD issue,
3 that's the kind of thing we need to answer in these
4 trials. And the MRD, I hope it's not deflecting
5 from the direction of these trials. We're not
6 trying necessarily to replace the gold standard.
7 It's just part of the menu for our patients.

8 DR. HILLMEN: I agree with John. We need to
9 compare against the gold standard, which is what I
10 believe he's saying. And we need to facilitate the
11 well-designed, randomized control trials in the
12 appropriate patients. And that's why MRD is
13 important.

14 So we have to beat FCR. I hope we can beat
15 FCR in terms of toxicity, and it looks like we will
16 be able to. But we have to do the trials in the
17 right patient group, and we can't at the moment.
18 It is very hard.

19 DR. DE CLARO: Are there any other
20 questions? Dr. Byrd?

21 DR. BYRD: I guess the question -- so FCR
22 for MRD is going to -- you don't have to do the

1 study to prove that FCR is going to beat ibrutinib
2 at six months probably for MRD. You're going to
3 have to follow those patients long term.

4 DR. HILLMEN: Yes, well --.

5 DR. BYRD: So for studies where you're
6 adding something to FCR, MRD is going to be very
7 fruitful. But the study that you put up that the
8 British group and the U.S. intergroup is doing in
9 young patients, that's going to take using the
10 traditional surrogate endpoint of progression-free
11 survival, in my belief, at least.

12 DR. HILLMEN: Yes. That's the primary
13 endpoint of that trial. It has to be. The MRD is
14 a surrogate. But we know that 25 percent,
15 20 percent of patients at two years with FCR have
16 progressed. We know from the 31 patients in the
17 frontline trial with ibrutinib, that 1 patient has
18 progressed. So it may be that we do get an earlier
19 readout because of that, but we have to do the
20 trials.

21 DR. BYRD: Absolutely.

22 DR. DE CLARO: So I guess I am seeing no

1 other further discussions. We will now break for
2 lunch. Boxed lunches are available at the food
3 kiosk outside for a fee. Tables are alongside this
4 room in the hall for lunch. We will convene again
5 in an hour, at 12:45. Thank you.

6 (Whereupon at 11:42 a.m., a lunch recess was
7 taken.)

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1 A F T E R N O O N S E S S I O N

2 (12:48 p.m.)

3 DR. WIERDA: So I'm going to be moderating
4 this next session, which is technical
5 considerations particularly relevant for flow
6 cytometry. And our first speaker is Andy Rawstron
7 from Leeds.

8 **Presentation - Andy Rawstron**

9 DR. RAWSTRON: Many thanks, and thanks for
10 the invitation over here. It's been an interesting
11 couple of days for people involved in flow
12 cytometry assays before this. Hopefully, I'll be
13 able to demonstrate that we have a fairly
14 standardized approach that's been in practice for
15 the last 10 years or so.

16 So the unusual thing about CLL, compared to
17 other hematological malignancies is, actually, the
18 aberrant markers have been known really since
19 almost the disease was identified, but from the
20 1970s, people knew those abnormal CD5 and weak
21 CD20 -- weak FC immunoglobulin, although they might
22 not have known not particularly what they were

1 until the '80s and '90s, when there were decent
2 antibodies. But over the years, we've seen
3 increasing depletion of disease with treatment and
4 better assays to cope with that increased
5 reduction-of-disease burden.

6 Now, the first point I just want to make is
7 that one of the things why that phenotypic
8 information has been useful is it means we haven't
9 had to rely on clonality assessments, which give a
10 really variable assessment of disease levels,
11 according to what the polyclonal background is.

12 So for somebody who has .1 CLL and .1 normal
13 B-cells, you may be able to detect it with a simple
14 clonality assay. But you take those normal B-cell
15 backgrounds up to 5 percent, and you can no longer
16 see the CLL. And if you have somebody who's got a
17 log lower CLL but no normal B-cells, suddenly they
18 become MRD positive again.

19 So having that ability to look at a surface
20 phenotype, a disease-specific phenotype, in
21 conjunction with clonality has been very helpful
22 until we got to the stage where, nowadays, we can

1 see that MRD is a clear predictor of outcome,
2 independent of the type of treatment. And this is
3 obviously sub-batches, Jim's CLL8 study group data
4 showing parity for FC versus FCR, whereas the real
5 difference is according to whether your disease
6 level is below 10 to the -4, or above 10 to the 2,
7 or higher.

8 Now, Peter Hillmen showed the follow-up from
9 the Leeds data, showing basically that MRD level
10 was an independent predictor of progression-free
11 survival and overall survival. And I'd purely like
12 to point out, in addition to the discussion this
13 morning, IWCLL response only fell out of the
14 equation after about eight years of follow-up.
15 Actually, it used to be in the multivariate
16 predictor of outcome as well. But you really need
17 the long-term follow-up to see that difference in
18 progression-free and overall survival for people
19 who have achieved a CR, MRD neg versus pos.

20 In the next few slides, I just want to
21 suggest why MRD might work. The way that we're all
22 assuming is that the CLL cells, once they've been

1 depleted by the treatment, then start to double at
2 a rate at what they would do when we see them in a
3 clinical setting. If that's true, then for
4 somebody with a six-month doubling time, for every
5 log depletion of disease you get, you should see
6 something like 20 months' improvement in
7 progression-free survival.

8 In fact, from that Leeds data that I just
9 showed you again, according to the log depletion,
10 if you're greater than 10 percent and so forth,
11 you're seeing about an 8- to 12-month improvement
12 in progression-free survival per successive log
13 depletion of disease. And we can also see from
14 some data that Seb provided me, for which I am very
15 grateful, that, actually, the level of MRD relapse
16 is about one year to increase per log of disease.

17 So here's the typical kinetics of relapse.
18 And I think it's worth just looking at this to get
19 a figure for when we should do MRD assessments and
20 what they might actually mean. And the first point
21 is to note that you get a very -- not very, but
22 certainly get a delayed clearance of treatment,

1 which means that sometimes B-cells are not in the
2 peripheral blood at all. The point where the
3 B-cells come into the peripheral blood, you can
4 start to make meaningful assessments of disease
5 levels in the peripheral blood as well. At that
6 point, the blood starts to become representative of
7 the total body.

8 At some later time, you start to see the
9 disease coming back. And in all our experience,
10 we've always seen, when you see residual disease,
11 it doubles from then on outside of the allogeneic
12 disease setting.

13 There's a little period, it seems, from here
14 where there's a slight delay in that relentless
15 progression and that happens about the crossover
16 between normal B-cell levels, but thereafter, you
17 also again see going back to doubling.

18 I've really only got about 18 patients with
19 valuable data, but basically, the correlation with
20 a doubling-type pattern is extremely good.

21 Sometimes there's a slight shift around that early
22 time point, but basically, once the disease is

1 there, it doubles from there on in.

2 So we have this situation where we've got
3 quantitative assays. That means we can get a very
4 specific measure of disease levels. We know that
5 the kinetics of relapse are exponential, even at
6 the lowest level of valuable disease. And we're
7 looking at per-logarithmic depletion of disease,
8 approximately 8 to 12 months' improvement in
9 progression-free survival.

10 So with that kind of biological background,
11 the other issue here is, have we got assays that
12 are suitable to be used in clinical trials, assays
13 that are suitably reproducible, that have been
14 tested against the gold standard. And we're very
15 fortunate in CLL to be able to do that.

16 I'm going to go through the experience over
17 the past decade or so with, effectively, the same
18 assay, but making it easier and easier to cope with
19 in individual laboratories. So the first effort is
20 with multiple laboratories in the E.U. and the
21 U.S., published on behalf of ERIC and the CLL
22 Research Consortium. And I guess the main point to

1 point out about this one is that we tested an awful
2 lot of different antibodies. Some of those aren't
3 really in current diagnostic use for the majority
4 of labs today. So that big amount of assessment
5 was actually done very early on.

6 From those, we identified some core markers,
7 some of which, as I mentioned before, have been
8 around since the '70s, are things that people are
9 very familiar with, CD20, CD5, and other things
10 which were maybe less familiar to some, CD81.
11 Maybe CD43 was variably used. And those form the
12 basis of the assays since then.

13 Now, one of the things that we did identify
14 was this contamination level, which is a false
15 positive, it's not a big problem above .1 percent.
16 It starts to become a problem at about .01 percent.
17 If you deal with it, you have a limited detection
18 of about 10 to the -4. If you don't deal with it,
19 you're looking at a limited detection somewhere
20 between that. But we identified it. We know how
21 to cope with it.

22 The other thing is the issue of can you

1 actually give this assay to a laboratory and expect
2 them to give you decent, quality results? So what
3 we did for a number of people who asked, "Can we
4 learn how to do the assay?" we said, "Sure. Here's
5 145 cases. Go analyze." And they would go off,
6 and analyze the case, and give a result against our
7 known reference.

8 As you can see, just using the assay itself,
9 the accuracy was only about 80 percent precision,
10 even worse. But then, what we'd ask them to do is
11 to read this 20-page protocol on how to do it
12 properly. They'd read that, redo it, and suddenly,
13 then, you see just with that simple exercise of
14 reading the protocol, the accuracy and precision
15 goes right up to the sort of levels that you'd want
16 to see for a clinical assay.

17 The other point is that we have a very good
18 gold standard to test the flow cytometry against.
19 The PCR and the flow cytometry, if you're using
20 quantitative assays, are both broadly equivalent
21 down to the 0.01 percent level. But the PCR is
22 just a bit problematic to introduce into a clinical

1 lab. It's a lot of work and has to be optimized
2 for each patient. But it's a perfect gold
3 standard.

4 Here, we can see, as also the German CLL
5 study group did independently, and as Seb showed
6 you, there's very good correlation between the two
7 methods.

8 So we have the assay. This was applied in a
9 number of clinical trials. It works fine. The
10 issues are that it requires a lot of cells for
11 analysis, and that limits your sensitivity. If you
12 only have 10 million samples, 10 million cells in a
13 sample, there's a limit to how far you can go.
14 It's fairly slow and difficult to analyze. People
15 have a lot of trouble with it. And the full panel
16 probably isn't required in all cases.

17 So the next thing to do just to keep
18 the same assay going, but to make it easier and
19 more applicable to different labs was to identify
20 situations where maybe a less time-consuming
21 approach would be sufficient for those laboratories
22 who haven't got six-color machines, who haven't got

1 time to process very high-sensitivity assays; and
2 also to develop a six-color panel that would be
3 quicker for those labs that did have it.

4 I think this is quite salient because, at
5 the time, even in the guidelines, the guidelines
6 that first came out in 2008, the published version
7 still had something about clonality assessment in
8 it, whereas the rest of the people involved in the
9 MRD setting already knew that basic clonality, just
10 looking at 19-5 kappa/lambda wouldn't really tell
11 you how much disease there was.

12 So we ran that and we compared in about 780
13 cases. And what you're seeing is that it's all
14 right for a threshold, for suggesting there's been
15 more probability of finding disease than not.
16 These standard levels or near-standard levels kind
17 of give you an indication. But, actually, if you
18 want 100 percent specificity for the prediction of
19 residual disease, you need very stringent
20 thresholds.

21 So if you want, from a basic kappa/lambda
22 analysis, to say there's definitely residual

1 disease there, you would need really things around
2 .05 to no greater than 32, one type of level, which
3 actually is applicable in a number of situations.
4 I mean, there's going to be a reasonable number of
5 cases where you definitely identify disease using
6 that simple approach. And for the others, we
7 worked through a variety of things looking for that
8 contamination level, looking for the level of
9 sensitivity, the balance between accuracy and
10 precision. But in the end, we're in the same
11 markers as we had before, just in a compressed
12 panel that's a lot easier to use.

13 The interesting thing here is that we start
14 to be able to maybe push the limit of detection
15 down a little bit further. Now, many people have
16 pointed out, do we need to do that? And the answer
17 is probably not. You know what you've actually got
18 is the level of disease per log depletion predicts
19 progression-free survival. But if you're very good
20 down to 0.001 percent -- if you can do it down to
21 0.001 percent, then you will be very good at the
22 0.01 percent, which is the aim, the target that

1 most people go for.

2 Now, we're currently working on the eight-
3 color, and this makes things very simple. It's
4 just one tube. There's the potential for basically
5 just adding blood to a tube and acquiring straight
6 off, which we hope will happen in the near future.

7 We had a few aims. And there are a couple
8 of bits of information that are probably worth
9 sharing with you from that. There's a number of
10 participants, again, across the E.U., across the
11 U.S., and Australia. And the first thing was the
12 dilution study, where we got down to 0.001 percent
13 reproducibly. Maybe not. It's a bit tricky.
14 There's a number of factors which could confound
15 that, but we'll certainly very easily go down to
16 0.01 percent and have an assay that could go
17 further, and we can evaluate further in the future.

18 What about the variation between different
19 laboratories? And again, that's at 0.01 percent,
20 this target. We're pretty good on the variability,
21 but below that, it starts to waiver. And you're
22 actually seeing up to half a log difference, which

1 makes some people think there's no way that could
2 possibly be applicable in a clinical setting. But,
3 of course, you're looking at logarithmic changes to
4 have an impact on progression-free survival, a
5 significant impact.

6 So if we go back to that Leeds data, looking
7 at the progression-free survival up there against
8 the tumor depletion level in logs, according to the
9 results we got, there seems to be a fairly linear
10 relationship, or an exponential relationship, if
11 you like.

12 If you add a .2 log error onto the result,
13 it doesn't change at all. If you add a .4 log
14 error onto that, it changes not one bit. If you
15 add a .6 log error, it doesn't change a bit. It's
16 only when you start to say we might have errors of
17 plus or minus nearly a log on that result that you
18 start to completely lose your ability to predict
19 progression-free survival.

20 So again, I'll show you that just with the
21 survival kits. That's the data set with those
22 errors added, looking at the progression-free

1 survival for the different levels versus the log
2 error added on. And you see it then starts to
3 decay about here.

4 So actually, in a clinical setting, we've
5 got some leeway. It's not really whether
6 somebody's got 5 percent or 1 percent disease left.
7 It's whether they've got 5 percent versus no
8 5 percent. So this is, actually, pretty
9 acceptable.

10 The final point for the development of the
11 assay is just to say that one of the advantages is
12 that we've got an internal control. A lot of the
13 problems that you see with clinical laboratory
14 assays, you need a whole heap of process controls.
15 They're all fine. And then something happens just
16 at the point you acquire the sample. You have no
17 idea what's happened there. You can't tell. With
18 this assay, you've got the internal controls set.
19 All the machines are working fine. The operator
20 has done the right thing.

21 I guess the final piece of the puzzle is
22 proficiency testing, and this is just sort of last

1 week that it was prepared by UK NEQAS. We got a
2 sample from Leeds. They've stabilized it. And
3 this is data on a sample that's more than 10 days
4 old, tested again, and all of the results are
5 looking good. So we should hopefully -- or
6 UK NEQAS should hopefully be able to offer a CLL
7 MRD analysis pilot proficiency testing approach,
8 maybe within the next few months.

9 So in summary, I think we've got very, very
10 good evidence that multi-parameter flow is
11 applicable to the quantitative detection of CLL
12 cells down to this target level. I mean, it's
13 quantitative, so whichever level you want to pick,
14 it can go there. Probably below to 0.01 percent
15 would be difficult -- sorry, .0001 percent.

16 We've validated a number of different
17 configurations, but effectively, it's the same
18 antibodies that we've been going through over the
19 years. In this laboratory, zero is within that
20 acceptable kind of range; proficiency testing
21 available soon.

22 I think I've got a few minutes left to say

1 just a couple of pointers that might be helpful for
2 trying to apply this in clinical trials. If we
3 look at this 207 trial that Peter spoke about
4 earlier, where people are getting alemtuzumab on
5 the basis of an MRD result -- now, obviously this
6 is something where you want to be absolutely 100
7 percent confident that the result you're producing
8 in the laboratory is correct, if that's going to
9 decide whether somebody gets treatment or not.

10 Reviewing that, we had a process to check
11 independently the result. So one person would set
12 up one component to the assay, and that would be a
13 different person, at a different time, and a
14 different batch of samples on a different
15 cytometer, who would set up the other components of
16 the assay.

17 The first two didn't match. The result,
18 they all had to start again. And what we saw in
19 that process is that somewhere on the order of
20 about 2 percent, there was some problem with one or
21 more parts of the assay, which we wouldn't have
22 known about if only one person had done that.

1 So I think there are problems with generally
2 producing laboratory results. It's not enough to
3 affect if you're looking at comparing two arms in a
4 clinical trial. This isn't something that would
5 skew the answer. If you're looking to decide on
6 introduction of treatment based on an MRD result,
7 you have to be extremely careful. Even after all
8 of that, we still found somewhere between 1 or 2
9 out of 500 where there's a discrepant result,
10 despite all those independent checks.

11 The other question that comes up a lot is an
12 atypical phenotype. Now, we don't tend to do
13 things like calling a disease CD5-pos, 23-pos,
14 20-week.

15 We look at a whole spectrum of antibodies
16 and see where it clusters together. And the thing
17 is, if the pattern doesn't fall into something that
18 you could monitor with that set of antibodies that
19 we've been using over the years, then all the other
20 markers in that panel also tend to fit without CLL,
21 outside of CLL.

22 If you've got weak CD5, it's not so

1 important, but if you've got weak CD43, it's
2 probably completely different to CLL in a number of
3 other parameters as well. So I think something
4 like about 1 to 2 percent of cases refer to trials
5 that are not typical. They wouldn't be optimally
6 monitored by this assay, and so you do need to
7 check before treatment starts. Usually, it's okay,
8 but it's better to check before treatment starts.

9 So I think, overall, what we would say in
10 recommendation for clinical trials, obviously, you
11 want a quantitative CLL-specific assay. The
12 clonality-based approach is anti-informative unless
13 they're also combined with other markers, which is
14 what most people do nowadays, but maybe not so much
15 in the past.

16 A pretreatment work-up, the reproducible
17 limit of detection, I would still say is
18 0.01 percent. We can maybe have assays that go
19 further, but that's just making it good at that
20 .01 percent level. There needs to be some
21 independent validation results, particularly if you
22 go to look at trials which introduce a decision to

1 treat based on the MRD result, probably looking at
2 multiple positive time points.

3 With that, hopefully, I've acknowledge
4 everybody on the previous slides that were all
5 involved in the various studies, but particularly
6 at Leeds: Peter, Emilio, Paolo, Michael, ERIC, all
7 the folk at BD Biosciences who provided the
8 antibodies for the various studies, and Matt and
9 David at U.K. NEQAS. And thank you very much for
10 your time.

11 (Applause.)

12 DR. WIERDA: Could you just briefly clarify
13 for me, the data that you show all relate to MRD
14 evaluation in blood?

15 DR. RAWSTRON: It varied, those mixtures of
16 blood and bone marrow. The assay works equally
17 well on blood and bone marrow. In terms of time
18 points, if you've got somebody who's on treatment,
19 particularly if they're with an antibody, then you
20 would need to assess in the bone marrow. But once
21 you've cleared treatment, two months to three
22 months after last therapy, the peripheral blood is

1 probably the preferred place to do this.

2 DR. WIERDA: Are there technical challenges
3 when developing an assay to evaluate for MRD based
4 on flow that are different between blood versus
5 bone marrow? Or is it --

6 DR. RAWSTRON: You have to account for
7 progenitors, additional problems with progenitors,
8 more plasma cells. It's cooked by the antibodies
9 that are in there. If we had a chance to go up to
10 nine colors, I'd stick CD38 in. That would make
11 things a little bit easier. But actually, you can
12 do it equally well in peripheral blood and bone
13 marrow with the current setup as it is on the four-
14 , six-, and eight-.

15 DR. WIERDA: Other questions, comments?

16 (No response.)

17 DR. WIERDA: Thanks. So we'll move on to
18 Gerard Lozanski, who will be reviewing for us the
19 experience at the Ohio State University with flow
20 cytometry and MRD.

21 **Presentation - Gerard Lozanski**

22 DR. LOZANSKI: Good afternoon. Thank you

1 for inviting me to present our experience with CLL
2 MRD in a busy clinical lab. I have nothing to
3 disclose. I will describe our experience with CLL
4 MRD from the point of view of a proctologist, who
5 is responsible for a laboratory which runs a lot of
6 evaluations on different samples, including bone
7 marrow and blood.

8 Since 2007, now, our lab analyzed about 3
9 and half thousand CLL samples, of which about
10 10 percent every year, 10 to 12 percent, is at the
11 level of less than 1 percent, which we then use our
12 MRD assay. I will describe our general outline of
13 staining, processing, analytic methods, utility of
14 prism plot in our MRD approach, and then describe
15 our approach to detect MRD using CD19 as a
16 Pan B-cell marker, and CD24 as an alternative to
17 CD19 in patients treated with CD19 antibody, where
18 CD19 is gone.

19 I will describe just our approach to blood
20 and bone marrow samples. We use fully automated
21 staining and processing using PrepPlus 2
22 workstation, with after-staining subsequent license

1 of red cells and post-fixation using a TQ prep
2 instrument with immunoprep reagents. And we are
3 using standard protocol suggested by the
4 manufacturer.

5 We acquired and analyzed our samples using
6 FC500, five-color flow cytometers. Our stops for
7 CLL MRD are set up on a half-million total events
8 or for leukopenic samples on 10 minutes per tube.
9 If we cannot acquire a half-million events,
10 acquisition will stop after 10 minutes.

11 We use basically exact combination of
12 antibodies, which was proposed by a paper by Andy
13 Rawstron and collaborators, with certain
14 modifications. We added as a fifth color CD45 to
15 these combinations. And we adjusted a combination
16 of some antibodies and colors, which serve best to
17 distinguish between B1A cells in peripheral blood
18 and hematogones in the bone marrow samples.

19 So use of CD45 allows accurate gating on
20 lymphoid cells based on CD45 and side-scattered
21 characteristics. The separation of lymphoid cells
22 in a singular population, based on side scatter,

1 allow us use of a Prism Plot, which is one of the
2 utilities of CXP software, which our FC 500s are
3 equipped. And in turn, Prism Plot allows precise
4 determination of percent of cells with phenotype,
5 which is consistent in this case with CLL, but one
6 can use it for any type of phenotype.

7 I will briefly describe the principle of
8 Prism Plot analysis. This is an algorithm which
9 allows summarization of multi-parametric data on a
10 one-dimensional Prism Plot, and depending on the
11 number of dividers will represent all possible
12 combinations of measured markers. Therefore, if
13 you have four-color or five-color, you can set up
14 specific immunophenotypes, of course supervising
15 gating strategy to come up with a specific
16 immunophenotype.

17 The dividers are set up based on a single
18 color plot or dual parameter plot, which creates
19 two dividers. And this is a scheme which
20 represents the principle of Prism Plot analysis.
21 After setting up your dividers, you can set up
22 hierarchy of these dividers, which clear the

1 specific subset of cells. And the combination of
2 dividers are combined to produce 2 to the P region,
3 where the P is the number of Prism Plot dividers.

4 This table represents the number of
5 phenotypes, which can be created by setting up
6 specific dividers. And this is an example of Prism
7 Plot analysis of cells with immunophenotype
8 informative, immunophenotype representing CLL MRD,
9 cells which are CD5 positive, CD19 positive, CD43
10 positive, and in this case, CD79b negative.

11 This is a very useful tool. It alleviates
12 the need for sequential gating because if you set
13 up your gating on lymphocytes based on CD45 and
14 side scatter, and you set up your regions in a
15 lymphocyte population, this always gives you a
16 reproducible immunophenotype.

17 Now, I will describe our approach to CLL MRD
18 panels using a backbone of CD5, CD19, and CD45. It
19 is comprised of four tubes. The first contains
20 informative antigens kappa and lambda. The second
21 contains CD43 in the context of 79b, cert 22 and
22 81, and last, 38 and 20. So as you can see, we

1 basically are transplanting on this panel to five-
2 color panels.

3 This is our tube number one. We changed a
4 little bit the colors around so it would suit best
5 our need, of our approach. And you can see we have
6 a backbone of 5, 19, and 45 and polyclonal cocktail
7 from Dako. We use polyclonal instead of monoclonal
8 antibodies to look for a large end restriction to
9 avoid problems with possible lack of detection of
10 restricted immunoglobulin by monoclonal antibody.

11 This panel shows an example of the residual
12 MRD in the background of recovering polyclonal
13 B-cells. As you can see, if we subgate on a
14 population of 95 cells, these cells end up in a
15 lambda-dim region if we look on CD19, total kappa
16 versus lambda. However, when we are asking what
17 light chain is expressed by 19 to 5 positive cells,
18 we can see the predominance of lambda over kappa.

19 This is represented here, where cells with
20 expression of 19-5 lambda represent 0.4 percent of
21 total lymphocytes and cells expressing 19-5 lambda
22 negative and kappa are basically zero. However, as

1 was mentioned before, the immature, especially in
2 recovering bone marrow post-therapy, as well as B1A
3 cells, often express a dim level of light chain.
4 Therefore, unless we found a discreet population of
5 such a cell, we proceed to the next tube to further
6 evaluate for MRD. Again, a backbone is comprised
7 of 19, 5, and 45. Informative antibodies here are
8 CD43 and 79b.

9 This is representative plots, informative
10 plots, where we see 19-5 cells, which are, as
11 expected, 43 positive and are 79 dim to negative.
12 Therefore, informative immunophenotype is obtained
13 by 19-5, 43 positive, 79b negative to dim. And if
14 we go to a Prism Plot analysis, the population of
15 cells with this informative immunophenotype
16 accounts for 0.4 percent of cells, so as much as
17 with the lambda-restricted 19-5 positive cells in
18 this case.

19 Now, this is our tube number 3. Again, the
20 backbone is the same, CD5, CD19, and CD45.
21 Informative antibodies in this case are CD81b and
22 CD22 in the FITC.

1 If we subgate on cells which are 19-5
2 positive, we can clearly see that this population
3 of cells is very dim to negative 481 and dim to
4 negative 422. Therefore, these cells represent
5 informative immunophenotype CD5, 19, 22 dim, and 81
6 dim to negative. And again, using a Prism Plot
7 analysis, we can see that cells with the most
8 restrictive immunophenotype, in this case 19-5
9 positive, 22, and 81 negative, represent .7 percent
10 of population of these cells.

11 The last tube of this panel is a tube with
12 informative antibodies CD20, 38. Analysis of cells
13 in this panel show that cells which are 19-5
14 positive are 38 negative to dim and 20 negative.
15 In this case, the patient probably was treated with
16 rixutimab because CLL cells are completely negative
17 for CD20. Again, the Prism Plot shows, as a
18 population of these cells, it represents .7 percent
19 of total lymphocytes, which can then be calculated
20 to total leukocytes.

21 The conclusion for this part of the
22 presentation is that a modified CLL MRD panel,

1 based on Andy Rawstron's 2007 paper, allows
2 reproducible and sensitive detection of CLL MRD in
3 peripheral blood and bone marrow samples. This
4 method is compatible with automated stain against
5 the sample processing method using an immuno-prep
6 reagent.

7 The addition of CD45 in site-scatter gating
8 allows a lymphoid separation for most leukocytes
9 and alleviates need for sequential gating because
10 we can use a Prism Plot to exactly enumerate cells
11 with the characteristic immunophenotype.

12 These four panels represent the situation of
13 CLL, residual CLL, in a patient who was treated
14 with humanized anti-CD19 antibody. As you can see,
15 with the CD5-positive cells, which are also CD3
16 negative, are clearly -- you have two choices, that
17 those are abnormal T-cells, which lost CD3 or that
18 they are CLL cells, which have lost CD19. And
19 clearly, these cells are negative for CD19.

20 However, when we add CD24, it identifies the cells
21 as the B cells apparently expressing CD5. And for
22 confirmation, CD24 cells are mostly negative for

1 CD19.

2 Based on a large number of samples from this
3 study, we observed that CD19 is effectively blocked
4 for weeks following the last dose of a CD19
5 antibody. We evaluated several CD24 and CD20
6 clones to find the best surrogate marker for CLL
7 detection. And one particular clone, ALB9, in our
8 case conjugated to Psi 5 (phonetic) was optimal for
9 our purpose.

10 This is a table representing our CLL MRD was
11 at 24 as a gating antibody. We're obliged to
12 switch CD5 and CD24. Therefore, a panel will be
13 comprised of a backbone of 5, 24, and 45 for gating
14 and other informative antibodies will be kept the
15 same.

16 This is an example of panel where CD19 was
17 blocked, but 24 effectively shows us the number of
18 CD5 24 positive cells, which are, as expected, 43
19 positive and 17ab negative, consistent with CLL.

20 This is the second tube, where again, the
21 informative antibodies are 81 and 22, and they are
22 ran against the backbone of 5, 24, and 45.

1 This panel, 5 24-positive cells are dim 481
2 and they are dim to negative for 22, rendering
3 informative phenotype, consistent with CLL MRD in
4 patients treated with CD19. The last tube in this
5 panel is a tube with CD20 and CD38. In this panel,
6 CD24, and CD5-positive cells are negative for CD38
7 and dim to negative for CD20, again consistent with
8 CLL MRD.

9 Before we use it for clinical study, we
10 evaluated the regular cases coming to our lab with
11 this particular CD24, not doing an extensive panel,
12 just comparing the number of CLL cells using CD19
13 versus CD24.

14 As you can see, with the exception of three
15 cases, which from the beginning were very dim for
16 24 and general agreement between CD19 and CD24, as
17 this particular clone was pretty good. We
18 evaluated two other 24 clones which were more
19 wobbly, all over the place in terms of level of
20 expression, and CD27, which didn't work at all in
21 our hands.

22 The conclusion from this part of the

1 presentation is that CD24 antigen represents a good
2 alternative to CD19 as a Pan B-cell marker in CLL
3 MRD detection, and CD24 can be used to measure CLL
4 MRD by flow analysis in patients treated with CD19,
5 and that this combination is also useful when using
6 automatic staining and cell processing, using an
7 alternative method recommended by the equipment
8 manufacturer.

9 This is the clinical flow laboratory crew,
10 without which this work would be impossible. I
11 wish to extend the acknowledgment to Dr. John Byrd
12 and his CLL team at OSU, which supplies us with
13 most of the clinical samples and engages us in
14 clinical studies, and of course, CLL patients
15 treated as OSU.

16 May I have one more minute?

17 DR. WIERDA: Sure.

18 DR. LOZANSKI: I just wish to mention, we
19 will try to develop 10-color CLL MRD panel. We're
20 updating our instruments currently to 10-color
21 right now. Right now, we are just evaluating
22 panels. We are running them side by side. The

1 problems which we encounter using our setup is that
2 PrepPlus cannot pipette for 10 separate colors,
3 therefore it forces us to create our own cocktails
4 because we need to reduce the number of antibodies,
5 which PrepPlus needs to pipette.

6 Another issue is that we don't have big
7 experience with many kinds of dyes, which we are
8 using in this setting. We are very rigorously
9 evaluating and analyzing the stability of stained
10 and processed samples for how good they behave if
11 the sample is not run the right way, because
12 there's a clinical upsetting.

13 Often, one sets up sample and will sit in
14 the refrigerator for a couple hours before it is
15 run. And it's very important that after staining
16 and processing the sample, it's stable for at least
17 several hours.

18 This is our proposed panel, which will take
19 full advantage like Dr. Rawstron mentioned. It's
20 got a number of cells, total number of cells, which
21 are required to analyze or total number of minutes
22 needed to acquire an adequate number of events to

1 be confident about CLL MRD. And it also brings a
2 considerable saving in terms of reagents because,
3 as you saw before, we used four times the same
4 backbone of antibodies. Cutting it down to a
5 single tube will allow compression and will lower
6 the cost of evaluation.

7 Thank you very much.

8 (Applause.)

9 DR. WIERDA: Thank you. Questions,
10 comments?

11 (No response.)

12 DR. WIERDA: Maybe you could clarify for me,
13 sir, I'm just sitting here thinking about reagents
14 and clones. Is everybody getting their reagents
15 from the same manufacturer? Are there differences
16 in clones, for example, monoclonal antibody clones
17 against CD5 or some of these other markers? And
18 can you make a comment on that, please?

19 DR. LOZANSKI: Yes. I think that this is a
20 very important question. It's why I mentioned in
21 my terrible description of our panel exact clones,
22 exact colors, and the manufacturers, and amount of

1 antibody added per action, because I think even it
2 sounds boring and is an integrated technical
3 problem. But it's very important to probably
4 choose the most effective clone for such purpose.
5 And the paper from 2007 described by Andy was
6 paying also attention to specific clones and weigh
7 how you process and how you stain your cells.

8 Because we are a Beckman Coulter lab, we're
9 using reagents which are compatible with our
10 platform. And I think that, in fact, we had an
11 opportunity to participate in this 2007 work. And
12 even when we used different clones, our outcome was
13 similar to what was published in this paper.

14 So I think that there are many ways to skin
15 the cat, but important is that everyone runs
16 rigorous evaluation. And there is a definite need
17 for a quality assurance program, which will
18 distribute just like CAP does not for evaluation of
19 CD4, CD8 counts, or stem-cell evaluation samples
20 with unknown level of CLL MRD. And labs,
21 independent, and whatever platforms they use, will
22 be compared to their peers, and it will be

1 objectively evaluated as falling within the
2 required level of detection.

3 DR. RAWSTRON: Just to answer that for the
4 current project, we're developing specifications
5 for each of the antibodies, so hopefully that will
6 be supplier-independent. They can say, does their
7 clone meet these specifications? If so, it's
8 suitable for inclusion.

9 DR. WIERDA: Other questions, comments?

10 (No response.)

11 DR. WIERDA: Okay. So we'll move on.

12 DR. BYRD: I think one of the things that's
13 just really notable through all the presentations
14 is how reproducible the numbers are, and less
15 minimal residual disease is clearly making an
16 impact. And Europe, and several sites in the U.S.,
17 that you've really been able to come up with a
18 reproducible assay that a lot of labs can do and
19 get the same answer.

20 So I think, looking at a surrogate endpoint,
21 it's really nice to have something that a lot of
22 places are going to be able to do, and it's going

1 to be able to be reproduced.

2 DR. WIERDA: Our next speaker is Maryalice
3 Stetler-Stevenson from the National Cancer
4 Institute, and she'll be showing us some data
5 related to the NCI work.

6 **Presentation - Maryalice Stetler-Stevenson**

7 DR. STETLER-STEVENSON: So I'm going to
8 start out with a sort of historical perspective for
9 us, and where we started, and where we ended up.
10 And there will be a lot of similarities with the
11 talks. And I'd like to also go into some technical
12 aspects that have not yet been discussed, because
13 one thing people should take away from this group
14 meeting is that the technical aspects are very
15 important. And you have to evaluate -- if your
16 laboratory is performing MRD studies for you, you
17 have to evaluate how they do that.

18 When you review papers, the methodology
19 section should contain detailed flow cytometric
20 methodology because, otherwise, you don't know if
21 they're doing a very good job or not. They may be
22 providing you with a number that has nothing to do

1 with other studies. So this is important.

2 So our basic approach to looking for MRD in
3 CLL is the basic approach we used in all mature
4 B-cell leukemia lymphomas; and that is that they
5 have aberrant antigen expression. And in CLL, not
6 only do you have CD5, but you also have dim CD20,
7 dim CD22, dim CD79b, frequently dim CD45, dim CD81,
8 partial dim CD11C, and CD 43 positivity. And the
9 second thing that we've always looked at is that
10 mature B-cell leukemias and lymphomas are clonal,
11 and, therefore, they have light chain restriction.

12 So based on that, the technical aspects that
13 we've always done is, we initially started out
14 acquiring only about 200,000 to 300,000 events and
15 realized that was not enough. We now acquire a
16 million events per two for these assays.

17 Another thing we noted was, you need to wash
18 between your MRD tubes because there's carryover
19 from tube to tube, and this can create false MRD.
20 And we focus on the B-cells by using some gating,
21 but we do not do extensive gating, sequential
22 gating, without looking at all the data together.

1 We use screening panels that include
2 cocktails that are specific for multiple diseases
3 to screen. And then we're able to follow them. We
4 use repeat panels with cocktails that are specific
5 for the disease and the therapy that the patient is
6 receiving. Again, you got to wash between the
7 tubes when acquiring.

8 When you look for an abnormal antigen
9 pattern, not just an automatic gating, each patient
10 may have slightly different abnormalities, but the
11 abnormal pattern will be present. And we like to
12 demonstrate light chain restriction in aberrant
13 B-cells. And we've used this successfully in
14 hairy-cell CLL, follicular lymphoma, and diffuse
15 large B-cell lymphoma.

16 So when we first started out, this is our
17 four-color panel. And we had one that was looking
18 at markers FMC7, CD79b, 19, and 23, and it was not
19 very useful. We also had a tube with kappa/lambda
20 19 to 5, and we had kappa and lambda in separate
21 tubes, looking at CD20 and CD22 with CD5. And this
22 was very useful because we could see abnormal

1 patterns of CD20 and CD22 in addition to CD5
2 co-expression.

3 We could achieve a level of 10 to the 4th or
4 .01 sensitivity with this, but with some variance,
5 it was difficult to pick it up. And we wanted to
6 try to improve our results.

7 With our eight-color panel -- I guess I
8 should briefly say that we had an intermediate six-
9 color panel that we patterned after the ERIC six-
10 color panel. And we validated that panel and were
11 able to detect down between 10 to the 4th and 10 to
12 the 5th sensitivity.

13 We do this with serial dilutions, and we
14 also did it with a blinded serial dilution. So we
15 were able to demonstrate that this worked, but we
16 always included kappa/lambda analysis. We did our
17 validation of our eight-color panel in comparison
18 to our six-color panel.

19 So we look at the combination of various
20 antigens that are expressed at abnormal levels, the
21 CD81, 79b, CD22, CD19, CD43, CD20, CD5, and then
22 CD3 to be able to discriminate T-cells. And we

1 also look at kappa/lambda with a backbone of CD19,
2 which will pick up all of the B-cells, and CD5, and
3 CD45, and CD38, because CD45 and CD38 are helpful.
4 In bone marrow, CD38 can indicate progenitor cells,
5 and it's also expressed at abnormal levels usually
6 in CLL cells. And CD45 is frequently abnormally
7 expressed.

8 But we also have a tube where we have kappa
9 and lambda in a single tube, and we have 20, and
10 22, along with 19 and 5, and CD11C, and CD45. And
11 with this, we're able to achieve reliable
12 sensitivities about 10 to the 4th, 10 to the 5th.
13 And it depends on the number of cells that we're
14 able to acquire.

15 So our basic approach is that we do not do
16 an automatic gating or bullying gating. We
17 basically look at our leukocytes. When we look at
18 CD19 and CD5, we identified B-cells. And we will
19 look at all of the data. And then when we're sure
20 that we have a gate that includes all the B-cells,
21 we'll also further interrogate just the B-cells.

22 So you see at the top, we have -- for

1 example, .14 percent of the leukocytes as CD19
2 positive, CD5 positive. And in the other one, the
3 .36 percent of the lymphocytes are CD19 positive,
4 CD5 positive. And when we look just at the
5 CD19-positive cells, you can see that we have all
6 these CD19-positive -- I don't know if I can reach
7 it from here -- CD38-negative, which is an abnormal
8 finding in peripheral blood, and furthermore that
9 they are kappa restricted, kappa positive and
10 lambda negative. So this is actually a lot of
11 minimal residual disease, so it's pretty easy.

12 When we go down to a lower level MRD, where
13 .01 percent of the leukocytes are CD19 positive,
14 CD5 positive, and you're looking at all of the
15 leukocytes, you can see that there are a few cells
16 that are 19 positive, 5 positive. And when we go
17 on to do CD19, restricted to further interrogate
18 those cells that are 19 positive, CD5 positive, you
19 can see that they're CD38 negative. They're
20 restricted to kappa, kappa positive, lambda
21 negative. They have a dim level of CD81
22 expression.

1 They're negative here for CD20. This is
2 post-treatment. But they're positive for CD22. So
3 actually, we can use this when there is still CD20
4 negativity because we have additional markers
5 present that allow us to detect abnormal
6 populations. So we are able to detect this quite
7 easily.

8 That was basically when all the B-cells
9 present were pretty much MRD. When you have
10 polyclonal B-cells, you have to use additional
11 methods to be able to tease out what are the CLL
12 cells and what are not. The B-cells in the first
13 panel, you can see, are purple. We're looking at
14 all the lymphocytes. And you can see that we have
15 the T-cells and we have the B-cells. And it's hard
16 to tell if we have CD19-, CD5-positive cells. This
17 is just looking at all the cells.

18 If we go to a lymphocyte gate, it's a little
19 bit more evident. And when we go and gate on the
20 CD19-positive cells, it's easier to see that we do
21 have, enclosed in the red circle, some
22 CD19-positive, CD5-positive cells. But a majority

1 of-the B-cells are CD5 negative.

2 When we look at those CD19-positive,
3 CD5-positive cells, they are all kappa positive and
4 lambda negative. So that's fairly easy to do. We
5 can also do this, however, with looking at CD22 and
6 looking at gating in CD22 cells. In looking here,
7 if we look at CD20, we can see that not only do we
8 have CD20-positive, CD5-positive cells, but we have
9 CD20 dim, which is abnormal CD5-positive cells
10 contained in that red box.

11 These cells, in this case, they're CD20
12 positive, but dim, and they're monoclonal for kappa
13 if you look at kappa versus lambda. So we're
14 looking for abnormal populations, CD19 positive,
15 CD5 positive. If polyclonal B-cells are present,
16 we can still manage to work around them. Usually,
17 they're not CD5 positive.

18 In the case below, there appears to be some
19 CD5 positivity falling into that gate, but we're
20 looking at the abnormal CD20. We can also look at
21 it normally, dim CD22, and dim CD45 to get a pure
22 gate on those cells that are CLL MRD and determine

1 further that they are monoclonal.

2 Now, I haven't gone into our use of the
3 panel that Andy Rawstron demonstrated because he
4 demonstrates it best. But we also do the same type
5 of analysis that he does alongside of this. So
6 basically, we're doing two different methods, and
7 we have found that they compare extremely well.

8 There has been just one case recently that
9 we seem to have discordant findings, and I'm going
10 to send it to him and figure out why there's a
11 difference. We'll figure out what it is. But I
12 used two methods to come to the same values.

13 So we've been using this for a long time in
14 our transplant patients, which was the CLL patients
15 that we initially had. And we've been able to
16 demonstrate in the transplant setting, we have
17 gotten to very high sensitivity. And we're able to
18 follow these patients, not just validating our
19 methodology through doing serial dilutions and
20 blinded serial dilutions, but the best validation
21 is to see what happens. And if these cells
22 continue to rise, eventually, the patient has a

1 recurrence and you have validated that the system
2 works. That's one method of validation.

3 We've also looked at patients who have had
4 anti-CD20 therapy of various types, and we've been
5 able to get some very sensitive results. It's the
6 number of cells that we acquire, and being very
7 stringent about washing in between, eliminating any
8 cross-contamination, and our analysis being rather
9 intense.

10 The main thing I want to point -- I want to
11 talk about in technical aspects in your reviewing
12 papers, and you're thinking about somebody who's
13 doing your analysis, the number of cells acquired
14 in MRD is vital. And this is not something that
15 everybody thinks about. But if we have .14 percent
16 of the leukocytes that are CLL MRD, and we have 1
17 million events acquired, it's obvious. It's a huge
18 population.

19 If we have 500,000 events, it's pretty
20 obvious. And it's harder when you get down to
21 50,000 events to determine this. And many
22 laboratories, certainly many reference

1 laboratories, will routinely do 20,000 events,
2 which is lower than this, so they would not be able
3 to detect what is a huge residual population. And
4 when you get down to a very small number, of course
5 with 1 million events, it's still obvious. We can
6 achieve at least 20 to 50 cells, which is your bare
7 minimum.

8 But at 500,000 events, now we're starting to
9 get down to a lower level of detection and, at
10 50,000 events, you can't detect it at all. So the
11 number of cells acquired is extremely important.
12 And if you go from 1 million to 2 million or
13 3 million events, you're going to increase your
14 sensitivity. But it takes more time and,
15 therefore, it's more expensive.

16 So conclusions from our studies, basically,
17 technical aspects are very important. The number
18 of events acquired, washing between MRD tubes,
19 these are the two most important things for
20 achieving high sensitivity, and reproducibility,
21 and high specificity.

22 We focus at looking at B-cells not through

1 looking at one marker, like just a CD19 backbone,
2 but we look at CD19, CD20, and CD22. And this is
3 important, since we not always aware that the
4 patients received rixutimab, for example, before we
5 get the specimen in the laboratory.

6 We identify antigen abnormalities. We
7 especially focus on first looking at CD5
8 positivity. However, CD5 negativity can also be
9 observed, and you will see aberrant CD20, aberrant
10 CD22, CD38 negative, or also aberrant CD79, CD81,
11 all of these things. So you have to identify that.

12 Then we like to confirm with a second test
13 with looking at monoclonality, and we don't look at
14 kappa/lambda ratios or just CD5-positive B-cells
15 kappa/lambda, but we look for aberrant antigen
16 patterns, and then confirm monoclonality. So it's
17 not a ratio. It's completely kappa or lambda light
18 chain restricted. And with this, we've been able
19 to get fairly sensitive and pretty specific
20 results.

21 One thing I would like to say, too, in
22 conclusion, I think that the panel that's been

1 developed by the ERIC protocol, by Andy Rawstron,
2 is an extremely robust panel that's been used in
3 multiple laboratories with good results.

4 People add additional things on, but I think
5 that one thing to consider is to have that a part
6 of CLL MRD, period, with the choice to add on
7 additional tubes that will answer the
8 investigator's questions or perhaps -- well,
9 personal. We all have our own personal favorites
10 and, in many ways, to skin a cat and still get the
11 same result, a cat without fur. But I would highly
12 recommend proceeding with having the tube that Andy
13 has developed as a backbone of any CLL MRD study.

14 DR. WIERDA: Thank you.

15 (Applause.)

16 **Clarifying Questions**

17 DR. WIERDA: Questions, comments? You kind
18 of addressed my question, which is, it sounds like
19 everybody agrees that the stuff that ERIC has been
20 doing is reproducible. Everybody does a little bit
21 different in terms of what their standard is. So
22 my question is, how do you conceptualize having a

1 technically harmonized assay? What is a harmonized
2 assay and process to you?

3 DR. STETLER-STEVENSON: So I think that
4 harmonization or standardization, I think that we
5 could standardize part of CLL MRD. And
6 harmonization would involve that there would be
7 additional studies that individual laboratories
8 would perform and could, perhaps, lead to
9 indications that they get more robust answers or
10 not. These need to be studied.

11 But I think that we could standardize part
12 of the panel, and harmonization would involve
13 having to have different platforms with different
14 fluorochromes, but the same basic panel. If you
15 can only do four-color, then you have to do a four-
16 color version; if you can do a six-color version or
17 a five-color version. And if you're doing 10-color
18 and you can validate against the eight-color panel,
19 if you want to add additional antibodies on, that's
20 also good.

21 So harmonization would be different
22 platforms, perhaps different fluorochromes. But I

1 think we have, really, an excellent, well-working
2 backbone.

3 DR. WOOD: In a similar vein, we've heard
4 today about at least five different variations of
5 assays designed to do CLL MRD detection. It's
6 clear to me at least that the ERIC assay they've
7 generated is by far the best validated assay in
8 terms of the number of samples validated, the
9 technical validation that's gone into it, and the
10 correlation of outcome data.

11 So it seems like this would serve as a
12 suitable kind of reference assay for this type of
13 work, at least at the present time. So I guess my
14 question is to the other people who have developed
15 assays. Have you tried to validate your assays
16 versus this type of reference method? And do you
17 know exactly how these compare?

18 I think this will be a very important point
19 going forward when one is conducting clinical
20 trials, particularly at multi-site centers.

21 DR. BOTTCHEER: If I could add a discussion
22 on behalf of EuroFlow, we do a standardized

1 instrument setup, so we are really set to
2 standardize even all the staining procedures, as
3 mentioned. And we will obviously validate -- and
4 have not done this on a large number of samples
5 against the ERIC consortium developed in
6 particular, because we currently use the ERIC
7 consortium panel in our lab, and we want to make
8 sure that we will be reproducible over time once we
9 introduce EuroFlow's color panel.

10 I think one thing about EuroFlow might be
11 that it's easy to transfer to different labs
12 because it always gives you the same MFI values,
13 regardless of which cytometer you are going to use.

14 DR. WIERDA: Yes.

15 DR. JORGENSEN: Sorry to get into the weeds
16 here, but this is Jeff Jorgensen at MD Anderson.
17 And can the three panelists comment on doublet
18 discrimination, whether they are pro or against,
19 using doublet discrimination to eliminate
20 aggregates and so forth?

21 DR. STETLER-STEVENSON: So we do doublet
22 discrimination as part of every analysis. And I

1 guess getting to how does one validate, I believe
2 the ERIC panel, or the tube, or the backbone has
3 been validated a number of different laboratories
4 with different instruments and somewhat different
5 antibodies.

6 It's not just the specific antibody. It's
7 more the approach. It's the idea that these
8 combinations reveal abnormal cells that are CLL
9 cells, and that it can be done in a robust manner.
10 So part of it is the intellectual approach. It has
11 to be done well. And, of course, whenever you
12 tried to standardize and disseminate any test -- we
13 just had a two-day discussion with FDA about
14 standardization in leukemia lymphoma, flow
15 cytometry in leukemia lymphoma.

16 When you have a poor-performing lab, they
17 will do a poor test. And there is nothing that you
18 can do to prevent a poor-performing lab from doing
19 a poor test. The main thing is for the clinician
20 to be an educated consumer. And the more education
21 people have as to what is required to do this test
22 well, then the better the result will be, and to

1 put into place, as Gerard said, proficiency testing
2 to have that available in this country.

3 I know that UK NEQAS is developing this for
4 the U.K. We need to do it here as well. We don't
5 have any. CAP does not provide this, really. So
6 have proficiency testing. And at this point,
7 there's no penalty for doing things poorly. Even
8 in CAP proficiency testing. They provide a test.
9 They sell a test. Then they come around and they
10 inspect you based on the test results that they
11 wanted you to pay for, which they make money from.
12 And they don't fail you on the test. They just say
13 you don't fall in consensus. And when they inspect
14 you for how well you perform, they don't penalize
15 you because you failed the test that they want you
16 to buy. It's sort of a commercial-interest issue.

17 So there are a lot of things to be
18 developed, but right now, we're talking about
19 biomarkers in clinical trials. One would hope that
20 you're not doing this in a little hospital out in,
21 I don't know, Alaska, wherever, where they get a
22 few per month. You're talking about good clinical

1 laboratories, and you're talking about clinicians
2 who know how to evaluate performance.

3 DR. BARNETT: Can I just respond to
4 Maryalice, three points? First of all, UK NEQAS is
5 available in this country. Anybody from this
6 country can join the UK NEQAS, but CMS has to
7 approve that's it's available in this country.

8 Secondly, I think your slide, which showed
9 the variability in the number of events collected,
10 all the way down, was actually well documented by a
11 robust limited CD34 analysis, showing the square
12 root of the number that you count, increases the
13 variability. So on the 50,000 events, you're
14 actually going to get close to 50 percent
15 variability on your absolute percentage values if
16 you're only counting 50,000. It's at no point not
17 1 percent.

18 Lastly, I'd like to say, I agree with Brent
19 that the data I'd like to see is the ERIC protocol
20 versus everybody else and how those fare given
21 that. And so far, I don't think that's been
22 presented.

1 DR. DE CLARO: Sir, could you identify
2 yourself for the record? Thank you.

3 DR. BARNETT: Sorry. David Barnett,
4 UK NEQAS, Sheffield, England.

5 DR. WIERDA: Other questions? Dr. Marti?

6 DR. MARTI: I want to follow up on this
7 comment about doublet discrimination. I don't
8 think that should be confused with what Andy
9 described in his CD3, CD19, eliminating those
10 cells. Those are not doublets. Those are cells
11 that are expressing both reagents. That's
12 different than a doublet in my mind. They could be
13 doublets, but I think it's some special case of
14 doublet discrimination.

15 Also, I was curious to know, they don't use
16 CD45 in Barcelona, in the two panels that
17 Dr. Montserrat showed this morning. Do they use
18 CD45 in the UK? And we do use it in Bethesda.

19 DR. RAWSTRON: I think, in both Barcelona
20 and Leeds, the 45 is there for enumerating B-cells
21 as a proportion of leukocytes. It's not in the MRD
22 component. It's in the basic assessment. In terms

1 of doublets, there's this background contamination,
2 which is one thing, and then there's a doublet
3 discriminator people are using flow cytometry to
4 stop counting T-cells as one as they go through.

5 We don't use it routinely. It probably
6 accounts for maybe up to a small fee percent of
7 error on your result, which when we're looking at
8 log errors as being significant, I don't think it
9 would make too much difference. But just for the
10 record, we don't do it routinely.

11 DR. WIERDA: Other questions?

12 DR. DE CLARO: This is a question for
13 Dr. Kay and Dr. Hillmen. Regarding the phase 3
14 trials that you had described earlier, wherein MRD
15 assessments were one of the endpoints being
16 assessed, could you comment on the number of labs
17 that are doing the assessments for MRD in those
18 phase 3 trials?

19 DR. KAY: So we are doing a central
20 laboratory, which we anticipate that all specimens
21 will come to the flow cytometry lab at Mayo Clinic.
22 And that flow cytometric assay is the one that I

1 described in rather crude terms. I apologize for
2 that, but Curt Hanson wasn't here. But that's the
3 one we intend to use.

4 Having heard the dialogue today, I'm
5 wondering if we shouldn't perhaps think about one
6 other central lab that might be a validation as
7 well, but our plan is to have one laboratory in an
8 academic center that is well-used, receiving
9 specimens.

10 DR. BYRD: So that's true for the ECOG
11 study, the Alliance. So both studies are going to
12 have central labs, because Dr. Lozanski's lab will
13 be doing the flow cytometry centrally for the
14 Alliance study.

15 DR. HILLMEN: Yes. We have used a single
16 central lab, our own laboratory, for the MRD
17 assessments in the clinical trials in the U.K. thus
18 far for the last 10 years, and they will continue
19 to do so in these studies as well.

20 I think the points were made, though, that
21 we have a robust assay, which, if we're going to
22 have a surrogate endpoint for approvals, is robust.

1 Now, it's a different question about them
2 using that in other places. We have the NEQAS
3 scheme, which we've used in similar assays, which
4 is very effective. And so part of it is getting
5 the right platform and making sure that all the
6 different approaches are as good as the standard,
7 but secondly, to make sure that the labs that are
8 doing it can actually do the tests with quality
9 assurance, and that's key.

10 DR. WIERDA: Yes?

11 ENRIQUE: Enrique (indiscernible). There's
12 another dimension about MRD, which is disease
13 relapse. Obviously, in CLL chronic disease, it has
14 a different dimension in time. However, the
15 patient disease recurs, and I wonder if somebody
16 can comment on any experience on disease relapse in
17 MRD related with disease recurrence, treatment
18 failure, or even disease reservoir.

19 DR. WIERDA: Who wants to tackle that one?

20 DR. HILLMEN: We showed some data on the
21 relapse of patients, both outside trials and within
22 trials. Our experience of MRD relapse is that

1 patients, apart from the allogeneic transplant
2 setting, when people are opposite MRD level, they
3 naturally increase the level of disease, which is
4 probably individual to the patient. But as Seb
5 showed previously, probably it stays the same or
6 similar for a patient until the patient relapses.

7 I didn't have the 10 to 207s today. We did
8 have within the protocol reinduction of MRD
9 negativity. So a patient who is consolidated with
10 CAM path may remain in MRD remission for over six
11 months could be re-consolidated three or four years
12 later to MRD negativity. And only a small
13 proportion of patients did that, but that was an
14 effective strategy.

15 So I think, at the moment, it's too early.
16 We haven't had trials that have looked at MRD
17 relapse and treating patients at that point, but I
18 guess that they will be coming.

19 DR. WIERDA: Emilio?

20 DR. MONTSERRAT: In line with that, maybe
21 this is a little bit what comes from the floor. I
22 think that we could elaborate a little bit more on

1 the comment by saying that MRD positivity or MRD
2 relapse is not a criterion, I mean, to treat the
3 patient. I think this is a very important mistake,
4 I mean, not only MRD relapse but also clinical
5 relapse.

6 When the patient relapses, unless the
7 patient has symptoms because of the disease, no
8 further therapy is needed unless this is done
9 within clinical trials.

10 DR. WIERDA: Dr. Lozanski?

11 DR. LOZANSKI: Gerard Lozanski from OSU. I
12 wish to comment just on being a hematopathologist.
13 We collect a lot of tissues from people treated in
14 our institutions, so we have several cases which
15 were MRD negative for a prolonged time in bone
16 marrow and in peripheral blood. However, a patient
17 for example has an acute gall bladder and was
18 removed. Then the GI person will bring it to us
19 and say, "Hey, this doesn't look normal." We stain
20 it using IHC, and it was clearly, gall bladder was
21 involved by SLL, or in another case, lymph nodes,
22 which were removed on occasion of removal of

1 thyroid nodule, and lymph node was involved.

2 We look two years back, and this patient has
3 every six months done CLL MRD on peripheral blood,
4 and it was completely negative. On one occasion,
5 the patient didn't have at all B-cells in
6 peripheral blood and no B-cells in the bone marrow.
7 It was a transplant patient. But he had, tissue
8 removed for different purposes, clearly involvement
9 of lymph node by SLL.

10 So the fact that MRD predicts outcome is not
11 equal that the disease is gone. The disease is
12 probably still there, but just on a level which is
13 not affecting the patient.

14 DR. HILLMEN: I mean, I agree that that's a
15 true input. If it was an ordinary transplant, that
16 is a different context. I mean, you cannot -- I
17 think everyone said that if ordinary transplant,
18 MRD behaves in a different way, opposed to allo,
19 you see compartment effects. You see a reinduction
20 of MRD-negative remissions with lymphocytes, et
21 cetera.

22 So there's always exceptions, but I don't

1 think we see many patients who are MRD-negative
2 prolonged and then have a lot of disease elsewhere
3 with CLL. That's not a common finding.

4 DR. MONTSERRAT: Yes. Again, to follow up,
5 those cases that you have mentioned do exist, but
6 they are extremely rare. In general, if there is
7 evidence of the disease, I mean, you can detect the
8 disease in blood. In the case of CLL, relapse is
9 related to lymph nodes, to other areas. It's a
10 phenomenon which is extremely infrequent.

11 DR. STETLER-STEVENSON: One thing about MRD
12 is, it's not just positive or negative. And
13 especially taking from the transplant setting, you
14 can have MRD positivity that slowly declines over
15 time, and it's an excellent prognostic sign. And
16 you can have patients who go to an MRD-negative
17 level, but then if you're following transplant
18 every six months, the patients every six months
19 over a five-year period, when it starts to
20 reappear, it's a bad prognostic sign.

21 As you move towards new novel therapies
22 where you may be treating patients over an extended

1 period of time, not just positive or negative, but
2 the numbers may be very, very important, and that
3 may be prognostically important.

4 DR. WIERDA: Tom, did you have a comment?

5 DR. KIPPS: Yes. I hope we can get away
6 from these anecdotes and try to come up with an
7 idea of what is the standard criteria for MRD
8 assessment. We have a problem of having different
9 panels being proposed. We mentioned the issue of
10 different levels of sensitivity being a cutoff. I
11 think it would be worthwhile for us to come to a
12 consensus.

13 I really think that you can maybe achieve
14 adequate detection with different panels. This may
15 have to be verified through sample sharing, like
16 has been done. Andy spearheaded the international
17 movement to actually look at this in different
18 laboratories. I think that effort could be done
19 again.

20 I think the point that was made earlier
21 about the laboratory technique and good labs have
22 to be involved, it's obvious. I think this is very

1 sensitive, but also time-consuming and meticulous
2 test that requires attention to detail. That's
3 clear.

4 So I would hope that we could maybe set the
5 standard, and then what anecdotes or changes in MRD
6 pattern may happen with and without disease will
7 come after we've adopted some form of criteria that
8 will allow us to go forward from this point with
9 MRD as being a useful surrogate marker, perhaps for
10 the assessment of treatment-free survival.

11 DR. WIERDA: Other comments? Go ahead.

12 DR. STETLER-STEVENSON: I'd like to follow
13 up on that in that there is a problem when you're
14 talking about different panels and harmonization,
15 in that everybody likes to do their own favorite
16 thing. And in moving forward, I think we're going
17 to have to reach consensus on certain items. And
18 probably everybody will be a little unhappy, but
19 overall the patients will be better served.

20 So moving to partial standardization with
21 some harmonization may be an approach. And
22 certainly, I think that technical recommendations

1 can be drawn up.

2 DR. WIERDA: Can you introduce yourself,
3 please?

4 DR. KHOURI: In MRD assessment, time removed
5 from the end of treatment and you pick up a clone,
6 is this always the primary clone or do you ever
7 find secondary clones?

8 DR. RAWSTRON: I suspect we have found some
9 secondary clones, but that's such at a level that
10 it's not possible to prove it yet. But that's
11 going to be an issue in the future, presumably, but
12 so far, the vast majority of patients, once a clone
13 appears, it progresses with a doubling exponential
14 rate. When you get the phenotype and the sequence
15 again, it's the same clone, so far.

16 DR. GHIA: If I may say something. Going
17 back to the technical issue, again, I don't think
18 it's a major goal of this workshop, because we
19 don't have to follow thinking that we can come out
20 today or any day in our life with one protocol that
21 can fit and suit everyone.

22 I think that we have to set the standard,

1 meaning in the level of detection, and then it
2 doesn't really matter how you get there. But if
3 you show that you are able to do that, then it's
4 okay. Of course, within international multicenter
5 studies, all centers have to use the same
6 technique.

7 But you cannot imagine -- you cannot even
8 think, that there would be one day one kit that you
9 just drop in any lab all over the world and the
10 result would be perfect. There will be always a
11 need of more than standardization, really,
12 experiences. So maybe we have to find a way of
13 quality controls or like they are doing for MRD in
14 CML to test the labs and give a sort of
15 certificate. And then it doesn't really matter
16 which panel you are really using if you get down to
17 10 to the -4.

18 DR. KIPPS: I would just like to echo that
19 because we just saw the discussion on CD19 antibody
20 therapy that would make the panel using CD19
21 difficult or challenging to use for MRD assessment.
22 So different therapies may require different

1 panels. And I think all the sensitivity that can
2 be achieved should be the benchmark that we try to
3 strive for.

4 DR. WIERDA: Along those lines, maybe you
5 can comment on the possibility of enhancing the
6 sensitivity of an assay by including novel markers
7 that are leukemia-associated targets, such as ROR1.

8 DR. KIPPS: I know I discussed this with
9 Andy, and I think that's a potential. There are
10 better antibodies out there that can discriminate
11 quite clearly. I think that this is also an
12 advantage, so ROR1 and other markers, there are
13 some other markers that could also be used that are
14 not being used in any of the panels today.

15 I do think, though, that whether this is
16 necessary for us to come up with a standard, I
17 would say not. Whether it's necessary to adopt
18 10-color flow and go down to levels below 10 to the
19 negative 4, I would say not.

20 So I think we can easily get fascinated by
21 the improvements in technology or the need to
22 standardize exactly doing the same thing

1 everywhere. I do think that there's a challenge
2 doing that because people are going to be doing
3 different panels, regardless of what we say here.
4 But can they validate those tests with any level of
5 certainty? And that may require some degree of
6 cross-standardization, similar to what's being done
7 in flow cytometry labs, where you have unknown
8 samples sent to you and you have to assess the
9 results, and then you get QC'd on those.

10 DR. RAWSTRON: Just to add, the real benefit
11 for CLL in particular is that you've got two gold
12 standards to test any assay against. You've got
13 the PCR. You've got validated ERIC panels. And
14 that gives you a lot of opportunity to move forward
15 if you so desire or to validate any panel that you
16 would particularly want to use.

17 DR. REAMAN: I think that is an important
18 point, and I think you're correct that it might be
19 beyond the scope of what we're trying to accomplish
20 here today to actually come up with what is the
21 standard technical approach for MRD assessment with
22 flow cytometry.

1 I think the key thing that we would like
2 consensus is that within a given trial,
3 particularly that's done in a multicenter fashion,
4 there is some standardization of the technique,
5 assays being used, maybe even centralized
6 performance of the test.

7 But I think you're right. To think that
8 we're going to come up with an international
9 standard probably is not something that we'll be
10 able to accomplish today, tomorrow, next year.

11 DR. STETLER-STEVENSON: From the U.S.
12 perspective, what does the FDA need to be able to
13 effectively allow this to be used as a surrogate
14 marker in clinical trials? Because we do not want
15 somebody who's not involved in these trials trying
16 to dream up criteria. So what type of guidelines
17 would be helpful to facilitate this process?
18 That's my question.

19 DR. REAMAN: Well, unfortunately, Dr. Becker
20 isn't here. He promised that he would be coming
21 back, but maybe anticipated that this question was
22 going to be asked.

1 (Laughter.)

2 DR. REAMAN: But seriously, I think there's
3 a requirement -- and maybe Dr. Marti can help here
4 also -- that there are performance characteristics
5 that are well-described, that there is proficiency
6 testing, that there is an active quality assurance
7 program that's adhered to. I think they are the
8 major things that are required.

9 DR. DE CLARO: From a regulatory
10 perspective, it might become challenging when we
11 have multiple sponsors coming in with their
12 different versions of MRD assays and wanting a
13 claim. And for us to be able to describe what is
14 the clinical benefit if you're talking about
15 different assays, we wouldn't want our labels to
16 start looking like the methodology section for flow
17 cytometry. I don't think that's the purpose
18 of -- that's not going to be very informative to
19 prescribers.

20 So I would recommend some level of standard
21 platform for us to be able to communicate
22 effectively from a public health benefit exactly

1 what benefit you're discussing.

2 DR. MARTI: Just a follow-up question about
3 what I think was a cleared FDA panel for MRD. This
4 morning, in Dr. Becker's presentation, there was a
5 tremendous amount of parsing out the difference
6 between, first of all, getting a biomarker
7 clarified. Certainly, clinical trials can be
8 conducted without a cleared panel for this. If you
9 want to take it a step further where it's going to
10 be used more widely, that's another issue.

11 I'll remind people that, yesterday, when one
12 of the people from the FDA decided to ask the most
13 burning question and said that she would throw
14 herself on the fire and dash herself with fuel,
15 asked the distinguished guest, "What panel do you
16 want?" And we never got an answer to the panel.

17 Dr. Becker, you're just in time.

18 (Laughter.)

19 DR. BECKER: Just in time, not during the
20 day --

21 DR. KIPPS: We surmise that you left the
22 room because of this.

1 DR. RAWSTRON: Would it make it a lot easier
2 if there was an IVD assay?

3 DR. FARRELL: Definitely, because any time a
4 phase 3 trial comes in for potential licensure and
5 you're looking at a novel endpoint, we're always
6 partnering with our co-centers to evaluate the
7 characteristics of whatever the device is, along
8 with the clinicians in CDER, looking at the drug or
9 monoclonal antibody.

10 DR. WIERDA: So we are going to take a break
11 now. Perhaps we can reconvene in 20 minutes, which
12 would be half past the hour, so five minutes later
13 than the scheduling, we'll resume. Thank you.

14 (Whereupon, a brief recess was taken.)

15 **Discussion and Questions**

16 DR. REAMAN: I think maybe we'll reconvene
17 and get started. And I may actually take this
18 opportunity to impose on Dr. Becker to answer the
19 question that I think Maryalice Stetler-Stevenson
20 asked and I sort of addressed. But I think you
21 could probably answer it much better.

22 DR. BECKER: What's the question?

1 DR. REAMAN: I'll let you repeat it.

2 DR. STETLER-STEVENSON: So what would be
3 helpful for the FDA in the way of guidelines or
4 protocols and things like that, that we could
5 develop, to be helpful for the FDA in evaluating
6 clinical trials in the specific use of MRD studies
7 as a surrogate marker?

8 DR. BECKER: So I would expect that there
9 are two aspects of it, the clinical implications of
10 that marker as it captures the disease process and
11 the effect of the potential range of treatments on
12 the disease process; and then the analytical
13 aspects that can give you confidence that the
14 marker, as it's going to be applied in a surrogate
15 endpoint situation, is up to snuff.

16 I can say that CDRH, the devices folks, are
17 very happy to give all the support we can in being
18 able to help develop and assess the analytical
19 performance characteristics that need to then be
20 rolled into the therapeutic center's decision about
21 the overall suitability of an instance of measuring
22 the biomarker or of a class of different kinds of

1 devices for measuring the biomarker to be used in a
2 surrogate endpoint application.

3 So did that capture it?

4 DR. STETLER-STEVENSON: So if, for example,
5 this group could come up with a preliminary set of
6 guidelines for evaluating, based on their
7 experience and what's in the literature, CLL MRD
8 studies, would that be helpful, a consensus
9 guideline?

10 DR. BECKER: Surely in a general sense, I
11 could never imagine it doing harm, and we'd like to
12 see it be as helpful as the information you bring
13 forward would allow it to be. But, yes.

14 DR. REAMAN: Okay. I think, if there are no
15 other questions related to the session on technical
16 considerations -- I'm sure they'll come up as we go
17 through these other discussion points. But I
18 thought that we would sort of frame the remainder
19 of our discussions, just sort of address the
20 questions that we've posed here.

21 The first is to discuss the advantages and
22 challenges with the assessment of MRD in CLL

1 clinical trials. Specifically consider the
2 definition of MRD. And I think that's still
3 something that, at least from a threshold level and
4 perhaps even broader consideration, we need to
5 think about.

6 The timing of assessments, sample source,
7 peripheral blood versus bone marrow. Are there
8 times when both are required, one preferable to the
9 other, or are they interchangeable when analyzing
10 for MRD?

11 So advantages. Does the panel really think
12 that qualifying MRD as a biomarker, a prognostic
13 biomarker, a response biomarker, and using that as
14 a surrogate endpoint -- would that, could that
15 facilitate new drug development and approval for
16 patients with CLL?

17 DR. KIPPS: Yes.

18 DR. REAMAN: Yes, but. Okay.

19 (Laughter.)

20 DR. KIPPS: I think that the data would
21 argue such, and provided that we also take notice
22 of these caveats that we have, too, whether it can

1 be used as the surrogate marker for response versus
2 a surrogate marker, I think that's important to
3 spell out.

4 I think that the data with regard to blood
5 versus marrow, it seems clear that a lot of the
6 data are patients treated with antibody trials.
7 The blood is typically a problematic source for MRD
8 analysis. And owing to the biology of the disease,
9 where the cells were homing to the marrow, it seems
10 that that's a more sensitive measure of MRD, at
11 least in the immediate post-treatment period.

12 In so far as you'd like to have the response
13 assessment within a sort of time window that is
14 practical, it would probably still be appropriate
15 to recommend marrow assessment to be sure that the
16 MRD assessment is going to be valid.

17 Now, this may not be necessary subsequent
18 data points farther removed from therapy to monitor
19 for maintenance or progression, MRD-free survival,
20 which would be another thing that could be adopted
21 as a strategy to look at when the disease may be
22 coming back to the blood that was not detected

1 there previously.

2 DR. REAMAN: So I think we probably all
3 should be in agreement that we are talking about
4 this as a potential surrogate marker, and clearly
5 not the surrogate marker, and certainly not the
6 gold standard endpoint for CLL. So I think we
7 really need to stress that, given what we have seen
8 with novel therapeutics, we're only talking about
9 this within the context of a specific drug that's
10 being evaluated.

11 DR. KAY: Greg, if I could just add one
12 thing?

13 DR. REAMAN: Sure.

14 DR. KAY: From the standpoint of specific
15 text, assessment of MRD in CLL clinical trials, so
16 obviously doing a lot. We're not just doing MRD.
17 We're doing now bone marrows. We're doing CAT
18 scans. We're doing all kinds of prognostic
19 factors.

20 I love the fact that we are doing MRD
21 because it's relatively easy to do, and, at the end
22 of the day, it may be -- and this would be one very

1 nice outcome -- that MRD, whether it's negative or
2 it's changing, would prove to be a wonderful marker
3 for how a patient is doing versus all those other
4 tests.

5 I have not heard too much about cost for
6 flow, but my guess is that once we standardize flow
7 in some reasonable way, it's not going to be nearly
8 as expensive as, say, some of the imaging studies.
9 So I like the idea of doing MRD, not just from the
10 standpoint of moving forward drugs quickly, but
11 that we could begin to answer questions with
12 potentially one test. I would argue that blood
13 might end up being as valuable in MRD assessment as
14 any other site.

15 DR. HILLMEN: I'm in agreement. I think if
16 we're going to -- we need to use MRD as a surrogate
17 endpoint for approval of drugs for our trials, for
18 the reasons I've pointed out before. I think, to
19 be conservative over making sure we actually are
20 using the data we have and appropriate, we should
21 be giving it at least three months' post-therapy,
22 for therapies that finish, because we have a lot of

1 data on that. And our guidelines say that we
2 should be assessing response in the marrow, for
3 example, at least three months after therapy. So
4 that's consistent with what we've been doing; so an
5 MRD-negative result at least three months' post-
6 treatment.

7 In the marrow, I think, at the moment, still
8 would be the standard, although peripheral blood,
9 if you're negative, is fine, but that's most
10 conservative. And I think we've heard a lot about
11 10 to the -4 as being validatable, reproducible,
12 and as an endpoint for a trial. So we have
13 clear -- all our data at least has 10 to the -4 as
14 the most important endpoint, and so I think we have
15 those criteria.

16 DR. BYRD: I would just add to what Tom
17 said, that, with adding new things to chemotherapy
18 or chemoimmunotherapy, we have not seen a study
19 where it didn't correlate with what we consider the
20 primary endpoints of progression-free survival or
21 overall survival. We're not going to be able to
22 develop drugs in CLL if we don't use MRD. I think

1 it's essential, unless we're going to give up, and
2 we don't want to do that.

3 DR. RAWSTRON: Do you not think you should
4 also have a six-month peripheral blood?

5 DR. HILLMEN: I think the data would support
6 MRD at that stage off-treatment, and the peripheral
7 blood is as good as marrow. Yes.

8 DR. RAWSTRON: Possibly non-reproducible?

9 DR. HILLMEN: Possibly, but we still have to
10 reproduce it in the trials that we're doing. So I
11 think, if you've been very conservative, you'd say
12 marrow at three months is what everyone's been
13 doing. And for approval of a drug -- I'm not
14 talking about for the assessment of trials, but for
15 the approval of the drug, you want to really be
16 robust and know that that data is solid. And I
17 think that's the most solid data we have. We might
18 have a better endpoint later, but like if it's an
19 early endpoint that we are comfortable with.

20 DR. REAMAN: Any other questions? A clear
21 understanding of the opinion of the panel here.

22 So we can go onto number 2. Initiation of

1 alternative therapy at the time of loss of MRD
2 might confound the analysis of progression-free
3 survival and duration of response if they happen to
4 be primary endpoints.

5 Can you just discuss or think about ways of
6 avoiding this confounding situation? Or are there
7 trials that could be designed such that initiation
8 of alternative therapy is an opportunity to
9 actually censor patients or call it a failure?

10 DR. GHIA: I think that, again here, the
11 question is not appropriate to the topic because we
12 are not going to change the management of our
13 patients, meaning that, as Dr. Montserrat said, but
14 it's according to the guidelines. When patients
15 relapse, even when they clinically relapse but even
16 more when they relapse at the MRD level, nobody
17 ever thinks to treat them. You treat them only
18 when they progress.

19 So again, I want also to answer the first
20 question. Now, we are talking about an endpoint,
21 meaning then one time point in which we are going
22 to assess MRD and say the drug works or not; stop

1 the trial, or go on. And we are not talking about
2 monitoring. That's another thing. It's a very
3 interesting thing. It's experimental. It will
4 probably change our landscape, but it has nothing
5 to do with assessing efficacy of a drug or not.
6 And again, when we get an MRD-positive or negative
7 result, we have nothing to do, and we should do
8 nothing.

9 DR. MONTSERRAT: Still Paolo, I fully
10 understand the question, because we accept MRD as
11 an endpoint for the treatment efficacy. So then it
12 seems reasonable, at least to argue whether -- I
13 mean, relapse in terms of MRD might be an important
14 additional endpoint to evaluate the efficacy of a
15 new drug. Do you see what I mean?

16 So to me, to address or to elaborate on
17 progression-free survival in CLL is one of the most
18 difficult things I ever encountered because I think
19 that they have a lot of definitions, but then when
20 you come to the reality, I mean, it is very
21 difficult to have consistent criteria to define, in
22 good terms, progression-free survival.

1 But I fully understand the question, and
2 maybe we should really try to think over this a
3 little bit more, and to include some kind of
4 evaluation of the duration of MRD, provided that we
5 accept MRD as a rating for efficacy.

6 Do you see my point? This is not about
7 management of patients with CLL. I mean,
8 management is quite clear and we shouldn't confound
9 that. But if we accept MRD negativity as an
10 endpoint, why then consider that the region of MRD-
11 negative status as a valid endpoint for the
12 efficacy of a new drug?

13 DR. GHIA: No, no. Of course, absolutely,
14 but what I want to say, that's experimental. It
15 will be your next publication, I want to say. So
16 it will be in a new trial, and probably these kind
17 of trials are already going on. But this does not
18 affect the evaluation or the efficacy of that
19 particular drug that you are testing.

20 Then, if you want to ask that question and
21 organize a trial in order to answer that question,
22 fine. You can already do it now if you want, but

1 then the FDA is not involved in approving or not
2 your drug. It's just a question that will foster
3 our knowledge.

4 DR. KIPPS: I think the way you have this
5 phrased is not correct. Do you mean loss of MRD
6 negativity rather than loss of minimal residual
7 disease? So when you become MRD positive after
8 having been MRD negative before, I mean, are we
9 going to necessarily try to adopt that as a
10 standard?

11 I don't know if that's necessary to do right
12 now. I mean, if you want to use the analogy of
13 CML, patients are treated and they have been
14 observed to become MRD negative. They may go off-
15 drug and they become MRD positive. Then they get
16 reinstated with drug rather than having full-
17 relapsed disease.

18 So we may be dealing with shifting treatment
19 paradigms that would require some adjustment in how
20 we address MRD. If MRD truly becomes an
21 appropriate surrogate marker, then there could be
22 strategies employed with consolidation and what

1 have you that seek to eradicate MRD.

2 Obviously, we'll have to follow patients and
3 look at those that have become MRD positive after a
4 certain type of therapy to understand the clinical
5 relevance of that, but I don't think we should be
6 wedded to the concept that we can't do that.

7 DR. REAMAN: Yes. I think that's what we're
8 just trying to ascertain, I mean, recognizing that
9 this is an evolving science, if you will, as
10 prognostic markers become more widely accepted, the
11 clinical community sometimes acts early on markers
12 and intervenes therapeutically, which clearly
13 confounds a trial and interpretation of results
14 when you're trying to use that endpoint, and that
15 endpoint is actually being used to make earlier
16 treatment decisions.

17 So if the consensus is that people now are
18 not acting on a change in MRD status as a "early
19 relapse," then that's probably not an issue. But
20 that's what we're really trying to ascertain with
21 this question.

22 DR. WIERDA: Just a comment that I would

1 make is, certainly, in the trial that I showed you,
2 the FCR trial for the prospective evaluation of
3 MRD, we're monitoring every six months in the blood
4 for relapse. And I think we're not acting on that.
5 We have to collect the data. In order to make any
6 assessment or recommendations, we need to have
7 data. So we don't do anything different for
8 patients who convert to MRD positivity other than
9 follow them as we otherwise would.

10 DR. KIPPS: Some patients are driven to
11 distraction to find out that they are MRD positive
12 again. And so this is a thing that we have to deal
13 with. So I'm not sure how to address that.

14 DR. HILLMEN: I think the CLL patients are
15 fully more sophisticated than that, because if you
16 look at what we do now, progression-free survival
17 is our endpoint, so that's usually a lymphocyte
18 count rising above 5. We don't treat patients
19 until they have active disease, and that can often
20 be a year or more after they have lymphocytosis.

21 So this is no different to what we're doing
22 at the moment. So I don't think we will robustly

1 say in our publications that we should not be
2 treating MRD relapses outside of clinical trial.
3 It's a very active area for clinical trials.

4 DR. MONTSERRAT: Absolutely.

5 DR. DE CLARO: Yes. I think we had posed
6 this question to address what we are observing in
7 the treatment of CML, when you have patients with
8 fluctuating molecular responses, and patients are
9 going off protocol therapy, and are taken off, and
10 are censored, and we lose follow-up on those
11 patients. So interpretation of the clinical trial
12 data becomes problematic. And I just wanted to
13 alert the experts that this might become a problem
14 in the future.

15 DR. REAMAN: The other place that we see
16 this is in ALL, where early MRD, after induction,
17 is such a poor prognostic indicator that patients
18 are getting therapy intensified early on. So how
19 do you interpret it or use it then as an endpoint?
20 But fortunately, we don't have that problem here,
21 so that's good.

22 DR. WIESTNER: Adrian Wiestner. I got a

1 little bit confused by the elements of this
2 discussion. I think assessing MRD status is
3 happening in the setting where you want to
4 eradicate the disease at the lowest minimal disease
5 level, not chronic management.

6 So if you have a clinical trial that has as
7 an endpoint MRD negativity, you reach the endpoint
8 once you assess that the patient is MRD negative.
9 And we know that that predicts for a better
10 progression-free time.

11 Now, the patient is being monitored, then
12 becomes MRD positive again. This is the ideal time
13 point to intervene and to test new drugs. And if
14 we make the case that MRD negativity, assessing MRD
15 status accelerates drug approval, that's exactly
16 where it accelerates drug approval, because you
17 would intervene in those patients. Conceivably,
18 you would intervene at the time where MRD becomes
19 detectible again. And you can test low-intensity
20 approaches and see if actually with a kinase
21 inhibitor, with one of the new agents, you can
22 re-induce MRD-negative status.

1 So I would actually think that over the
2 lifetime of the patients, several times, MRD
3 assessment contributes to clinical endpoints.

4 DR. REAMAN: That might actually accelerate
5 the evaluation of new drugs in that it would make
6 the patient population a little bit larger for
7 testing. But what we're really trying to discuss
8 here is using MRD as the endpoint of the trial.

9 DR. DE CLARO: Now, in those situations, the
10 benefit/risk evaluation would be different because
11 you're starting with a population of patients who
12 are just MRD-positive and probably do not have
13 symptoms related to the disease. So I think, from
14 a benefit-risk standpoint, that would have to be
15 considered.

16 DR. MONTSERRAT: Yes. I think it's very
17 important. Yes, I fully agree. And not to mix up
18 things, the questions that we have been asked,
19 it's -- so the recommendation of investigating MRD
20 within clinical trials, this has already been made
21 by a number of bodies.

22 I mean, the issue, which is the measure

1 issue, why we are here, as to whether or not MRD
2 negativity achievement should be considered for
3 certain drugs, a surrogate, an endpoint surrogate,
4 I think that we are in favor of that.

5 So in terms of the technique, I think that
6 there is an agreement of the threshold of the level
7 of the residual disease that should be detected, 10
8 to the -4. This should not be confounded and mixed
9 up with treatment strategies which are a completely
10 different thing. And we should be very cautious
11 when writing down about all these items, I mean,
12 not to convey confusion to the medical community
13 because there are many things that can only be
14 proved on trials. These are the realm of trials.
15 We have to be very cautious and not mix up these
16 things.

17 DR. KAY: I would like to add one other
18 aspect. I mean, it's a very challenging question,
19 but probably ahead of its time. One thing that I'd
20 like to say, I think everyone agrees with, is, CLL
21 is not CML in many, many ways.

22 Looking at that question, right now, if we

1 had done those trials -- and I knew MRD was a
2 surrogate for PFS. It's just a hypothesis. But if
3 a patient had MRD-negative conversion to
4 MRD-positive and they were 17p, I would surely be
5 inclined to want to put that patient in a clinical
6 trial and see if I could prevent what I know would
7 happen.

8 So I think it's a great question. I'd love
9 to return to that at some point. We're clearly not
10 there yet.

11 DR. REAMAN: Enough. We will go on to
12 number three. So discuss the challenges with
13 implementation of a standardized method of
14 assessment of MRD. I think we've touched on that.

15 Should testing be centralized, regionalized?
16 And if performed at a local institutional level,
17 should the assays and the performance of assays be
18 certified and audited? And how could, should
19 proficiency testing be conducted?

20 I think that's yes to all of these.

21 (Laughter.)

22 DR. RAWSTRON: We are still probably in a

1 situation where we need centralized testing, aren't
2 we?

3 DR. MONTSERRAT: The only one that remains a
4 question mark is the very last question, how should
5 proficiency testing be conducted. The answer to
6 this is definitely yes. I mean, if we are going to
7 accept MRD negativity as an endpoint -- I mean, to
8 accept a drug, then, you have to make completely
9 sure that the data are robust and solid.

10 DR. STETLER-STEVENSON: I think the idea of
11 certification and auditing is important. I was
12 involved in evaluation of a large multicenter trial
13 evaluating drug therapy for cutaneous T-cell
14 lymphoma. And we did a large number of the
15 testing, but the testing was performed in multiple
16 countries in multiple institutions in the United
17 States as well. And then all the data was sent to
18 me for review, and the quality of the data varied
19 greatly.

20 It came to the point where, if it was one
21 institution, I'd smile because I'd know there'd be
22 beautiful data, easy to evaluate, and a lot of it I

1 had to say non-evaluable because the data was just
2 so horrible, yet they were allowed to proceed
3 throughout the trial.

4 So it is big. If it's going to be performed
5 at local institutional level, there has to be some
6 sort of certification and central review of the
7 data.

8 DR. REAMAN: But actually performing it
9 centrally would be superior to institutional
10 performance and central review.

11 DR. HILLMEN: For regulatory approval, we're
12 talking about.

13 DR. REAMAN: Yes.

14 DR. HILLMEN: Yes. Definitely.

15 DR. REAMAN: John?

16 DR. BYRD: So having the experience of being
17 a reference lab for some older studies with agents
18 that didn't move forward, I think most studies can
19 be done internationally. And so you have to have
20 two or three central labs. When you send samples
21 overseas, they arrive in bad shape, particularly if
22 they're coming to a high alt because they're

1 frozen. So two- or three-day-old blood that's
2 frozen, you don't get the best flow cytometry from.

3 But I can't see any way in a registration
4 study you could get by without doing it central and
5 not lose a lot of your events to the quality issues
6 that are going to come up for auditing.

7 So even if we said it could be done at
8 multiple centers, I think a company would be -- if
9 they're investing in the cost of a trial, would be
10 crazy to do it outside of a central lab.

11 DR. GHIA: One thing I just want to
12 underscore, it's true that the major question is
13 the last one because I think there is no difference
14 between centralized or regional. The problem is
15 who is doing the test. So even if it is only one
16 center, it is even worse because you have to really
17 be sure that that center is doing things properly.

18 So I think the proficiency testing is
19 inevitable. And then you can do it one center, or
20 more centers. And of course, as John said, it's
21 better to have more centers, especially
22 international studies, because otherwise we cannot

1 do flow.

2 DR. HILLMEN: Sorry. There is a long
3 history over the last 10 years of standardization
4 across the globe for these flow assays. And so
5 they have to follow the same quite stringent
6 criteria. I've seen this in other disease areas,
7 where a commercial trial lab, because it's
8 accredited, comes in and does a test, which they
9 can't do, whereas the academic labs are very good
10 at doing it.

11 So standardized in a way that's been
12 standardized for the focal of flow would be the way
13 I would recommend.

14 DR. JORGENSEN: Jeff Jorgensen, MD Anderson.
15 Yes. Just to play devil's advocate, there are
16 transport issues in Texas as well. We can cook our
17 samples by chance transporting them across Texas.
18 So I'd put in a plug for regionalized rather than
19 insisting on centralized and, again, with careful
20 certification, auditing, and good quality control.

21 DR. REAMAN: I guess centralized doesn't
22 necessarily mean that it has to be a single center.

1 Points all well made.

2 Maryalice?

3 DR. STETLER-STEVENSON: One of these sticky
4 issues of standardization is that there are several
5 standardized protocols. And one way to approach is
6 that there are excellent standardized protocols
7 that one could compare one's methodology to.

8 It doesn't have to be the ERIC approach. It
9 could be the EuroFlow approach. They've done the
10 studies. So those that have been validated, if a
11 centralized laboratory wants to perform a new way
12 of doing the testing, to validate that it compares
13 to these others --

14 DR. REAMAN: In parallel.

15 DR. STETLER-STEVENSON: In parallel, yes.

16 DR. RAWSTRON: I was just going to say, in
17 the absence of proficiency testing, maybe some
18 independent validation of the data.

19 DR. REAMAN: In the situation where
20 centralized testing may actually be performed in
21 multiple laboratories or more than one laboratory,
22 sharing of blinded specimens and evaluating

1 results. Yes, sir?

2 MR. FAHAM: So you mention --

3 DR. REAMAN: Identify yourself, please.

4 Sorry.

5 MR. FAHAM: Sorry. Yes. Malek Faham from
6 Sequenta. So you mentioned initially there's
7 obviously a difference between developing an IVD
8 and a biomarker for clinical trials endpoint. And
9 I assume we're talking about the latter. And I
10 guess I want to understand from this perspective, I
11 think I can still imagine you end up with a
12 spectrum of things to do.

13 One is, there is only one standard panel of
14 these specific flow markers that you would do, and
15 that's what we're going to use all the time in one
16 lab or several labs and that's always. On the
17 other spectrum of things, it would be a set of
18 criteria. The test is done three months from
19 blood, three months after treatment, like the 1 in
20 10,000 sensitivity. And using these, it may need
21 to be validated with these analytical performance
22 metrics. And I know this is a specific way to

1 validate this.

2 That's a spectrum of things. The latter end
3 of the spectrum would be you could use any number
4 of flow markers, but even you could imagine you
5 could use other techniques, like for example,
6 sequencing. That's obviously where Sequentia is
7 coming from, sort of biased in that direction.

8 But my point is, do you imagine that at the
9 end of this process, we end up with -- you're
10 talking about standardization, harmonization. Is
11 it a specific set of protocol or is it a specific
12 set of criteria that only detects cancer cells 1 in
13 10,000 at that time point, against these
14 performance metrics, and these validation ways to
15 do that?

16 DR. REAMAN: I think, for the purpose of a
17 given trial, evaluating a given drug, we would
18 probably expect both of those, so not only the
19 qualifications, but the specific assay and
20 performance characteristics as well.

21 MR. FAHAM: So you are not imagining that
22 there will be -- I mean, because you mentioned for

1 a specific trial, but you also mentioned initially
2 that this could be valid across several trials. I
3 mean, you don't imagine that there will be a
4 criteria over a marker across several trials that
5 we will use. That's like BCR-ABL. That is a
6 marker they use not just per trial, but over a
7 bunch of them.

8 DR. REAMAN: But what we are really
9 addressing here is the potential use of this as an
10 endpoint for accelerated approval of a specific
11 drug, which usually comes as the result of a given
12 trial. So use across multiple trials, we wouldn't
13 have as much a concern with. But from the
14 standpoint of approval considerations of a specific
15 drug and a specific trial, or even set of trials
16 that are submitted as part of an application, I
17 think we would really expect to have both of those
18 considerations adequately addressed.

19 DR. DE CLARO: Yes. I think maybe the
20 criteria for MRD does not necessarily need to be
21 exactly the same in all the trials, but having some
22 commonality, I think, would help. For example, we

1 have standardized response criteria in CLL. So
2 when we approve drugs, it's easy to describe the
3 response, and definitions for response to
4 progressions were consistent with IWCLL criteria.

5 We would allow some modifications, but if
6 you're starting from the definitions of MRD with
7 different methodologies, it can't be that MRD was
8 assessed by flow cytometry and that's all we put in
9 our label. It would be a benefit to have some sort
10 of a backbone that the people have identified as a
11 useful starting point.

12 DR. FARRELL: I just want to make a point
13 that, when we write a label, the consumers, the
14 physicians, and the patients who are reading the
15 label have to get an understanding of what they can
16 expect if they were enrolled in the trial. And
17 that's why we usually go to great lengths to work
18 on what is the test object that we are going to use
19 to define success.

20 DR. GHIA: I think I also need a
21 clarification following the question, because as I
22 showed today, we have flow cytometry, which is well

1 established. PCR is well established. In two
2 years, maybe we will have next-generation
3 sequencing.

4 So does that need -- can one come with
5 another technique, next-generation sequencing or
6 whatever it will be in two years, just showing that
7 it has the same level of limit of detection, the
8 same sensitivity? Or it's not possible because --

9 DR. REAMAN: Yes. I think what we're trying
10 to avoid is the assessment being done by PCR in
11 some institutions by next-gen sequencing and other
12 institutions' data being consolidated as to MRD
13 status. That's problematic. But obviously, this
14 is all going to be evolving and changing.
15 Sensitivities will hopefully increase in the years
16 to come methodologically, so we would anticipate
17 that.

18 DR. DE CLARO: Yes. And similar with
19 evolution of response criteria in lymphoma, with
20 the integration of FTG PET scans with current
21 criteria, we did not require that all the trials
22 had to include -- but some sort of bridging the

1 comparison of how the performance is using the old
2 (indiscernible) and new would be helpful.

3 DR. GHIA: Yes.

4 DR. REAMAN: Do we have to talk more about
5 proficiency testing or do you think we're in
6 agreement that it needs to be done?

7 DR. DE CLARO: I guess we don't know how --
8 done.

9 DR. RAWSTRON: That's the thing. We'll
10 hopefully find out within a few months, but I mean,
11 it looks good that there will be some form
12 available. And the other alternative is data. If
13 you can assume that the laboratory checks that they
14 can run a machine properly, are okay, if CAP is
15 suitable for that, then you can check how the
16 individuals analyze their data. And so there will
17 be some form of proficiency testing even if it's
18 not perfect.

19 DR. GHIA: Probably the question more than
20 how is who.

21 DR. REAMAN: I think I would assume the -
22 (off mic.)

1 DR. GHIA: No, no. So, I mean, we have all
2 the tools to do that, even in remote.

3 DR. HILLMEN: Just to follow up on Andy's
4 point, the initial validation of follicular flow
5 was by sharing data, and you can look at data and
6 say whether a lab has made a complete mess out of
7 the test, are misinterpreting it, or happen to have
8 T-cells and often normal B-cells in there that have
9 a specific pattern.

10 So you have internal controls. It's not
11 like PCR or other tests where you don't have those
12 internal controls and you don't have an electronic
13 record of the test. So it's a bit more like CT
14 scans, that you can actually send the images to
15 somebody in Australia, and they can interpret the
16 data, to some extent. And so it can be validated
17 on a number of different levels.

18 DR. REAMAN: So is there any way to go on to
19 number 4?

20 DR. DE CLARO: We only have three.

21 DR. REAMAN: Three.

22 DR. RAWSTRON: Could I just ask another

1 question? What would you accept as proficiency
2 testing?

3 DR. REAMAN: Well, I was going to
4 actually -- I thought we had another question, so I
5 didn't want to prolong this discussion. But in
6 addition to sharing data, which I agree is
7 certainly very feasible in the setting of flow
8 cytometry, what about actual sharing of specimens,
9 blinded specimens?

10 I think, personally, doing both would
11 probably be better than doing just one, that is,
12 sharing data. I mean, is that something that
13 people feel would be feasible? I know, in other
14 situations from personal experience -- and Dr. Wood
15 is there, so I can call on him to comment. But I
16 know it can be done.

17 DR. WOOD: It certainly can be done. We do
18 something similar for the Children's Oncology Group
19 ALL MRD testing, where there are two centralized
20 reference labs, and we exchanged and prepared
21 samples between the two labs in a blinded fashion
22 to ensure that we're getting similar results.

1 As the number of labs increases, the
2 complexity and difficulty will increase in a
3 variety of different levels. But as long as the
4 number of labs is relatively small, one can
5 certainly exchange data as well as samples and
6 improve the quality, I think, for the whole group.

7 DR. HILLMEN: I mean, The NEQAS
8 switched -- mentioned before. We've used the
9 national quality control scheme in the U.K. with
10 sera samples and sent samples around the world for
11 various specific testing. And that is a very
12 robust way of doing it. And we've done this with
13 hundreds of labs, I think, over 100 labs for a
14 certain test.

15 Of course, they all are compared with each
16 other, so you get a very good proficiency testing.
17 So I think Andy's comment about the fact that we've
18 actually got stabilized samples that remain stable
19 means it's feasible to send samples around for
20 testing.

21 DR. RAWSTRON: I just wondered, is UK NEQAS
22 recognized for American regulatory bodies?

1 DR. REAMAN: Repeat your question.

2 DR. RAWSTRON: So if somebody is doing a
3 proficiency test with UK NEQAS, is that recognized
4 to the FDA?

5 DR. MARTI: It's not, but it could be.

6 DR. GHIA: But who will take the
7 responsibility to assess the proficiency? Because
8 I don't think it's only a matter of sharing. You
9 can share as many data and samples as you want, but
10 if you are sharing among people who don't know what
11 they are doing, steal the data.

12 (Laughter.)

13 DR. GHIA: No, no.

14 DR. REAMAN: I think that's a good question.
15 I mean, I don't necessarily have an answer for it.

16 DR. GHIA: Yes, no. I thought the FDA -- I
17 don't know.

18 DR. REAMAN: I don't think we're suggesting
19 that we should be the body responsible for --

20 DR. GHIA: No, no.

21 DR. REAMAN: -- oversight of the proficiency
22 testing. I think what's important is that some

1 element of proficiency evaluation be part of the
2 trial and proof of that be part of the application.

3 DR. STETLER-STEVENSON: I think the UK NEQAS
4 would provide evaluation of your performance in
5 proficiency testing. You could probably get David
6 Barnett to comment further. And within the U.K.,
7 you use UK NEQAS results actually to affect whether
8 or not labs are allowed to continue to do testing.

9 Am I correct?

10 DR. RAWSTRON: Yes. Theoretically, although
11 they're mostly educational, so if somebody is
12 underperforming, their first role is to go in and
13 improve things.

14 DR. HILLMEN: Yes. But that leads to
15 accreditation of the laboratories, so this is
16 outside trials. This is in routine use. And so if
17 you failed twice in a row, then the boys come
18 around and sort you out. And if you fail again,
19 then your accreditation is jeopardized.

20 DR. MONTSERRAT: What I would anticipate is
21 to the benefit of everybody and those willing to
22 investigate new agents, novel therapies based on

1 MRD status -- if you take what happens nowadays
2 with the current trials, there are a number of
3 biomarkers included already.

4 I think that it is going to be highly
5 optimistic, and I think that this is going to be
6 relatively easy by gaining experience with
7 biomarkers, genetics, IGVH mutations, and many
8 others, and to extrapolate all those to the MRD
9 evaluation as well. I mean, as long as there is a
10 strict requirement of the intent of the
11 sensitivity. I mean, it should be really very,
12 very, very strict.

13 DR. DE CLARO: We can all open the floor for
14 questions from the panel or from participants in
15 the audience.

16 (No response.)

17 **Workshop Summary**

18 DR. DE CLARO: All right. Hearing none, I
19 think we can move on to the summary; then again,
20 just give it from here.

21 So I think we had a broad discussion today
22 of the clinical experience to date of MRD and CLL

1 from multiple international sites. I mean, in
2 general, we saw that there's an association between
3 MRD status and longer-term endpoints such as
4 progression-free survival and overall survival.
5 And MRD emerged as a significant independent
6 predictor on multivariate analyses for these
7 endpoints.

8 I think, regarding the definitions of MRD
9 with regards to the threshold, I think we are in
10 general agreement that a .01 percent level is a
11 reasonable threshold. A majority of the methods
12 that we discussed today were flow cytometry-based.
13 We had some discussions regarding samples of
14 peripheral blood or bone marrow and discussed some
15 caveats on use of both.

16 On timing of assessments, in general, what
17 we heard was probably an end-of-therapy assessment
18 would be preferred. And with regards to monitoring
19 for MRD, that's not the topic for today, but it was
20 identified as an interesting topic for future
21 discussion.

22 Now, regarding the settings wherein MRD was

1 assessed, it was typically in our older approved
2 therapies such as alkylating agents, purine
3 analogs, chemoimmunotherapy. It will be
4 interesting to see how MRD assessment is performed
5 with novel therapeutics coming in the future.

6 In general, I think we have a consensus that
7 MRD would be a useful endpoint to assess in
8 clinical trials, with a caveat. It is a surrogate
9 endpoint, not the surrogate endpoint, for all
10 clinical trials.

11 With regards to technical considerations, we
12 had discussions regarding having our reproducible
13 assay and in regards to that having standardized
14 methods. I mean, currently, I think what we're
15 taking from the panel with the phase 3 trials here,
16 at least for registration purposes, is having
17 centralized, assessing laboratory would most
18 facilitate any application, as this would give the
19 most assurance that the tests were reliable and
20 reproducible.

21 I think we had some discussion regarding
22 proficiency testing and, in general, the panel was

1 recommending that such should be done, the details
2 of which remain undefined.

3 In regards to moving forward, I think there
4 are several unanswered questions that probably
5 would need further data and perhaps come with a
6 follow-up at a later time. With that, I would like
7 to thank the FDA and all of the co-sponsors, ASCO,
8 ASH, the Leukemia Research Foundation, LLS.

9 Did I miss anybody?

10 **Adjournment**

11 DR. DE CLARO: I would like to thank all the
12 participants, especially the planning committee.
13 I'd like to thank the people on site and also
14 online. We have details regarding if you need a
15 cab to go to -- if you're leaving for the airport,
16 Christine Lincoln will have details regarding
17 contacting cab companies, and thank you.

18 (Applause.)

19 (Whereupon, at 3:21 p.m., the meeting was
20 adjourned.)

21

22