Treatment Evolution in Acute Lymphoblastic Leukemia: Insight on Novel Antibody Technologies and Immunotherapy Advances

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Activity Information

Activity Description and Educational Objectives
In this activity, leading experts offer key insights on the evolution of acute lymphoblastic leukemia (ALL) care, and how the present and future of patient management will change in its wake. The experts address important topics such as how minimal residual disease (MRD) monitoring will be integrated into the use of novel therapies, the impact of antibody-based approaches—including antibody-drug conjugates and bispecific agents—on patient care, and the emergence of other immunotherapy strategies, such as chimeric antigen receptor (CAR) T-cell therapy. Survey responses from patients on the emergence of this new generation of therapies, collected via a survey, are featured alongside the science-based presentations. This activity highlights the emerging science that may mark a new road in the care of patients with ALL, who may not benefit from standard chemotherapy, and a change in how optimal care is defined.

Upon completion of this activity, participants should be better able to:
- Describe the mechanisms of novel immunotherapies and antibody-based treatments in ALL, including antibody-drug conjugates, bispecific agents, and other options
- Cite clinical evidence on the efficacy of immunotherapy and antibody-based treatment in ALL, including in relapsed/refractory disease and other populations with unmet clinical needs
- Integrate antibody therapy into the management of ALL patients with relapsed/refractory disease and other relevant treatment settings
- Manage treatment-related toxicity in patients with ALL receiving antibody-based therapy
- Identify patients with ALL eligible to receive novel treatments, including antibodies or immunotherapy, in the setting of a clinical trial

Target Audience
This activity has been designed to meet the educational needs of hematologist-oncologists, hematologists, oncologists, advanced practice oncology nurses, and other healthcare professionals involved in the care of patients with ALL.

Requirements for Successful Completion
In order to receive credit, participants must view the activity and complete the post-test and evaluation form. There are no pre-requisites and there is no fee to participate in this activity or to receive CME credit. Statements of Credit are awarded upon successful completion of the post-test and evaluation form.

Media: Enduring Material
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Time to Complete: 120 minutes

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Treatment Evolution in Acute Lymphoblastic Leukemia: Insight on Novel Antibody Technologies and Immunotherapy Advances

Introduction

Dr. DeAngelo: Good evening, everybody. Thanks for coming to this educational symposium on the treatment evolution of acute lymphoblastic leukemia. I’m Dr. Daniel J. DeAngelo from the Dana-Farber Cancer Institute, Harvard Medical School in Boston.

Joining me in this PeerView Live Symposium are Dr. Monika Brüggemann from the University of Kiel in Germany, Dr. Elias Jabbour from the University of Texas at MD Anderson, and Dr. Stephan A. Grupp from the University of Pennsylvania, the Perelman School of Medicine, as well as Children's Hospital of Philadelphia.

Narrator: After completing the activity, access the post-test and evaluation form by clicking the red “Get certificate” button.

I encourage you to download the slides, Practice Aids, and any other activity features that may interest you.

As a preface to Dr. Monika Brüggemann’s talk, I’m just going to show a few slides. As we all know, for those of us who treat ALL, there’s been a dramatic improvement over the last few decades, at least in pediatric patients—we do have a pediatrician here who can attest to that—with remission rates in the 95% range and a 5-year event-free survival of 80% to 85%.

Unfortunately, adult patients have not fared as well, although the CR rates are in the 85% range, the 3-year disease-free survival and the overall survival are around 40% to 45%. Higher rates of relapse, more adverse cytogenetic features, and persistent positive MRD, as we’ll learn from Dr. Brüggemann, as well as poor tolerance to chemotherapy—specifically in the older age groups—really leads to more patients with relapsed and refractory disease.

Long-term survival of these patients, who suffer from relapsed acute lymphoblastic leukemia, is short, with a long-term survival of less than 5%.

Moving Beyond (and Augmenting) Chemotherapy in ALL

- Recent evidence suggests the potential of monoclonal antibodies and immunotherapy to improve outcomes in ALL and augment standard treatment options
- Several approaches in various stages of development

Approved
- Blinatumomab (relapsed B-cell Ph-negative ALL)

Phase 3 trials
- Inotuzumab ozogamicin (INO-VATE)
- Rituximab + chemotherapy; GRAALL-R 2005

Phase 1-2 trials
- Combotox
- Ofatumumab
- Denintuzumab mafodotin
- CAR T-cell therapy
- PD-1 inhibitors

Moving beyond standard chemotherapy, recent evidence—and we’ll show that today—suggests that the development of monoclonal antibodies and immunotherapeutics can provide outcomes for patients with ALL that can improve standard options.
Many of these will be discussed today—including blinatumomab, which was approved, at least in the United States, last year—as well as a discussion of CAR T-cell therapy, and from Dr. Grupp on the CAR T-cell experience.

We'll hear from Dr. Jabbour on blinatumomab and bispecific antibody therapy, and then from Dr. Grupp on the CAR T-cell experience.

So as I've already alluded to, we'll have a discussion on MRD monitoring, what are the current standards, and what would be the impact of newer therapies on MRD—Dr. Brüggemann will lead that. Then I'll come back and discuss with you some of the developments in monoclonal antibody conjugates and emerging strategies, including some of the newer therapies.

Dr. Jabbour will speak on the bispecific antibody technology and the data with blinatumomab in patients with relapsed acute lymphoblastic leukemia. And then to round out the talk will be Dr. Grupp on CAR T-cell therapy, obviously one of the more exciting and novel immunotherapeutic approaches that we have.

Understanding MRD Monitoring and Implications for Novel Therapy in ALL

There was a poll sent out to patients—I find this very interesting—what do patients think of the developments in acute lymphoblastic leukemia? I know what we all think, but these were trends from patients with ALL. And these are just some excerpts from what we've learned.

The vast majority of patients had only received chemotherapy as part of their therapy and were unaware and had never heard of the newer therapies. Most reported very limited awareness of these agents. Very few said that there was a discussion of treatment options with patients by their physician or oncologist. Many had not received education on clinical trial-based therapies. And some patients felt that their doctors were reluctant to offer information about available clinical trials.

Obviously, the important issues to most patients are the ability of the therapy that they're receiving to extend their life and, obviously, to control their leukemia. So patients are speaking out and they want to know more about what's being developed in acute lymphoblastic leukemia.

Dr. DeAngelo: And with that, I'll introduce Dr. Monika Brüggemann from the University of Kiel in Germany. Monika?

Dr. Brüggemann: Thank you very much. So, I want to start with a case presentation of Paul, a 23-year-old male with Ph-negative ALL. He did not have Philadelphia chromosome or MLL...
rearrangement. His white blood cell count was 23 per nanoliter, and his differential blood count showed 80% blasts.

Treatment was started according to the German Multicenter ALL protocol 07, and after induction 1, the patient reached a complete remission. So, according to all the parameters mentioned above, he was stratified as standard risk ALL and was intended to receive induction consolidation treatment followed by maintenance therapy.

So, as per protocol, MRD was monitored using IGH RQ-PCR during induction and all consolidation treatments. And, as you see here, MRD was highly positive at first, at a level of $2 \times 10^{-2}$, then $5 \times 10^{-3}$, and after consolidation 1, at the level of $5 \times 10^{-4}$.

Paul proceeded to stem cell transplantation. And after allogeneic stem cell transplantation, he became MRD-negative already at day 30 and remained MRD-negative until day 100.

So, very early MRD identified patients with a very rapid tumor clearance and an excellent prognosis, whereas patients with persistent MRD positivity above a level of $10^{-4}$, as Paul had, were patients with chemoresistance and a particularly poor prognosis with a relapse-free survival of only 12%.
So these data were updated in the next trial, in the 07 trial, including 580 patients not only standard-risk, but now also high-risk patients. And here, MRD was the only variable with independent prognostic relevance. Patients who had persistent MRD above levels $10^{-4}$ at week 16 had a probability of continuous complete remission of only 26%.

### MRD for Tailoring Treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>No SCT</th>
<th>SCT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR</td>
<td>120</td>
<td>60</td>
<td>57</td>
<td>$60.0 \pm 9$</td>
</tr>
<tr>
<td>DFS</td>
<td>120</td>
<td>60</td>
<td>57</td>
<td>$44.0 \pm 8$</td>
</tr>
<tr>
<td>Overall survival</td>
<td>120</td>
<td>63</td>
<td>57</td>
<td>$54.0 \pm 8$</td>
</tr>
</tbody>
</table>

Within this protocol, an MRD-based treatment was given to these patients with MRD persistence after consolidation phase 1. So, these patients were allocated to stem cell transplantation.

From these 120 patients with MRD persistence after consolidation 1, 47% received allogeneic stem cell transplantation, whereas 53% did not receive stem cell transplantation.

And as you can see here, the probability of CCR was highly, significantly better in the patient group that received stem cell transplantation compared with those MRD persisters who continued chemotherapy.

### MRD for Tailoring Treatment (Cont’d)

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>No SCT</th>
<th>SCT</th>
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</tr>
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<td>CCR</td>
<td>120</td>
<td>63</td>
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</tr>
<tr>
<td>Landmark analysis</td>
<td>60</td>
<td>35</td>
<td>25</td>
<td>$73.0 \pm 10$</td>
</tr>
<tr>
<td>DFS</td>
<td>120</td>
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<td>57</td>
<td>$44.0 \pm 8$</td>
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<tr>
<td>Landmark analysis</td>
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<td>36</td>
<td>25</td>
<td>$50.0 \pm 10$</td>
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<tr>
<td>Overall survival</td>
<td>120</td>
<td>63</td>
<td>57</td>
<td>$54.0 \pm 8$</td>
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</table>

And also, in a landmark analysis, where all patients who had a relapse prior to the median time of transplantation were excluded, and even there, this difference remained. So also here, patients who went to transplant did significantly better compared with patients who continued to have chemotherapy.

However, you see here that also the outcome of patients who received allogeneic stem cell transplantation was far from being optimal, with a CCR rate of 66% and a disease-free survival of 44%.

And unfortunately, this also happened to Paul. He reconverted to MRD positivity at month 18 after transplant, and 3 months later, he suffered a relapse.

### Impact of MRD on Outcome After Allo-SCT: Selected Major Published Trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of SCT</th>
<th>N</th>
<th>Method</th>
<th>Estimate</th>
<th>MRD-negative</th>
<th>MRD-positive</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Nwol (2013)</td>
<td>Aku (P)</td>
<td>54</td>
<td>PCR</td>
<td>$L_1$</td>
<td>73%</td>
<td>36%</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Chemi (2002)</td>
<td>Aku (P)</td>
<td>63</td>
<td>PCR (CR)</td>
<td>$L_1$</td>
<td>41%</td>
<td>71%</td>
<td>.04</td>
</tr>
<tr>
<td>Nwol (2013)</td>
<td>Aku (P)</td>
<td>149</td>
<td>PCR</td>
<td>$L_1$</td>
<td>73%</td>
<td>$41%$</td>
<td>&lt;.0001</td>
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<tr>
<td>Chemi (2002)</td>
<td>Aku (P)</td>
<td>37</td>
<td>PCR</td>
<td>$L_1$</td>
<td>8%</td>
<td>44%</td>
<td>2.02</td>
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<tr>
<td>Chemi (2002)</td>
<td>Aku (P)</td>
<td>50</td>
<td>PCR</td>
<td>$L_1$</td>
<td>87%</td>
<td>44%</td>
<td>1.01</td>
</tr>
<tr>
<td>Sali (2002)</td>
<td>Aku (P)</td>
<td>26</td>
<td>PCR</td>
<td>$L_1$</td>
<td>87%</td>
<td>30%</td>
<td>.03</td>
</tr>
<tr>
<td>Liu (2001)</td>
<td>Aku (P)</td>
<td>63</td>
<td>Flow</td>
<td>$L_1$</td>
<td>6%</td>
<td>56%</td>
<td>.13</td>
</tr>
<tr>
<td>Sali (2002)</td>
<td>Aku (P)</td>
<td>110</td>
<td>PCR</td>
<td>$L_1$</td>
<td>0%</td>
<td>40%</td>
<td>.001</td>
</tr>
<tr>
<td>Sali (2002)</td>
<td>Aku (P)</td>
<td>110</td>
<td>Flow</td>
<td>$L_1$</td>
<td>0%</td>
<td>3%</td>
<td>.001</td>
</tr>
<tr>
<td>Zanz (2014)</td>
<td>Aku (P)</td>
<td>110</td>
<td>PCR</td>
<td>$L_1$</td>
<td>67%</td>
<td>22%</td>
<td>.00</td>
</tr>
</tbody>
</table>

And this was seen in several trials that analyzed MRD prior to stem cell transplantation—those patients who are MRD-positive prior to transplant do significantly poorer compared with those who are MRD-negative. So what do we do with these patients? Continuing chemotherapy is not an option, because they are chemoresistant, and they hardly ever become MRD-negative.

Going for allogeneic stem cell transplantation seems to improve the outcome, but still the outcome is suboptimal. So here, there is the potential of new drugs that are non–cross-resistant—like immunotherapies or antibodies.

### Blinatumomab in MRD-Positive BCP-ALL: MT103-202 Trial

<table>
<thead>
<tr>
<th>Response category</th>
<th>N</th>
<th>MRD responders, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluable</td>
<td>20</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>MRD persistence</td>
<td>15</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>MRD relapse</td>
<td>5</td>
<td>4 (80%)</td>
</tr>
</tbody>
</table>

And this was exactly what was done in the blinatumomab 202 trial, where patients in first remission but with MRD persistence or MRD relapse were included and received blinatumomab—a bispecific cocktail.
T-cell engager. From the 20 patients who received blinatumomab, 80% became MRD-negative after one treatment cycle.

This translated into a favorable outcome in these patients. Nine patients proceeded to stem cell transplantation afterwards, but 11 patients did not proceed to stem cell transplantation, and nevertheless, the outcome of these patients was quite favorable.

So afterwards, there was the BLAST trial that was intended to confirm these results. Again, MRD positivity was an inclusion criterion, but here MRD levels above 10^-3 was the inclusion criterion.

And here, not only could patients in first remission be included, but also in second or later remission. And one-third of the patients were in second or later remission. MRD response was evaluated after one cycle of treatment. Here, a total of 116 patients were enrolled, and 113 were evaluable, ie, MRD markers were identified.

Outcome of these patients was as follows. The 18-month relapse-free survival was 54%.

But most of the data that have been gained for adult patients with ALL for MRD are gained for first-line treatment, and only a few data exist on the value of MRD in relapsed/refractory ALL.

This was, among others, done in the INO-VATE trial, where inotuzumab ozogamicin was administered to half of the patients, whereas the other half was randomized to the standard of care treatment.

Among the responders, MRD was measured with multicolor flow cytometry. And the group with inotuzumab had a complete MRD response in almost 80% of patients, whereas in the standard of care arm, only 28% became MRD-negative.

And looking at the outcome, you can see that the MRD-negatives in the inotuzumab arm performed best and had the highest probability of retaining the remission. However, the relapse rate was also considerable in this group of MRD-negative patients, so the meaning of MRD negativity in this setting seems to be different from the first-line setting.
And this was very nicely shown in a study by Dr. Jabbour and colleagues, who analyzed a series of patients with relapsed/refractory B-cell precursor ALL, who received either inotuzumab, blinatumomab, or a combination of chemotherapy and inotuzumab.

Among the 130 patients who received this treatment, 78 had a complete response, and these were monitored for MRD. Sixty percent of them were in first salvage treatment and 40% in the second salvage.

And, looking at MRD, patients who became MRD-negative had a trend to perform better compared with those patients who were MRD-positive.

But now, looking at the salvage status, you can see that if the patients are MRD-positive, it doesn’t matter whether they are in first salvage or second salvage.

But, if they are MRD-negative, this translates only to a better prognosis if they are in the first salvage—arguing for an earlier treatment and showing that conversion to MRD negativity seems to improve outcome, mainly earlier during therapy.

So you have to know about the clinical setting where MRD is assessed, and you also have to know about the technique with which MRD’s measured. So there are basically two different methods to analyze MRD in Ph-negative ALL. This is one, multicolor flow cytometry, and real-time quantitative PCR of immunoglobulin or T-cell receptor gene rearrangements.

Here you analyze leukemia-associated immunophenotype inserted during follow-up. And you identify a leukemia-specific immunoglobulin or T-cell receptor gene rearrangement and diagnosis, which works like a kind of molecular fingerprint of each individual leukemia. You establish a patient-specific real-time quantitative assay and use this during follow-up.

Both methods have their pros and cons. This method is quite time consuming, because you have to establish an RQ-PCR assay for each individual patient, and it’s quite expensive. But so far it has the highest sensitivity and a very high degree of standardization.

An international consortium of flow labs first tried to apply this point of the lack of standardization of multicolor flow cytometry to MRD assessment, but also tried to increase the sensitivity of this approach.

And they just published an eight-color flow cytometry assay for B-cell precursor ALL and compared it with real-time quantitative PCR. They reached a very high concordance between flow cytometry and RQ-PCR, but only if at least 4 million events were acquired.
So, this is a very important point that is also stressed on the next slide.

You see here, a comparison of the sensitivity of molecular versus flow cytometry, and the fraction of samples that were, when positive for PCR, also positive for flow cytometry. And you can see, if only 1 million events are acquired, there is a high degree of discrepancy.

In particular, at later time points where regeneration hampers the sensitivity of flow cytometry—and also, the MRD levels are lower compared with very early time points—there’s a high degree of discrepancy if you only analyze 1 million cells. This higher degree of concordance between the results of flow cytometry and PCR is only reached if at least 4 million or more cells are analyzed. This is a very important point; that the number of acquired events is crucial for the sensitivity of the analysis, and MRD negativity is not the same depending on the method you apply and the number of cells you analyze.

And as MRD is a quantitative variable, it tends to be that the lower the MRD value is, the better the prognosis. It is good to be even more sensitive than the current approaches.

There is one method coming up that seems to be more sensitive compared with the current standard, and this is the IG/TR-NGS.

There, you perform at initial diagnosis, multiplex PCR to amplify all potential immunoglobulin gene rearrangements, not only of the leukemia, but also of accompanying polyclonal B cells. And then, you sequence all the amplicons.

At initial diagnosis, the dominant sequence will be the leukemia sequence. And then you apply exactly the same multiplex PCR at follow-up. Again, sequence all the amplicons, and then you will probably have much more polyclonal rearrangements. You can also re-identify the leukemia-specific sequence bioinformatically.

And with this approach—as it is very specific and you can analyze millions of reads in parallel—it also has the promise to be more sensitive. The beauty of the approach is that first you get rid of this need for clone-specific assays, and you can also gain insights into the polyclonal repertoire and also oligoclonality or clonal-evolution phenomena.

If you analyze enough DNA—so, again, this is the prerequisite for high sensitivity—then you can reach a sensitivity of $10^{-6}$ with this approach.

Comparisons with the current molecular gold standard, RQ-PCR, show that there is a high concordance between both methods. However, there are some samples that are positive with one method and negative with the other, and the other way around.
The group of Jan Trka, who performed this analysis on children with B-cell precursor ALL, did not only compare the methods, but they also evaluated the prognostic value of MRD measured with NGS compared with RQ-PCR at day 33 in a BFM-based treatment. They saw that NGS predicted relapse was even more precise than RQ-PCR. And interestingly, the rate of MRD positivity in the NGS group was lower compared with the RQ-PCR—so, there is the hypothesis that RQ-PCR potentially included some false positives; so that NGS is the more specific method here compared with RQ-PCR.

Jan Trka’s group also analyzed the polyclonal B-cell repertoire, which, as I said, can be nicely done with this approach, and they saw that patients who did not relapse had a broader B-cell repertoire at the time of MRD measurement, and that depending on the B-cell repertoire, outcome was different.

However, this is only a first analysis, and of course standardization and validation of this approach has to be performed in a multicenter and multidisciplinary setting in the context of clinical trials. And again, there is an international consortium that focuses on this.

So what is the best method to use in ALL? It depends. It depends on the aim, if you want to identify high-risk patients with high-level MRD, a method that is less sensitive will be sufficient. If you want to identify very low levels, of course, you have to have a more sensitive method.

The number and time points of MRD assessment are relevant, and also, of course, the formally used method to measure MRD, because risk group stratification might be different depending on the technique you use, and also, the availability of the resources.

But does MRD tell the whole story? First, what is very important to know is that MRD negativity is not identical to eradication of the disease, but only to the level of residual disease below the limit of the MRD technique. Therefore, knowledge of the respective methodology is important to correctly interpret the results.

Second, MRD negativity at different time points does not have
the same meaning. So, very early MRD assessment has a different impact than a late MRD assessment, where you identify the MRD persistence. It’s a time-dependent variable.

And third, the treatment elements that are given after MRD assessment influence the prognostic impact of MRD. Of course, if you perform stem cell transplantation, or continue with chemotherapy, or even stop treatment, this influences the impact of the MRD value.

So, summarizing, MRD has the beauty that it integrates different patient-specific, treatment-specific, and leukemia-specific factors and allows a refined assessment of treatment efficacy prior to MRD measurement.

But you have to be aware of limitations of the method. The sensitivity is important. It’s a time-dependent parameter, and also, the efficacy of treatment elements after MRD measurement influences the prognostic impact.

However, MRD after induction therapy in first-line treatment is the most important independent prognostic factor in adult and childhood ALL. MRD-guided treatment is feasible, but a prerequisite is a standardized measurement and interpretation.

Dr. DeAngelo: I will proceed with a discussion of some of the monoclonal antibody–drug conjugates. But of course, I also have a case. This is Robert, who is actually one of my patients—a male patient with relapsed/refractory ALL.

Back in 2009, when I first met Robert—a 42-year-old man diagnosed with Philadelphia-negative pre-B ALL—he had a white blood cell count of less than 50,000—so, standard risk, normal cytogenetics. He was treated as per ECOG 2993, the international trial that was published by Gladstone and colleagues. He achieved a remission status.

Two years later, he relapsed towards the end of maintenance actually—all too common a feature. He had no sibling donors. He was treated on a clinical trial at that point with clofarabine-based therapy—clofarabine plus high-dose Ara-C—and unfortunately, failed to enter remission. He then received hyper-CVAD, and again, failed to enter into remission.
Treatment Evolution in Acute Lymphoblastic Leukemia: Insight on Novel Antibody Technologies and Immunotherapy Advances

So let’s try and review this. What ended up happening at this time was there was a clinical trial open back in 2011, and he was re-induced with inotuzumab, he achieved a CR, and then he went in for an unrelated fully-matched transplant. He’s actually still alive now—almost 5 years out.

So, as I alluded to at the very beginning of my preface, before Dr. Brüggemann’s talk, there’s been dramatic improvements in the outcomes of children. This is just one slide showing 5-year event-free survival in a series of trials done at the Dana-Farber Cancer Institute from 1981 to 1991. And there are others.

Every cooperative group or large center has large studies like this, showing a dramatic improvement, really without a lot of changes in the therapy, just by re-stratification of standard versus high-risk and dose intensification of those children with high-risk disease.

Unfortunately, the adult patients, those 18 years and older, have not held pace, and the study here on the right that I elected to show is from the CALGB by Dr. Richard Larson—the CALGB 9111—showing about a 40% to 45% 3-year disease-free survival—really, dramatically different.

One of my favorite slides that I like to show that addresses the ultimate clinical need for new agents in the relapse setting is this one from the ECOG 2993 study. This shows 609 patients who had relapsed on the study, and the simple question was asked, “Well, how did they do?”

Well, they do poorly. They do really, really poorly, with a 5-year overall survival of 7%. And it was regardless of whether these patients were consolidated with an allogeneic—either related or unrelated—transplant, chemotherapy, or an autologous transplant. All three modalities did not salvage these very difficult-to-treat patients.

So, with the new therapies and monoclonal antibodies, which is the topic that I’ve been assigned, there are a lot of agents out there.

### Monoclonal Antibodies and Their Targets in ALL

<table>
<thead>
<tr>
<th>Antigen Target</th>
<th>Antibodies</th>
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<tbody>
<tr>
<td>CD19</td>
<td>Blinatumomab</td>
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<tr>
<td></td>
<td>SGN19A</td>
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<td></td>
<td>SAR3419</td>
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<tr>
<td>CD20</td>
<td>Rituximab</td>
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<tr>
<td></td>
<td>Ofatumumab</td>
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<td>CD22</td>
<td>Epratuzumab</td>
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<td></td>
<td>Indolizumab</td>
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<td></td>
<td>Combizumab</td>
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<td></td>
<td>BL22, H22</td>
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<tr>
<td>CD52</td>
<td>Alemtuzumab</td>
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So, let’s start with CD20. CD20 is expressed in about 40% of patients with B-cell ALL. It has been shown by the group at MD Anderson to be associated with an adverse prognosis, which obviously suggests that targeting this agent may affect outcome.

### CD20 in ALL

- CD20 is expressed in about 40% of patients with B-cell ALL
- CD20 expression is associated with an adverse prognosis in adult ALL
- This suggests that targeting CD20 may affect outcome

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One of the things that has been noted is that CD20—although it is expressed at the time of analysis—when you initiate therapy, the CD20 density or expression goes up, which also enhances its ability to be targeted.

A retrospective analysis by Debbie Thomas at the MD Anderson Cancer Center (MDACC) showed that, based on their hyper-CVAD regimen, patients who were CD20-negative had a better outcome than those who were CD20-positive.

This was also done—and shown to be helpful—by the German group with the GMALL study. And here, rituximab was added to those patients with CD20-positive standard-risk ALL.

It was almost 300 patients: 200 of them received rituximab—as compared with the other 66 patients, who did not—in a randomized fashion. You can see in the upper curve that the remission duration clearly favored those patients who received rituximab as compared with those who did not, and this is shown in the lower curve, as well, in terms of survival.
Those of you who were at ASH last year saw Dr. Maury present in the plenary talk, the addition of rituximab to the GRAALL regimen, which is a pediatric-inspired regimen for younger patients. So these patients were 18 to 60 years with pre-B ALL who were CD20-positive. So this is, as I mentioned, pediatric inspired. Sixteen to 18 doses of rituximab were infused, with a median follow-up of about 30 months.

And what Dr. Maury reported last year—and this was just published a few weeks ago in the *New England Journal of Medicine*—is that the CR rate was not dramatically different, nor was the MRD rate at the end of remission, nor at the end of consolidation. But what was improved was the event-free survival and the overall survival. Of course, that’s what matters.

There are other anti-CD20 antibodies. Ofatumumab—approved for chronic lymphocytic leukemia—was added in a very similar fashion as rituximab in hyper-CVAD; that is, two doses per cycle for the first four cycles of hyper-CVAD.

This led a small nonrandomized study to a very high remission rate, a very high MRD-negative rate at the end of CR, and a high MRD rate eventually at the end of consolidation, which was extremely well tolerated, with very few early induction deaths.

This led a small nonrandomized study to a very high remission rate, a very high MRD-negative rate at the end of CR, and a high MRD rate eventually at the end of consolidation, which was extremely well tolerated, with very few early induction deaths.

And here you have both the overall survival as well as the remission duration. The overall survival is in brown, the CR remission duration is in blue—showing, again, nonrandomized—just in comparison with the historical control—really dramatic improvements in both the 3-year overall survival, as well as remission duration.
So, in conclusion of this part of the talk with CD20, these are encouraging results. It hasn’t been established—the use of rituximab in older patients—defined as over 60 years, the GRAAL capped patients at 59 years. The GRAAL-2005—that was the plenary talk last year and in the *New England Journal of Medicine*—really established the addition of rituximab in these pediatric-based or pediatric-inspired studies.

Other anti-CD20 antibodies are in development—and probably deserve some attention—for use in these difficult-to-treat patients.

Moving on to CD19-targeted monoclonal antibodies; denintuzumab mafodotin. This is one addressed against CD19. This monomethyl auristatin compound is conjugated to a monoclonal antibody against CD19.

It’s the same idea. The antibody gets bound to the surface, gets internalized. In the acid environment of the lysosome, the monomethyl auristatin compound gets released. It’s a tubulin inhibitor—hopefully, leading to cell cycle arrest and apoptosis.

And you can see here, the composite complete remission rate on the weekly schedule was about 20%, and on the every-3-week schedule it was about 38%. Interestingly, there was about a 50% response rate in refractory Philadelphia-positive ALL—that probably deserves some more attention for this disease.

The maximum tolerated dose was never established, but one of the interesting toxicities is this keratitis or ocular toxicity, that on first impression was dose-limiting, but steroid eye-drop prophylaxis starting several days before was able to ameliorate much of the toxicity.
This is the overall survival of the phase 1 study, with a median survival of 18 weeks. And so this will be in further development.

### CD22 Is an Attractive Therapeutic Target

- **CD22** is expressed on the malignant cells in >90% of B-lymphoid malignancies.
- **CD22** is internalized upon antibody binding.
- **CD22** is not shed into the extracellular environment.

Moving on to CD22—CD22 is a reasonable target. It is expressed on the vast majority, but not all malignant cells in B-lymphoid malignancies—about 90%—whereas CD19 has a more ubiquitous expression. CD22 is internalized upon antibody binding, and it is not shed into the extracellular environment. Therefore, it seems to be an attractive target based on these three qualities.

### Epratuzumab in Pediatric ALL

- **Idea:** Introduce new agents at first relapse along with VCR/PEG/PRED/DOKO backbone.
- **COG study A3910D2:**
  - Add weekly or q2w epratuzumab, and anti-CD22 mAb.
  - Compare to historical controls.
- **CR rate of 67% with either schedule:**
  - No better than historical CR rate = 66%.
  - More pts were MRD-negative than expected.
- **IntReALL (International Study for Treatment of Childhood Relapsed ALL): phase 3 trial in children with relapsed ALL.

Epratuzumab is a naked monoclonal antibody against CD22. It’s been studied primarily in pediatric patients. There were several large TACL (Therapeutic Advances in Childhood Leukemia & Lymphoma) children’s cooperative group studies. In the TACL, what they have as a backbone is this four-drug re-induction for kids with relapsed disease consisting of vincristine, pegylated asparaginase, prednisone, and doxorubicin. And what they’ve done very successfully is they just added a novel agent to this four-drug background.

When epratuzumab was added to that, the remission rate was about 67%, which is about the same as the historical controls. But the MRD negativity was much higher, arguing that there may be a deeper remission in these children.

There is the IntReALL study, which is being done primarily in Europe—adding epratuzumab to a regimen for relapsed pediatric ALL. So, obviously a randomized study is going to be important.

### SWOG0910: Cytarabine and Clofarabine + Epratuzumab for Relapsed/Refractory ALL

- **Phase 2 Study**
  - **Cytarabine** 40 mg/m²/day iv days 2-6 and **Cytarabine** 1 g/m² day on days 1-5
  - **Epratuzumab** 380 mg/m² iv days 4, 11, 18, and 25
- **CR/CRi of 52%; compared to 17% in a prior trial of cytarabine plus clofarabine alone**
- **Encouraging results, but a randomized trial is needed to answer this question**

Dr. Advani from the Cleveland Clinic presented and has recently published a small phase 2 from SWOG—adding epratuzumab to a regimen that’s been studied by the SWOG group—clofarabine plus cytarabine. In her study, they had a remission rate of about 52% compared with 17% on the prior trial of cytarabine and clofarabine, suggesting an advantage from the addition of epratuzumab. But obviously, randomized trials will be necessary to sort this out.

### Inotuzumab Ozogamicin (InO)

- **IntAct Linker:**
  - 4-(4-acetylephenoxy) butanoic acid dimethyl hydrazide

- **Intact ADC**

- **N-Acetyl + Calicheamicin**

- **Average loading of calicheamicin derivative on mAb is 5-6 moles of calicheamicin/mole of mAb (range, 3-9) for InO**

- **~100% of mAbs conjugated**

What about antibody–drug conjugates against CD22? Well, here we have inotuzumab. Dr. Brüggemann has already introduced the topic of inotuzumab. Inotuzumab is a monoclonal antibody against CD22 that is linked to calicheamicin. Calicheamicin is the agent used in gemtuzumab.

The average loading of calicheamicin is about 5 to 6 moles of calicheamicin per mole of antibody. Within the antibody complex, almost 100% of the monoclonal antibodies are conjugated to at least one molecule of calicheamicin.
Similar to any antibody–drug conjugate, just like the SGN-CD19A, when it’s bound to CD22, it gets internalized, and the calicheamicin derivative is then released from the acidic lysosome environment, which then intercalates DNA, leading to apoptosis.

There were several phase 1/2 studies that were done. There were two studies done at MD Anderson Cancer Center, and those have been published already. One was an every-4-week regimen with a dose of 1.8 mg/m² per cycle. The overall response rate was about 57%.

A second phase 2 study was done at MD Anderson Cancer Center, but this time using a weekly dosing, where that 1.8 mg/m² was spread out over 3 weeks out of 4—showing a very similar overall response rate.

Then, there was a multicenter trial led primarily in the United States that really looked to see, “What was the maximum dose that could be delivered?” The dose-limiting toxicity was 1.8 mg/m², which was the highest dose that was achieved, and we were able to demonstrate a 68% overall response rate. Dr. Advani confirmed that in patients with salvage 2 or higher—also showing a very high overall response rate.

What Dr. Brüggemann alluded to was not only that these patients were able to achieve a high remission rate, but that the immunotherapeutic response was that they got a deep remission, as well, and that—at least in this phase 1 multicenter trial—of those patients who responded, 86% were MRD-negative.

Now, the way that MRD was detected in this study was by multicolor flow cytometry, with 10⁻⁴ as the bar. And this was done in the lab in Seattle at the University of Washington, as a reference lab.

This led to the randomized phase 3 study—the INO-VATE study—which is inotuzumab versus chemotherapy. This is a 1-to-1 randomization for patients in first or second salvage. It did include patients who were Philadelphia-positive, which is an important thing to remember.

A total of 326 patients were enrolled in this study. The inotuzumab dosing was on a weekly schedule as the phase 1 at 1.8 mg/m² per cycle. And then in patients who went into remission, the dose was reduced to 1.5 mg/m².

And then patients were either randomized to inotuzumab or investigator’s choice. The chemotherapeutic options that they could choose from were FLAG, high-dose Ara-C, or cytarabine-mitoxantrone. Most physicians chose FLAG.

Patients were stratified based on one of three criteria—duration of first remission (less than 12 months or greater than 12 months);
salvage 2 versus salvage 1; and aged younger than 55 years or older than 55 years.

### INO-VATE: Treatment Response

<table>
<thead>
<tr>
<th></th>
<th>INO</th>
<th>SOC</th>
<th>1-sided (P)</th>
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<tbody>
<tr>
<td>RF</td>
<td>100</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>CR/CRi (%)</td>
<td>89.7</td>
<td>33.3</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>CR</td>
<td>38.8</td>
<td>10.8</td>
<td>.0086</td>
</tr>
<tr>
<td>CRi</td>
<td>45.0</td>
<td>13.7</td>
<td>&lt; .0001</td>
</tr>
</tbody>
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MRD negativity among responders, in % [95% CI]:

- CR/CRi: 68.8 (98.6-88.7) vs 13.2 (81-81) \(P < .0001\)
- CR: 36.9 (98.7-79.2) vs 19.1 (93-96.7) \(P < .0001\)
- CRi: 54.0 (88.4-58.5) vs 15.3 (3.1-54.5) \(P = .0014\)

1. In both arms, most patients achieved CR/CRi in cycle 1 (80, 73%; SOC, 91%).
2. Kaplan-Meier curves now, this is the remission duration curve, which favored inotuzumab—4.6 versus 3.1 months with a one-sided \(P\) value of .0169.

And so, the primary endpoint was the remission rate. You’ve seen this slide. The inotuzumab arm had 81% of patients achieve a complete remission or CRi, compared with 33% of patients receiving standard of care chemotherapy, with a high level of statistically significant responses.

And of those patients who achieved a remission, almost 80% in the inotuzumab arm achieved an MRD-negative status compared with 28%. And there were similar responses in the CR and CRi.

### Kaplan-Meier Curves

- Remission duration curve, which favored inotuzumab—4.6 versus 3.1 months, with a one-sided \(P\) value of .0169.
- Progression-free survival, also favoring inotuzumab, with 5 versus 1.8 months, which was heavily statistically significant.

If you look by stratification factor, in favor of inotuzumab, it was regardless of if patients had early or late relapse—defined as 12 months. Both patients who were in salvage 1 or salvage 2 seemed to benefit, as well as younger and older patients—both had a very similar benefit to inotuzumab over standard-of-care chemotherapy.

### CR/CRi by Stratification Factors

<table>
<thead>
<tr>
<th></th>
<th>INO</th>
<th>SOC</th>
<th>1-sided (P)</th>
<th>% Rate Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>80.7</td>
<td>23.3</td>
<td>&lt; .0001</td>
<td>47.4 (34-61)</td>
</tr>
<tr>
<td>&lt;12 mo</td>
<td>77.7</td>
<td>27.9</td>
<td>&lt; .0001</td>
<td>40.5 (34-67)</td>
</tr>
<tr>
<td>≥12 mo</td>
<td>90.9</td>
<td>45.6</td>
<td>&lt; .0001</td>
<td>41.4 (18.6-64)</td>
</tr>
<tr>
<td>Salvage 1</td>
<td>87.7</td>
<td>31.3</td>
<td>&lt; .0001</td>
<td>65.3 (41.4-72)</td>
</tr>
<tr>
<td>Salvage 2</td>
<td>88.7</td>
<td>37.9</td>
<td>.0134</td>
<td>20.7 (2.68)</td>
</tr>
<tr>
<td>Age ≤55</td>
<td>80.3</td>
<td>36.1</td>
<td>&lt; .0001</td>
<td>44.2 (27.6-62)</td>
</tr>
<tr>
<td>Age &gt;55</td>
<td>81.4</td>
<td>28.6</td>
<td>&lt; .0001</td>
<td>52.8 (3.5-78)</td>
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* Analysis of CRIROC based on a modified ITT population excluding 15 patients from the SOC arm (143 in the SOC arm and 148 in the INO arm).

This is the overall survival. Although there was a \(P\) value of .02, the \(P\) value that was necessary, because there was some a burn in the CR, was supposed to be .01—although, it’s still statistically significant when you look at the 2-year probability of survival, which was 23% versus 10% for the standard of care arm.
One of the problems with calicheamicin, as you all remember from the gemtuzumab days in the United States, is veno-occlusive disease, which was seen rarely, but was seen in the inotuzumab arm—13% for inotuzumab patients versus 1% for standard of care.

There were only five patients who developed veno-occlusive disease during therapy. Most of the cases occurred after transplant—specifically after second transplant—because some of the patients who were enrolled had already failed transplant.

The factors that contributed to the development of veno-occlusive disease were the use of a double-alkylating regimen during chemotherapy conditioning prior to transplant and age.

Once you have an active drug, it’s nice to combine it with therapy. And my colleagues at the MD Anderson Cancer Center—Dr. Jabbour specifically—were able to add inotuzumab to a dose reduction in what was called mini-hyper-CVD.

In this regimen, the cyclophosphamide dose has been reduced, the dexamethasone has been reduced, the anthracycline has essentially been replaced with inotuzumab, and then the patients on the even cycles, as you know, get methotrexate and cytarabine. Rituximab is added for those patients who are CD20-positive as well.

The remission rate was about 53% of CR. CRp was 19%, for an overall remission rate of 77% in this relapsed-refractory group, with MRD negativity of about 37%.

And if you look at the 2-year progression-free and overall survival, they were 60% and 32%, respectively, in this combination therapy of inotuzumab plus mini-hyper-CVD.
Inotuzumab plus mini-hyper-CVD greatly benefited those patients who were salvage 1 versus salvage 2 or 3. Again, earlier treatment with these novel agents seems to be better—that’ll be a theme.

And, if you remember, from the single-agent data that we talked about, it seems, based on historical controls, that the addition of this low-dose chemotherapy, or mini-hyper-CVD, to inotuzumab seems to improve survival over single-agent therapy.

This has led us to propose the new successor to the US Intergroup CALGB 10403 trial. This was a large pediatric-inspired trial that Wendy Stock led and presented at ASH a couple years ago in patients 16 to 40.

And so, in this study, it’ll be the same chemotherapy backbone as CALGB 10403, with just some minor tweaking. Patients will be stratified based on age and on the presence or absence of a Philadelphia-like signature—something that we’re not discussing too much today, but that has clearly been an adverse feature—and on CD20 status.

Patients will then be randomized to receive two cycles of inotuzumab or not during consolidation, prior to receiving the rest of their consolidation therapy.

We think that the addition of inotuzumab will improve the deepening or the MRD-negative rate, and hopefully improve survival. It’ll be done early enough not to hinder, if patients are going to go to transplant.

So, in conclusion, monoclonal antibodies have high overall response rates. I would argue that ALL has been treated with multi-agent chemotherapy for decades, and so, we’re now seeing very high single-agent therapy rates with high MRD-negative rates.

There are some interesting toxicities—I showed you inotuzumab, which has thrombocytopenia, some liver function abnormalities, and veno-occlusive disease, and the SGN-CD19A, or denintuzumab, which has some ocular toxicity.

Randomized phase 3 studies show that inotuzumab seems to be superior to standard of care, with improvement in CR and MRD rates, as well as in progression and overall survival, and really needs to be added to chemotherapy for improvement in overall response rates.

So with that, I’ll end. I thank you for your attention. I’d like to introduce Dr. Jabbour from the MD Anderson Cancer Center, and he’ll be discussing for you the current role and future of the bispecific antibodies in ALL. Thank you very much.
Treatment Evolution in Acute Lymphoblastic Leukemia: Insight on Novel Antibody Technologies and Immunotherapy Advances

The Current Role and Future of Bispecific Antibodies in ALL

Elias Jabbour, MD
The University of Texas MD Anderson Cancer Center
Houston, Texas

Case: Jane Experiences Toxicity but Achieves a CR

So, before we start discussing blinatumomab, I’ll go over the program. So, essentially, this patient, 36 hours—a day and a half—after starting blinatumomab, had some fever—39.3°C—and slight tachycardia. You get a call from a nurse, “What’s going on?” Blood culture and x-ray were done, and urine culture. She was started on an antibiotic. We gave her dexamethasone 8 mg every 8 hours, and then tapered it for 3 or 4 days.

She did well. In addition to that, during cycle 1 she had some headaches, grade 2. We coded the cytokine release syndrome, grade 1. She had tremors and some fatigue.

She received a second course of therapy: 4 weeks on, 2 weeks off, 4 weeks on, 2 weeks off. And during cycle 2, she again had neurotoxicity, grade 2. She had increased LFTs and some dizziness.

So, what was her response to therapy? We did a bone marrow at day 15 of cycle 1, which showed hypoplastic marrow. And when we assessed for MRD, she was MRD-negative already at day 15.

At day 29, she still had 1% of blasts. She started recovering her count, although she did not reach CHR (complete hematologic response). And therefore, she was coded as complete remission with partial hematologic recovery.

At day 42, 6 weeks from the first cycle of blinatumomab, before starting the second cycle, we repeated the workup—0% of blasts. We started cycle 2 at 28 mcg/day. She did well, and at the end of the cycle she achieved a CR, with a platelet count above 100And

Dr. Jabbour: Good evening, everybody. I want to thank the organizers for inviting me to this symposium tonight.

So my patient is Jane, a 27-year-old. She has refractory ALL. She was diagnosed back in December 2010 with B-cell ALL. She was treated in the community setting with a linker regimen. She achieved a CR, had consolidation, and then maintenance for 17 months.

Then in October 2011, she was found to be in relapse. She received clofarabine, etoposide, and cyclophosphamide—she was refractory to this regimen.

And then, she had an asparaginase-based regimen—the MOAD—and she was refractory. And then in May 2012, she was referred to a clinic in Houston, where, at that time, we had the blinatumomab trial available to us.

So, we screened her. She was found to be eligible. And therefore, the patient was enrolled, and we started her on blinatumomab therapy, where we started at 9 mcg/day for the first 7 days, and from day 8 and beyond, we did escalate to 28 mcg/day.
therefore, Jane had a donor and received transplantation. A success story.

But a couple of years ago, we had nothing to offer this patient. You could give anything you want, and they'd live very shortly. Now, we live in an exciting time in ALL, because we want to cure this patient, and we're going to make cancer history. You know, I work with MD Anderson Cancer Center, and the slogan of our hospital is, "Making cancer history." So we have a big challenge, and we need to match it.

What do we have for that? I think today the major breakthrough in ALL therapies are the tyrosine kinase inhibitors in Philadelphia-positive ALL, and the monoclonal antibodies—the bispecific and the regular monoclonals. Dr. DeAngelo went over the CD20 and CD22, and I'm covering CD19, essentially blinatumomab. And Dr. Grupp at the end will enlighten us on CAR T cells.

So CD19 is expressed in almost everybody with B-cell ALL, and it's normal to go after this target.

Dr. DeAngelo showed you this curve. As I said to you, in 2011 we had nothing to offer these patients. So you can give them any chemotherapy and the survival will be only 4.3 months, and they spend half of them, at least, in the hospital, and the other half in the EC room before, unfortunately, they pass away.
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**Salvage Therapy for ALL: Treatment Options**

- Depends on CRD1 duration
- Approved drugs: nelarabine (T-ALL), clofarabine, liposomal VCR

<table>
<thead>
<tr>
<th>Regimen</th>
<th>% CR</th>
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<tr>
<td>Monoclonals (inotuzumab ozogamicin, blinatumomab)</td>
<td>50%-70%</td>
</tr>
<tr>
<td>CAR T cells</td>
<td>70%-60%</td>
</tr>
<tr>
<td>Hyper-CVAD (± augmented), augmented BFM</td>
<td>30%-70%</td>
</tr>
<tr>
<td>FLAG-IDA, BIDA ± asp</td>
<td>30%-40%</td>
</tr>
<tr>
<td>MOAD</td>
<td>30%</td>
</tr>
<tr>
<td>If T-ALL: Nelaarbine</td>
<td></td>
</tr>
<tr>
<td>If Ph-positive ALL: Add TKI</td>
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So, definitely, we’re witnessing great success, and people may underestimate it, but just if you compare it with what we had before—if you put things in perspective—you can appreciate the success we’ve so far achieved.

So historically, what did we have for ALL when they relapsed? The chemotherapies—and all of them can induce about 30% responses and very short survival. Today, in immunotherapy, we have the monoclonal antibodies and the CAR T cells.

At least with the monoclonals, we can have responses up to 70% or 80%, and if used in first salvage, we can have survival at 3 years of 50%. The median survival is still 7.7 months for everybody, but if you use them early enough, we can have a better outcome.

**Bispecific Antibodies Work in Several Different Ways**

- Recruiting of T cells or NK cells via binding to tumor cell surface antigens, as well as to immune cells (TregMabs, BiTEs, DARTs, and TandAbs)

So, blinatumomab, or bispecific antibodies—essentially, these are constructed antibodies engineered to capture T cells or NK cells and to induce killing B cells—what we call the “serial killers.”

**Mode of Action of BiTE Antibody Blinatumomab**

Here, the blinatumomab was anti-CD19 and anti-CD3. And here’s a cartoon showing you the antibody with two arms to capture the T cells and put them in contact with the B cells and induce T-cell cytotoxicity. That’s why it’s called “serial killer”—killing one B cell at a time, and finally killing all the B cells available and curing this leukemia.

**ALL: Probability of Survival by Molecular MRD**

So first, the drug was assessed in MRD, in Europe, mainly in Germany, under the leadership of Dr. Max Topp; they assessed the drug in minimal disease, where 20 patients were treated.

And we know that these patients had a very bad outcome. These are the data from GMALL reported by Dr. Gökbuget—where patients with MRD positivity had a survival of 6 to 7 months.

So in the United States and in Europe, and across the world, we don’t have any drug approved for MRD-positive disease, although we know that these patients do very poorly—and I think that they are even equivalent to relapses, or worse. In the United States, we were lucky to get the drug, because our payers approved it for relapses, and patients do die very quickly; so we can get the drug, in our hands, for the benefit of the patients.
In Spain, Dr. Ribera assessed the outcome of transplantation because we did not have anything for them. You assess for MRD at different time points, and those who are MRD-positive, they have a very poor outcome, so you go for transplantation. The question is: Can we save them with transplant? Can we improve their outcome?

The answer is no. In fact, in this study, we had more than 300 patients assessed for MRD by flow cytometry. Those who were MRD-positive received transplantation with the aim to see if we could improve the outcome. And in fact, those with a transplant did not have a better outcome or were equivalent to those with good features who did not receive a transplant.

Therefore, we needed something for these patients, and, as I said, blinatumomab was assessed for MRD first. There was the pivotal trial at the beginning with 20 patients, and later on there was a confirmation, what we called the BLAST trial by Dr. Gökbuget. In fact, two-thirds of the patients were able to achieve MRD negativity—the majority of them through one cycle of therapy, less so in the second remission and third remission. But these patients were referred to transplantation. And the question is, is it helpful or beneficial? What are the data on survival and outcome?

Here are the endpoints that were met—113 who had blinatumomab, and 103 who had the full course. You have 80% responses seen in first CR, and in second CR, and beyond. Most of the responses were achieved after one course of therapy.

If you had a donor, you went for transplantation. Seventy-five percent did get a transplant done. The primary endpoint was MRD negativity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>% MRD-negative</th>
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<tbody>
<tr>
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<td>82</td>
</tr>
<tr>
<td>CRD2</td>
<td>70</td>
<td>67</td>
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<td>MRD10^-1</td>
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<tr>
<td>MRD10^-2</td>
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1. Gökbuget (et al.) ASH Annual Meeting (2016); Abstract 175

<table>
<thead>
<tr>
<th>BLAST: Complete MRD Response Within 1 Cycle (MRD-Positive B-ALL)</th>
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<tr>
<td><strong>Primary endpoint full analysis set (n = 113)</strong></td>
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<tr>
<td>Patients with evaluable MRD</td>
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<tr>
<td>Primary endpoint</td>
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<td>Exploratory endpoint</td>
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1. Gökbuget (et al.) ASH Annual Meeting (2016); Abstract 175

Blinatumomab in MRD-Positive ALL (Cont’d)

<table>
<thead>
<tr>
<th>Median, mo</th>
<th>Overall</th>
<th>MRD-negative</th>
<th>MRD-positive</th>
<th>P</th>
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<tr>
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<tr>
<td>RFS</td>
<td>19</td>
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<td>DOR</td>
<td>NR</td>
<td>NR</td>
<td>15</td>
<td>.015</td>
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| No difference in OS (HR = 1.39; P = .37) and RFS (HR = 0.89; P = .73) between allo-SCT vs no allo-SCT |

1. Gökbuget (et al.) ASH Annual Meeting (2016); Abstract 37
The median follow-up was 3 and a half years, and the survival for the whole cohort was 3 years. Keep in mind, MRD-positive disease patients—their survival is 6 to 7 months. So, whole cohorts who have 3 years of survival—that is a great achievement. And obviously, if you do respond, you do even better with improvement in survival.

So the investigators did assess with the landmark analysis, the impact of transplantation in first CR and second CR. Here's a Kaplan-Meier curve for survival, highlighting the advantage for responders, where the survival has been almost 40 months, and, as is normal, those who did not respond had a shorter survival.

But then they did the landmark analysis to assess the impact of transplantation in first CR and second CR. In first-CR patients, transplant did not seem to improve the outcome. We had a higher rate of transplant-related mortality and morbidity that may negate the benefit of blinatumomab, while in second CR and beyond, you have an advantage favoring blinatumomab.

Now, here, I'm not saying that transplant is not helpful, but hopefully one day we will not need transplantation. Do not transplant a patient with MRD positivity. Try to convert them into MRD negativity before you go for transplantation, if needed.

Blinatumomab was then taken to the relapsed/refractory setting with a first trial on 36 patients, most of them in salvage 1, and showed great efficacy.

That led to a phase 2 study on 189 patients, which led to the approval of blinatumomab 2 years ago—exactly 2 years ago, in San Francisco at ASH—where patients, relapsed/refractory, salvage 1, 2, and beyond were given blinatumomab in a dose-escalation fashion; 9 mcg for the first week, then 28 for the rest of the 4 weeks, and then 28 per day moving to the second week and beyond.

You got up to two cycles to induce a response, although most of the responses did take place after one course of therapy. And if you had a donor, you went for transplantation. If not, you went for further cycles—up to three—to consolidate the response. The primary endpoint was response rate. This was a phase 2 study.
A 43% response rate—CR and CR without complete hematologic recovery. The median survival was 6 months. You have to keep in mind, in this trial, most of the patients enrolled were salvage 2 and beyond. We know—and I will show the data from the TOWER trial—that the earlier you use the drug, the higher your response rate, and the more durable the response.

So, based on these data, the drug was approved by the FDA for patients who have Ph-negative disease with a relapsed-refractory setting.

Therefore, there was a confirmation of these findings in what they called the TOWER trial. It’s a randomized study where patients with relapsed/refractory Philadelphia-negative ALL were randomized 2:1 to either blinatumomab or investigator’s choice, mainly FLAG, HIDAC, or high-dose Ara-C.

In contrast, with the INO-VATE trial reported by Dr. DeAngelo, patients who had Philadelphia-positive disease were not enrolled in this study, and patients who received blinatumomab did get up to two cycles of induction, then consolidation. Those who did not get transplantation and responded could benefit from a maintenance program where blinatumomab was given 4 weeks, every 8 weeks for the first year of treatment. The primary endpoint was overall survival.

This study was an international multicenter study in the USA and in Europe, and then in 2014, the drug was approved in the USA. Therefore, the study shut down in the USA and kept accrual elsewhere.
How about prior salvage therapy and transplantation? I mentioned to you that most of the patients enrolled were mainly in Europe, where patients do receive transplantation in first remission more often than in the United States. Here, 35% of the patients enrolled have already received transplantation and failed—whether they received it in the refractory setting or in consolidation. These are mixed here.

You have only a minority who were primary refractory, which is typical in this disease, where most of patients respond to first salvage. And that was true, as well, in the intent-to-treat, as well as in those who were treated.

You have the overall intent-to-treat as well as treated. Responses were seen at a higher rate with blinatumomab. Whether you consider all responses, CR, or CRh—44% versus 25%—treated, similar numbers can be seen for both arms, proving the superiority of blinatumomab compared with standard of care.

I’d like to remind you, the rate of responses observed in phase 2 were exactly the same, and patients enrolled here were salvage 2, 3, and beyond.

Now, survival was 7.7 months for the blinatumomab arm. With 2-year survival, we’re at 23%. Similar numbers to what you’ve seen from Dr. DeAngelo with the INO-VATE trial, with the standard of care arm—historically, this was the chemotherapy with a survival of 4 months—exactly what we have here.

In the INO-VATE trial, we had better survival for the standard of care arm, which was 6.7 months, and the P value was significant. So, we have a higher response rate, we have high MRD negativity, and we have better survival in patients who are in salvage 1, 2, 3, and beyond.
So, a forest plot to see whether there are any subsets of patients who did not benefit from blinatumomab—as you can see, across the board, blinatumomab was better than standard of care.

Now, as you heard from my colleagues, salvage 2 and beyond, they do benefit, but not to the same extent as patients treated in salvage 1. What that means is that if you have somebody relapsing, if you’re going to go for heavy drugs or at least expensive or fancy, do it in first salvage, because if you’re going to cure this patient, you will cure them in the first salvage. Do not wait for multiple relapses—then, no matter what you do, the outcome is going to be dismal.

How about safety concerns? What do we see with blinatumomab? There’s some concern about myelosuppression—seen at a lower rate. And you see with the standard of care, when you give chemotherapy, you’re going to have myelosuppression with hyper-CVAD, FLAG, or whatever. We do see it at a lower rate with blinatumomab, and we do see fewer infections.

There was concern about the neurologic events and the cytokine release syndrome—seen only at 5% and 9%, respectively, which is not bad at all.

Now, where are we going with that? Okay, blinatumomab is a great drug. In the relapsed setting we can cure patients—maybe in salvage 1.1 want to take these drugs to the front line. Ideally, if these are so effective, they should be effective in the front line too, correct?

And if you use them, they are not myelosuppressive. They don’t cause enormous safety concerns. Therefore, we can combine them with chemotherapy. The idea is to minimize chemotherapy and minimize safety concerns, and to have better, deeper responses—what you call eradication of minimal disease—and then cure them.

In Houston, this is our front-line trial right now—instead of giving eight cycles of hyper-CVAD, we give four—half of them—and then we add four weeks of blinatumomab regardless of MRD. We can stratify them by MRD negativity or positivity at the start of blinatumomab, and then, instead of 3 years of maintenance, we have 1 year.

Well, there are some risks taken here, and we have stopping rules. So the study will be monitored to see whether with minimal chemotherapy we have a high risk of relapse or not. But if we succeed, we can prove that we can minimize chemotherapy, minimize safety concerns, and optimize the chances for curing this patient.

In the United States, the ECOG-E1910 is enrolling patients. Again, these patients who have Philadelphia-negative ALL are randomized. They start with induction, consolidation, and are then randomized regardless of MRD status to blinatumomab or no blinatumomab, and then to transplantation. The study is enrolling, and the PI is Dr. Litzow. So, the excitement is to use this drug in a front-line setting, rather than just waiting for multiple relapses.
One last area in ALL is Philadelphia-positive disease. Blinatumomab was assessed in these patients. These patients have failed TKIs—multiple TKIs—and acquired certain mutations. Blinatumomab was given in a phase 2 study. Responses were seen in 36% of these patients, with a survival of 7 months, which is not bad at all.

What’s next? Next, is to treat this patient without chemotherapy. In APL, we moved from chemotherapy to a chemotherapy-free regimen. Maybe in this disease, we can move from chemotherapy to TKI, from TKI to blinatumomab, and cure them without any chemotherapy. Wouldn’t it be good? Just four cycles of blinatumomab, take pills, and you’re cured. Well, this would be amazing. Hopefully we’ll get there. Studies are ongoing in the United States and in Europe, starting in 2017. Stay tuned.

Well, where we’re going is, to use the drug in salvage 1—this is my whole message—and front line. You’ve see in the design of the trials with inotuzumab and blinatumomab—these are the front lines.

Then CAR T cells are great. They are one of the most innovative, exciting areas in ALL. I don’t see them in the multiple relapse setting as well; they are to be used earlier on once we establish safety.

Transplantation—they do very poorly with everything we have today—maybe it will be front-line for these patients. MRD studies are critical. No transplant with MRD-positive disease.

Also, exploring the immune system further with PD-1 inhibitors, checkpoint inhibitors—we’re looking to combine the checkpoint inhibitors with blinatumomab, for example. That is an exciting trial. Thank you very much for your attention, and I will take questions later.

Expanding Immunotherapy Vistas in ALL: CAR T-Cell Therapy

**Stephan A. Grupp, MD, PhD**
University of Pennsylvania Perelman School of Medicine
The Children’s Hospital of Philadelphia
Philadelphia, Pennsylvania

Dr. Grupp: I’ll gently and respectfully disagree slightly with Dr. Jabbour on one point. If you remember that slide of iterative improvements in outcome in pediatric ALL, that was mostly randomized trials, so there’s definitely a little bit of value in doing that. And of course, in the pediatric community, we’re very used to having a large fraction of our patients on trials of one sort or another.

This is, I think, very reminiscent of data that you’ve seen in the earlier talks. These are pediatric data, so while we do very well in patients with pediatric ALL, the patients who relapse do very poorly. This is outcome in second or greater relapse, regardless of whether the patient went to transplant. In the pediatric world, we only transplant patients who are in remission.

We agree with Dr. Jabbour’s comment about the importance of MRD prior to remission. So this is really the state of the art as of 2013, with really dismal outcome for these patients.

And then the other aspect of it is that we only get a third of these kids in remission, so it’s very hard to treat these patients.
So, David, a 21-year-old male. Standard-risk B-ALL, a late extramedullary relapse, was treated with radiation and chemotherapy, and then relapsed again—now, in the bone marrow, which does happen, obviously.

And now, the patient is refractory to re-induction—refractory to two of the drugs that we’ve heard about, which were CD19-based blinatumomab and CD22-based moxetumomab, which was an experimental therapy in the same general notional class as inotuzumab.

So, the patient goes to a clinical trial for CD19-directed CAR T cells.

This is the CAR T-cell approach. On the top, you see a CD19 target, which is what we go after, just like with blinatumomab. On the bottom, we see an engineered T cell that expresses this complex molecule, this CAR—chimeric antigen receptor—with various pieces.

And if you’re a maven in this area, you know that one of the major differences between various CAR T-cell products right now is their costimulatory domain. Some people use 4-1BB, which is what is used in the product that we have studied at Penn, and others use CD28. So that’s of interest, because there are some differences in performance between these two costimulatory domains.

So this is what this looks like in the test tube. So the green cells here are drug-resistant ALL. The gray cell at the bottom is a T cell—just a normal donor T cell from somebody in the lab. And that has been transduced with a CAR. Ordinarily, the T cell will just wander by the ALL cell and would have no interest in it whatsoever.
But in this case, I want you to focus on the lower green cell and look at what happens to the cell membrane. So the T cell comes in, docks with the ALL cell, and then that bubbling that you’re seeing right there is the destruction of the cell membrane and the formation of the membrane attack complex.

And the T cell does not care at all that this is a drug-resistant ALL cell, because it’s physically attacking the ALL cell in a completely orthogonal method that is not particularly informed by whether it has high-risk cytogenetics, whether it’s multiply relapsed, or whether it’s chemotherapy-refractory.

So, this is what proliferation looks like. In the era of modern CAR therapy, which started in 2010—really, it happened at that moment—we started to see these very significant degrees of proliferation of these engineered T cells. Without proliferation, there is no response.

And so what you see here—is this just flow cytometry, and I really like that, because you can actually see the individual T cells and whether they’re expressing the CAR protein. A day after you give these T cells, you don’t find them in the peripheral blood, either they’re gone, or they’ve distributed to the tissues, and that’s what they’ve done, because 2 weeks and 3 weeks later, you see a huge number of CAR T cells. The red box is the CAR-modified cells, and the cells outside the box are just regular old T cells.

And, in this particular case, we had given this patient a CAR-modified product that was 11% CAR T cells, and now we’re seeing 71% CAR T cells at peak—showing that this is antigen-driven cell proliferation. This isn’t just generalized T-cell proliferation in an immunosuppressed patient.

Patients who have received this particular product have remained in remission and continue to have B-cell aplasia, which indicates functional persistence of the cells after 5 years.

What does the proliferation look like? So this graph is all the individual QR-PCR curves of patients from our CAR T-cell trial, the CTL019 trial. And what you see is that it’s up to 100,000-fold expansion of these T cells in these patients. So, it’s an enormous level of expansion that’s driven by the leukemia at the peak. It’s a very tight peak.

The leukemia cells are disappearing in the patient and the T cells rest down very rapidly. And then one of two things happens. In a small number of patients, the cells go away in a few months, and in a much larger number of patients, the cells stick around for several years—we see both of those. And so, we’re very interested in what happens to these patients over time.

Now, there’s been a lot of discussion about, treating patients early, and the importance of salvage 1 versus salvage 2—which is not so much of a pediatric concept. And then, of course, we can look at the issue of disease burden.

The bar sizes here are just the number of patients, so you can get a sense in our large CHOP trial of how many patients fall into each of these buckets. And so, all the way on the right, you have patients who are already MRD-negative just from their lympho-depleting chemotherapy. So, 100% of them go into remission. Big deal.

Of patients who are MRD-positive—out to 5% blasts—100% go into remission. Over 5% blasts, 88% go into remission. Over 50% blasts—now, these are absolutely refractory patients—83% go into remission. So this is pretty powerful in terms of ignoring disease burden, in terms of the probability of response.
Disease burden has another role to play here and that’s toxicity. And the other aspect of this—and I’ll show you some data on this—is that this seems to work on CNS leukemia, which we’re really surprised by.

So in this trial, our relapse-free survival at 12 months is 60% and at 24 months, 53%. And most of these patients just got CAR T cells. Most of them did not go on to transplant. Only seven of these patients subsequently went to stem cell transplant, and one got a DLI infusion.

That was an interesting patient who actually had T-cell ALL that was inappropriately CD19-positive, whom we treated on the trial because I hadn’t actually said in the eligibility criteria, “B cell.” I just said, “CD19.” So we actually treated the patient. She went into remission. She had a very tiny amount of MRD, and actually that cleared up with DLI.

Overall survival was 79% at 1 year, with a median follow-up on this particular trial now out past 16 months. Clearly, there are patients who experience recurrence, and any death that happens later on trial is related to disease.

So, typically in pediatrics, we tap patients and we look for disease, because the CNS is such an important sanctuary site. And so, we were surprised when we did our routine disease evaluations to find these odd-looking cells in the peripheral blood and in the spinal fluid. And those aren’t blasts, they’re large granular lymphocytes—they are CAR T cells.

Overall survival was 79% at 1 year, with a median follow-up on this particular trial now out past 16 months. Clearly, there are patients who experience recurrence, and any death that happens later on trial is related to disease.

So, 98% of the patients whom we’ve treated have CAR T cells in their spinal fluid. And in the beginning, since we weren’t treating patients with overt CNS disease, we were like, “I don’t know if that’s doing anything. You know, they’re just swimming around in the fluid. Maybe they aren’t doing anything. Maybe they’re not getting into the brain.”

But now, we’re beginning to get a little bit of a signal. We’ve had multiple patients who were CNS 3 at some point, prior to their CAR T-cell treatment, have zero CNS relapses. We had one patient—Ph-positive ALL, CNS relapses, bone-marrow relapse, transplant, six CNS relapses—and that patient subsequently got CAR T cells 3 years ago and remains in remission with no further therapy.

We’ve now seen several patients with CNS 3 disease—including brain parenchymal disease—that disease controlled by CAR T cells with no further therapy for out to 18 months in one case. These are all somewhat anecdotal, but they at least show proof of concept that this is a powerful way of controlling CNS disease.
Now, toxicity is an extremely important point. So Dr. Jabbour was talking about cytokine release syndrome in blinatumomab—we see cytokine release syndrome, and it’s worse in these patients.

And so, the first thing we notice in these patients is that patients with severe cytokine release syndrome—and by severe, I mean grade 4, and by grade 4, I mean they’re in the ICU. So, patients with severe cytokine release syndrome have, in addition to their cytokine release syndrome, a macrophage activation syndrome that’s characterized by very high ferritin levels. That was an interesting observation to us, and that sort of set off a bunch of studies looking at various cytokines. And we’re very interested in this.

So, we started to ask the question, of the 44 different cytokines that we were testing in these patients over time, which of these cytokines was associated with the toxicity in these patients? And two of them are really, strikingly elevated. So, if you look at these patients who do or don’t have severe CRS on a log scale—and all of these graphs have log scales—you can see 100-fold higher levels of interferon-γ—not surprising because that’s a T-cell cytokine.

But then, there’s interleukin-6. We were not expecting that interleukin-6 would be this elevated in these patients, or so closely associated with severe ICU-level toxicity. And that observation, as many of you are probably aware, has been absolutely transformational in being able to safely control the toxicity associated with these CART T cells, because our friends in the rheumatoid arthritis community have a drug that targets IL-6.

It wasn’t really designed to be given to transplant patients or cancer patients or cell-therapy patients, but when we saw the signal of high IL-6 associated with our first patient who had very severe cytokine release syndrome—life-threatening without a question—we gave her a dose of this drug, tocilizumab, and abrogated her cytokine release syndrome literally within hours.

She went from being on three pressors—100% O₂, oscillator, 20% of nitric, really calling the family and saying, “This is about all we can do”—she’d already gotten steroids, and unfortunately, they didn’t do much for her—she got this single dose of tocilizumab, by the next morning she was off all of her pressors. Her chest x-ray, which had been whited out, was back to black. She was on 21% O₂ within a day and a half.

This sort of response is very striking, and really is why we’re able to control the cytokine release syndrome in these patients with an IL-6-based strategy.

And this is a patient of David Porter’s who had high, spiking fevers, was starting to get soft blood pressure and a second liter of normal saline, who headed up to the MICU and got a dose of tocilizumab, and the fever went away, and the other sequelae went away. And I’d say two-thirds of the patients have this sort of very quick response, and a third of the patients require multiple doses of IL-6–directed therapy, and occasionally steroids, as well.

So, we can control CRS in these patients—in all pediatric patients, actually—but patients can be quite ill during this period of time.
And what drives this is the disease burden. So, again, the yes/no—did you go to the ICU or not? Look at the disease burden here. And this is roughly estimated by bone-marrow blasts. At over 50%, these patients essentially all go to the ICU. If you see the three patients who didn’t go to the ICU, two of them didn’t respond. So, this is clearly a situation where disease burden is extremely prognostic.

On the other hand, if you look at the no group, if you treat patients in MRD, they’re not going to the ICU. I would estimate that the likelihood of ICU-level toxicity for a patient with MRD-level disease is probably less than 2% to 5%.

IL-6 is associated with a peak of toxicity. It does not predict toxicity. We have been able to develop a predictive model using multiple cytokines, and we have a three-cytokine model that’s 93% predictive that could be used to predict severe toxicity and possibly drive earlier intervention.

It includes interferon-γ, and does not include IL-6. It includes IL-1 receptor α, and soluble gp130, which is actually very important in IL-6 trans-signaling, so that points back, again, to the importance of IL-6 in this toxicity response.

These patients also get coagulopathy. Not all of them, but maybe 20% or so. And it’s a very characteristic coagulopathy that’s associated with a crash in fibrinogen, when the CRS is getting better. And so, these patients can be at bleeding risk, unless the hypofibrinogenemia is recognized and is very aggressively corrected with cryoprecipitate. But fortunately, if you do that, it works very well.

### Toxicity Summary

- **Cytokine release syndrome**
  - Correlates with T-cell proliferation and efficacy
  - Severity related to disease burden
  - Reversed with anti-IL-6 therapy
  - Severe CRS mirrors HLH/MAS

- **Neurotoxicity**
  - Seen in several CD19 immunotherapy trials: CAR T cells (NCI, CHOP/UPenn, MSKCC, Seattle) and blinatumomab
  - In our experience: generally untreated, fully resolves

- **Chronic B-cell aplasia requiring IgG replacement**

So, I would say that cytokine release syndrome is our major toxicity. It correlates with both degree of T-cell proliferation—and you can see most of the patients who have efficacy, have CRS. The severity is not related to anything other than disease burden, which is to say, you don’t have to be very sick to get better, but if you have a lot of leukemia, getting very sick seems to be part of getting better, unless we can prevent CRS.

The IL-6 blockade has really been very important, as well as the observation that there’s a macrophage activation syndrome.

Neurotoxicity is interesting. So, this has been an issue of a lot of discussions across all of the CAR T-cell platforms that have highly active clinical results. And so the experience in the U Penn, CHOP, trials is that there are neurotoxicities, and those include confusion, encephalopathy, and seizures in very small numbers of patients.

What it does not include is cerebral edema, which has not been reported in any of our trials. So we haven’t seen fatal CRS in the pediatric populations, and we haven’t seen cerebral edema in any of the patients on the three clinical trials that we’ve done in pediatric ALL.

These CAR T cells, if they remain in the patient, cause chronic B-cell aplasia, and these patients require IVIG replacement. So the good news is that the cells remain on the hunt. They’re in the patient. It has emboldened us not to transplant the vast majority of these patients to see if they can remain in remission. Two-thirds of the patients whom we treated had already had a transplant anyway, and if you want to do another transplant, that’s awesome. I’m a transplanter. But I’m also very happy not to do that.

But we also have patients for whom they finally are in remission with their CAR T cells, and we tell them transplant is the standard of care, and they’re like, ‘Do we still have CAR T cells?’ And, ‘Yes, you do.’ And they say, ‘Well, we’d like to wait.’
I want to acknowledge the fact that there are patients who have relapses that are associated with the loss of CD19, and there, the persistence of the CAR is not helpful.

If you look at the three CAR platforms that are the most clinically advanced today, there are two CD28-based CARs from Memorial Sloan-Kettering Cancer Center and from the National Cancer Institute. And then the U Penn 4-1BB CAR, which is a lentiviral transduction approach.

I think the CR rates are very similar across these platforms: 70%, 80%, 90%, depending on how you count and whether you’re doing an intent-to-treat analysis. I think it’s all pretty much the same, but persistence is really different—1 or 2 months with the CD28 CARs, and years with the 4-1BB CAR.

But what are the mechanisms of relapse in the patients who do relapse? And so CD19-positive relapse is associated with losing your CAR cells before 4 months. And so that means that the T cells have petered out and we haven’t completely depleted the disease, in my opinion.

In patients who develop CD19-negative relapse, they are universally still CAR-positive, but now the CAR T cells tragically can’t see the ALL. And so, it’s either one or the other—you lose your T cells quickly with CD19-positive relapse. You keep your T cells and you’re at risk for CD19-negative relapse. And of our relapses, about two-thirds are CD19-negative.

I think there are a variety of ways to do these sorts of combination approaches, and right now, the idea is that of treating CD19-negative relapse with a CD22-directed agent or CAR. But of course, we’re pediatricians. We believe in prevention.

So we’re most interested in dual targeting eventually, that being a step or two beyond where we are right now, and really seeing whether we can decrease the risk of CD19 escape, which is, for our patients, the principal problem that we’re dealing with in about 20% to 25% of the patients.
This has actually rolled out to multicenter trials, and the global registration trial—25 centers, 11 countries—will be presented tomorrow afternoon in the ALL session, and so you can see some of the updated data on how this works outside of a single-center setting.

So, there are a number of centers in the United States, and then centers across the EU, Canada, Japan, Australia. And this has been a great group to work with, and we'll be talking about these data tomorrow.

So, here we are. We've probably improved off this baseline. We have some prospect for longer-term disease control. We hope that we can start to roll this out earlier in therapy, where we know that patients with lower disease burden won't have the risk of severe cytokine release syndrome.

So that's where we are right now, and I thank you very much for your attention.

**Dr. DeAngelo:** Steve, that was great. And I want to thank my other panelists for their great talks. We do have a lot of questions. There are microphones. I'll try and get to some of them.
of potential immunogenicity for that purpose. But we need longer-term follow-up.

Unfortunately, post–CAR T-cell relapses are highly problematic. In these kinds of trials, we’re sort of the last stop on the subway, and there’s not a lot that we’ve been able to do in terms of salvage for these patients, and the overall survival of the patients who experience relapse is really fairly poor.

Dr. DeAngelo: Right.

### Audience Q&A: Question 2

What is the preferred method of monitoring MRD between flow cytometry, PCR, and next-generation sequencing?

Dr. DeAngelo: The next question is about the choice of monitoring MRD, and the question is: What is the panel’s—and I’ll have Monika start with this—the panel’s preference of flow cytometry versus PCR versus next-generation sequencing for MRD?

Dr. Brüggemann: So, it really depends on what is the aim of your trial. If you only want to go for high-level MRD, flow cytometry is definitely, I think, the most convenient method. But also here, reference diagnostics are really important. And molecular is still the more sensitive method. I think the method with the largest published experience here is RQ-PCR, but I’m convinced that in a couple of years from now, NGS will substitute.

Dr. DeAngelo: All right. I think in the United States, it may be a little different. We don’t have the beauty of the centralized centers that you do, and I think, at least from a cost perspective, most of our patients are getting flow-based strategies.

### Audience Q&A: Question 3

In which patients would you consider a transplant following first remission?

Dr. DeAngelo: The next question—there are two questions like this, and this is a very important question that I think troubles a lot of folks and that is: Which adult patients should go to transplant in first remission? And there are two questions addressing that. And I’ll start with you, Elias. Who would you transplant in CR 1?

Dr. Jabbour: Well, traditionally, we had the high white blood cell count being a prognostic factor. We had hypodiploidy and complex karyotype being prognostic, as well. And then the French published their paper with Ikaros being a prognostic factor to be considered, karyotype 4;11 translocation to be added. And then finally, MRD, as well, in a dynamic fashion.

Dr. Grupp: Pardon me. Sorry about that. Somebody disagrees with you.

Dr. Jabbour: So all these criteria are essentially “if your patient is not doing well—he may relapse, and you can add the Philadelphia-like phenotype, as well.”

Then the question is: If you have somebody with bad features who turns out to be MRD-negative, will MRD negativity trump the baseline factors? With the knowledge we have today, I’m not sure it does. Maybe if we do NGS and we can have a higher sensitivity of minimal disease that may change. I think today, still, somebody with, for example, 4;11 translocation, hypodiploidy, I will still go for transplant in first remission.

Dr. DeAngelo: Anything you’d like to add, or—

Dr. Brüggemann: —Yeah, I fully agree.

Dr. DeAngelo: Yeah. That’s been our approach, as well. I think the biggest difficulty is knowing what to do with the Philadelphia-like patients, and whether to add a TKI or whether to use MRD-based stratifications.
Dr. DeAngelo: There are a lot of questions from Steve's talk on CAR T cells, and a couple of them that I'd like to address are on the use of tocilizumab. And the two questions that were asked by two different folks—and I'll ask both, if you don't mind, Steve, were—one is: Does the use of tocilizumab decrease the successfulness or the efficacy of the CAR T-cell approach, and can it be used prophylactically? Number one. And number two: Is there a role for using it prophylactically?

Dr. Grupp: So, those questions are absolutely two sides of the same coin. So, the approach we're using right now is to wait until the patient starts to get fairly ill, which is to say they're at least on pressors. The rationale, and it's not a very good one, is that we want to make sure that the expansion phase of T-cell proliferation is well established before we treat the patient with anything that might interfere, although we don't think that IL-6 matters that much.

And so, that's been our approach to this point, and in that approach, patients who require tocilizumab on our single-institution study, ie, patients who require tocilizumab for grade 4 CRS have 100% CR rate. And there are a lot of data that show that it doesn't affect the PK. The PK doesn't drop after tocilizumab administration. So then, that opens the question up of why aren't we giving this stuff earlier?

And so that's being tested. The group in Seattle is testing this. We have a trial open at CHOP where we're looking at this question of giving preemptive—I would say not prophylactic, but preemptive—as soon as the patient gets high fever and we know they have a high disease burden and that they're at high risk, we'll give them tocilizumab. We've had that study open for about 5 months, and we should be in a position to talk about that in about 6 to 12 months. So it's a great question. We have to be answering this question. It's really important for the future.
Dr. DeAngelo: Monika, a question for you: Since the PCR-based MRD assessment is individualized, how does clonal evolution impact its sensitivity over time?

Dr. Brüggemann: It can lead to false negativity. This is well known. This is why precautions should be taken. So it’s highly recommended to analyze at least two different targets to avoid this false negativity, and also to focus this on the stable V(D)J stem of the rearrangement. But still, there might be false negativity. In the series of the German trial, we saw that in 2% of samples MRD was negative with one target, whereas it was clearly positive with the other one, so—

Dr. DeAngelo: —Interesting.

Dr. Grupp: And I would add to that, that this may be an area where the next-generation sequencing approach may pick up some of these allele-specific PCR false negatives. Certainly, if you get a signal, that’s either picked up initially or gets involved at a certain level. So that may be something that’s useful in some of these patients.

Dr. DeAngelo: So, one of the questions that was asked is: Are there any experiences from the panel on using defibrotide for veno-occlusive disease after antibody–drug conjugates in ALL?

I’ll take that question. Our center has been very active in using defibrotide in patients who develop VOD for a multitude of issues, and I can say that both gemtuzumab, when it was available, and now inotuzumab, that the use of defibrotide really has been able to treat these patients effectively and reverse the VOD phenotype. So it’s a very effective way of treating the VOD that’s seen in inotuzumab, which has slightly different properties than those that are seen with conventional therapy—after transplant.

Dr. DeAngelo: One of the questions that was asked to the group is about the role of monoclonal antibodies in CNS relapse. Is there any role for using it? Elias?

Dr. Jabbour: You know, there are some data on rituximab in CNS disease in a pediatric setting—a small study. We tried to reproduce this study in adult ALL using 10 mg/kg of rituximab as a first step, and went to 25 mg/kg. In the pediatric setting, above 50 mg/kg, they couldn’t succeed to deliver. It was inconclusive.

The other antibodies—with inotuzumab we don’t have any experience. With blinatumomab—the T cells being in the CNS, it’s more in the CAR T cells, and blinatumomab remains to be proven. I do not rely today on antibodies for CNS disease.

Dr. DeAngelo: Okay.

Dr. Jabbour: So I need systemic chemotherapy, and intrathecal chemotherapy as well.

Dr. DeAngelo: So although this is a question about the monoclonal antibodies, I’m going to ask Steve, because you’re the pediatrician: What’s the outcome in infant ALL with blinatumomab, inotuzumab, or CAR T cells?
**Dr. Grupp:** So, I’m not aware of any inotuzumab data. So, infant ALL would be a great potential target for CAR T cells, because transplant doesn’t work very well in these patients, even if they achieve remission.

Unfortunately, we have not been able to make products in these babies. Patients who are under 2 years of age have a very high manufacturing failure rate. We saw that on the UPenn trial. They’re actually not even eligible for the multisite trials. And this has been studied in some detail by my colleague, David Barrett. The baby T cells are perfectly capable of proliferating and making good CAR T cells until you give them cyclophosphamide, and then they’re just knocked out.

And this is our experience with infant ALL. These patients look like they have profound immunodeficiency from moderate to intensive chemotherapy.

**Dr. Jabbour:** Just speaking of this, the CAR T cells, can we get “on-the-shelf” cells—so, we cannot collect in infants, but can we get normal people to donate cells, engineer them, and give them back to the patients?

**Dr. Grupp:** So, the answer to that question is that there are technologies that are being developed for off-the-shelf approaches with third-party allogenic T cells, and what they depend on is the various gene knockout strategies, either TALENs or CRISPRs, to remove the T-cell receptor at least, if not other aspects of the T-cell signaling machinery, so that there’s no risk of allogeneity and GvHD.

And so, there is a clinical trial where two babies actually have been treated with some early potential efficacy signal, but also clearly a GvHD signal, because the TCR-positive cells are not completely eliminated. And these patients are unmatched entirely with the donor of their T cells.

So I think that off-the-shelf is a great approach, but I think that we need to see what the persistence looks like, and we need to know that there isn’t a risk of GvHD.

**Dr. DeAngelo:** All right—thank you. The next question is for Monika. MRD status is important, we all know. Does it matter how you get there? So, for example, is the quality of the MRD-negative status different in patients who receive chemotherapy, blinatumomab, or transplant? Is there a difference?

**Dr. Brüggemann:** For chemotherapy versus transplant, we know that for patients who are MRD-positive before and become MRD-negative after transplant, that they nevertheless have a high risk of relapsing. Chemotherapy versus monotherapy, we do not fully know about this.

**Dr. Grupp:** Yeah. Well, let me ask you an unanswerable question, which is: We know that MRD negativity before going to transplant is very important, but what about patients whom you have to really beat into remission or into an MRD-negative remission to get them there? Do we know that MRD negativity after extensive attempts to get to that point is as good as MRD negativity in patients who get there fairly easily?

**Dr. Brüggemann:** The earlier the MRD negativity and the less amount of treatment you have to give, the better it is. In our series where we did this analysis, already at day 11, the outcome of the MRD-negative patients was very much better than the outcome of the patients who became MRD-negative at later stages. There, the relapse risk was considered very high. So, the time point is also crucial.

**Dr. DeAngelo:** And you showed data from the MD Anderson Cancer Center, Elias, where the MRD-negative versus MRD-positive in salvage 1 was prognostic or predictive of outcome, but in salvage 2 or higher was not.

**Dr. Jabbour:** And even in the INO-VATE trial and the TOWER trial, if you get MRD negativity by blinatumomab or inotuzumab, you have more durable responses than just chemotherapy alone.
But I'll actually ask that question, and follow it up with: What's going on with the neurotoxicity?

**Dr. Grupp:** So, 98% of our patients have CARs in their spinal fluid when we look, so it's very hard to say that that's associated with neurotoxicity. The number of CAR T cells may be in the 5 to 20 cells per microliter range. So they have a mild pleocytosis in some cases. And then you do a PCR analysis, and you see that most of the cells early on are CAR T cells. They persist out to a year, which was as late as we've looked in anyone.

So, what's up with the neurotoxicity? All of these CD19-directed agents— blinatumomab has a modest degree of neurotoxicity, but CARs have more. There seem to be differences among the second-generation CARs in terms of the nature, severity, and incidence of neurotoxicity, where the 4-1BB experience is more focused toward confusion, aphasia, very rare seizures, and the general encephalopathy picture.

Whereas, of course, there's been discussion around the 4-1BB CAR, and the discovery relatively late in the development of the drug, and of having not seen previously—cerebral edema as a major toxicity. And that we haven't seen in the pediatric trials.

**Dr. DeAngelo:** Any concern, Steve, of using blinatumomab before CAR T-cell therapy?

**Dr. Grupp:** Well, that's a great question. And so, if CD19 escape is an important mechanism of relapse—which it clearly is—the anecdotal sense is that there's clearly CD19-negative escape after blinatumomab, and there's clearly CD19-negative escape after CAR T cells, and it's higher in persistent CAR T cells. Those are all true things.

Does getting blinatumomab before CAR T cells set you up for CD19 escape? We really don't have the data to address that. In our single-institution trial, we've allowed patients to go on, and there have been several patients who had gotten prior blinatumomab who then had CD19 escape. But it's all anecdotal. In the multicenter trials, we haven't actually allowed patients with prior blinatumomab.

**Dr. Jabbour:** How about CAR T cells targeting CD19 and CD22 at the same time and how that will eventually overcome?

**Dr. Grupp:** So your comment about single therapies versus combination therapies fits right into that thinking, and so that may be where things are going in a couple years. Of course, we have to show safety and efficacy of both before you combine them. But that may be the way to go.

**Dr. DeAngelo:** Well, I know I learned a lot. It's 8:30. My job is to get you guys out of here on time. So thank you for everything—the panelists. I hope everybody has a good time in San Diego, and enjoy the rest of your ASH.

Thank you very much.
Treatment Evolution in Acute Lymphoblastic Leukemia: Insight on Novel Antibody Technologies and Immunotherapy Advances

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