5 Minute JournalClub

POST-ASH Issue 7, 2014

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CME Information

LEARNING OBJECTIVES

- Develop an understanding of the mechanism of action of chimeric antigen receptor T-cell therapy, and evaluate the emerging efficacy and safety data with this therapeutic approach under evaluation in the front-line and relapsed/refractory settings for B-cell lymphoma and leukemias.
- Evaluate the benefits and risks of the addition of gemtuzumab ozogamicin to standard chemotherapy and of other emerging agents such as novel FLT3 inhibitors or hypomethylating agents for the treatment of acute myeloid leukemia.

CME Information (Continued)

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FACULTY DISCLOSURES
The following faculty (and their spouses/partners) reported real or apparent conflicts of interest, which have been resolved through a conflict of interest resolution process:

CME Information (Continued)

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No real or apparent conflicts of interest to disclose.

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Contracted Research: Novartis Pharmaceuticals Corporation; *Other Remunerated Activities:* Genentech BioOncology (faculty and spouse).

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On this final issue of our review of select key papers presented at the American Society of Hematology annual meeting, we focus on a handful of fascinating early clinical reports on CART and a smattering of what's new in AML.

David L Porter, MD

CART clinical trial data

Although we heard about the seemingly miraculous effects of this novel therapeutic approach in 2012 in Atlanta, New Orleans was the true coming out party for CART-based approaches, specifically those targeting CD19. Led by the powerhouse team at the University of Pennsylvania, which includes Dr David Porter, we were treated to numerous fascinating presentations that have generated great excitement and enthusiasm.

Since ASH I have been fortunate enough to interview Dr Porter on 2 occasions, and it is impossible to hear about this dramatic story without getting goose bumps.

The concept behind Penn's CART-based approach is intriguing. Patients undergo leukapheresis, after which their T-cells are transduced with a lentivirus encoding an anti-CD19 single-chain variable fragment linked to 4-1BB and CD3-ζ signaling domains. The genetically modified cells are then expanded ex vivo, and soon after a course of lymphocyte-depleting chemotherapy they are then reinfused into the patient where, as documented previously and in new reports at ASH, they expand (up to 10,000-fold), persist functionally (beyond 3 years) and exert a direct antitumor effect.

Early efforts by the group have focused on very advanced chronic lymphocytic leukemia (CLL) and B-cell acute lymphoblastic leukemia (ALL), and although they hoped to see some discernible benefit in early testing, as related by Dr Porter, the initial clinical responses were stunning in rapidity and depth. Much of this work was updated and expanded on in New Orleans. Dr Stephan Grupp **presented longer follow-up outcomes** from 17 adults and children with relapsed/refractory ALL revealing that 82% achieved a complete response (CR). Similarly, Dr Porter **provided an update** from their Phase I study in which 8 of 14 patients with extensively pretreated CLL had objective responses, including 4 patients with CRs, none of whom have yet experienced relapse. He also unveiled data from their dose-finding randomized Phase II study demonstrating that 7 of 18 patients responded, including 3 CRs, with no correlation between dose and outcome or toxicity.

Perhaps the highlight of these presentations, at least in my mind, was an impressive set of scans that Dr Porter displayed from a patient with del(17p) CLL treated on their original pilot study. Amazingly, 3 months after receiving the

CART infusion this individual, whose disease had progressed through 10 prior therapies, including ibrutinib and radiation therapy, was pretty much disease free in peripheral blood and bone marrow.

In discussing this work with Dr Porter, I was eager to learn more about the profound and rapid tumor lysis/cytokine release syndrome (CRS) that has been described with this therapy. I was wide-eyed as he detailed the team's early experiences with this scary complication that generally occurred within the first few weeks of treatment at the peak of initial T-cell expansion and initially led to life-threatening multiorgan failure. What was perhaps most compelling was that the team was able to quickly determine that the key cytokine causing this syndrome was IL-6, and they were able to successfully intervene with a novel IL-6 receptor antibody approved for rheumatoid arthritis (tocilizumab).

Penn is not alone at the forefront of this research, and at ASH the Memorial group also reported activity with a slightly different anti-CD19 CAR platform across several diseases. In high-risk CLL, Dr Jae Park **presented preliminary data** from a Phase I study evaluating this approach as consolidation after up-front rituximab-chemotherapy. While the data set was quite small — 8 patients with 2 CRs and 2 partial responses — it provides an important proof of principal that ultimately may allow some patients with CLL to receive a short-term treatment that will lead to prolonged tumor control. Relevantly, the study results suggest greater benefit in patients with lower tumor burden, and Dr Porter believes that an important future strategy will be initial cytoreduction, particularly with novel B-cell inhibitors like ibrutinib, followed by an attempt at cure with CART.

Memorial's Dr Marco Davila **also reported** on CAR therapy as a bridge to allotransplant in patients with B-cell ALL, including those with Philadelphia chromosome-positive disease. Importantly, responses were rapid, occurring as early as 7 to 14 days, CRS was manageable and 10 of 12 patients with detectable disease before CAR therapy became minimal residual disease-negative. Four patients went on to allotransplant with 5 more being prepped for it.

The NCI, too, is very involved in this field, and at ASH they provided us with more data on the **use of this technology in ALL** as well as our **first look at it in B-cell lymphomas**, where 5 of 8 patients with chemotherapy-refractory diffuse large B-cell lymphoma or primary mediastinal B-cell lymphoma experienced objective tumor responses.

The next step for this fascinating and innovative treatment strategy is to not only obtain more data but also to investigate the feasibility and reproducibility of doing this on a larger-scale basis.

AML: Life beyond 3 plus 7

The dismal current landscape of this important cause of mortality is reflected by the fact that the only compound approved for this disease in the last 20 years, gemtuzumab ozogamicin (GO), was removed from the market by the FDA 4 years ago due to toxicity concerns.

As such, for far too long conversations about AML management have revolved around optimal induction and consolidation chemotherapy doses and schedules and the role for various transplant strategies. However, the rapidly emerging

translational and related clinical science that permeates ongoing AML research has many optimistic that brighter days are ahead (click for summary slides of studies discussed below).

One of the more talked about areas is management of the 30% of patients with FMS-like tyrosine kinase 3 (FLT3) mutations, and in the Big Easy Dr Jorge Cortes presented a provocative study on the effects of the as yet unapproved FLT3 inhibitor quizartinib. One of the proposed benefits of this agent is that it is more specific for the target than tyrosine kinase inhibitors such as sorafenib, which is sometimes employed off label when no other alternatives exist. In this Phase II study using lower doses of quizartinib in 76 patients, a 47% CR rate with acceptable toxicities was reported. All eyes are on the ongoing Phase III study evaluating this compound in hopes that it may end up as a useful tool in practice.

The MD Anderson group also reported on SGI-110 — a novel molecule that combines decitabine with guanosine to produce a longer half-life and potentially higher areas under the curve than decitabine. Importantly, in this Phase II study this subcutaneously administered hypomethylating agent resulted in an encouraging preliminary objective response rate of 53% in elderly patients with treatment-naïve AML. Dr Hagop Kantarjian and his group at MD Anderson are interested in doing a study comparing this agent to conventional decitabine or azacitidine.

Because activating KIT mutations are present in 25% to 30% of patients with core binding factor (CBF) AML, it has been hypothesized that KIT inhibition might provide therapeutic benefit. In this regard, the CALGB reported on a single-arm trial evaluating the addition of dasatinib to induction chemotherapy in patients

with molecular confirmation of CBF AML. Reported at ASH, the trial resulted in an encouraging CR rate of 92% in 59 evaluable patients, but many are reserving judgment about this approach until further follow-up is available.

Given its activity in a multitude of hematologic cancers, it should probably come as no surprise that lenalidomide is also being evaluated in AML. Significantly, preliminary results look encouraging with a CR rate of 43% in 37 elderly patients over age 70 with low-dose lenalidomide added to low-dose Ara-C (LDAC). Perhaps even more importantly, a 5-gene molecular signature has been identified that appears highly predictive of treatment response with 87% overall accuracy.

Additionally, although the previously mentioned anti-CD33 antibody-drug conjugate GO is no longer available, at ASH we saw data reinforcing its evidence-based benefit most specifically for patients with CBF AML. Described in **a meta-analysis, a pediatric study and a longitudinal analysis** of trials of the UK MRC/NCRI group, these results all point to a modest advantage with limited toxicity. Whether GO will make it back into the clinic, however, remains unclear.

Finally, in terms of the FDA and AML, one of the more interesting and positive recent developments from the agency has been the 2012 implementation of the "Breakthrough Therapy" designation pathway, which fast-tracks promising agents in diseases with important unmet needs. Over the past year on our CME programs we have discussed many exciting oncology compounds that have earned this designation, and just 2 weeks ago we saw this novel pathway in

action as the second-generation ALK inhibitor ceritinib was granted accelerated approval in ALK-positive non-small cell lung cancer based on the results of a single-arm, open-label clinical trial enrolling 163 patients.

Last fall, the selective and potent polo-like kinase (PLK) inhibitor volasertib became the only "AML drug" to join this short "Breakthrough" list. Volasertib inhibits PLK1 — the best characterized of the 5 known human PLKs and a critical enzyme regulating mitosis — resulting in cell cycle arrest and ultimately cell death (apoptosis). At ASH 2012 a randomized Phase I/II trial reported an impressive response rate advantage when the agent was added to LDAC in patients not eligible for intensive induction. These encouraging results led to the ongoing Phase III POLO-AML-2 trial with a similar randomization in patients age 65 or older with previously untreated AML not eligible for intensive induction. Although the future of this interesting agent is unclear, it seems plausible that in the next few years AML will join the other myeloid cancers in seeing the documented benefit of important new and clinically useful treatment strategies based on the evolution of understanding the underlying disease biology.

This concludes our ASH review series. If you're heading to Chicago this month, join us at the end of each day for a series of evening symposia as we review what's happening in **lung cancer**, **gastrointestinal cancers**, **non-Hodgkin lymphoma/multiple myeloma** and **HER2-positive breast cancer**.

Neil Love, MD
Research To Practice
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T Cells Engineered with a Chimeric Antigen Receptor (CAR) Targeting CD19 (CTL019) Produce Significant in Vivo Proliferation, Complete Responses and Long-Term Persistence without GVHD in Children and Adults with Relapsed, Refractory ALL

Grupp SA et al.

Proc ASH 2013;Abstract 67.

Background

- CARs combine a single-chain variable fragment (scFv) of an antibody with intracellular signaling domains into a single chimeric protein.
- A previous study of CTL019 cells expressing a CAR with intracellular activation and costimulatory domains demonstrated that (*NEJM* 2013;368:1509):
  - Infusion of these cells results in 100 to 100,000 times in vivo proliferation, durable antitumor activity and prolonged persistence in patients with B-cell tumors, including 1 sustained complete remission (CR) in a patient with acute lymphocytic leukemia (ALL).
- **Study objective:** To report on longer follow-up outcomes of patients with relapsed/refractory ALL who have undergone infusion of CTL019 cells.

Grupp SA et al. *Proc ASH* 2013;Abstract 67.

Study Methods

- 20 patients with relapsed/refractory CD19-positive ALL underwent treatment.
  - Children (n = 16); adults (n = 4)
- All patients received an infusion of T cells that had been lentivirally transduced with a CAR composed of anti-CD19 scFv/4-1BB/CD3ζ and activated/expanded ex vivo with anti-CD3/anti-CD28 beads.
- 17 of 20 patients received lymphodepleting chemotherapy the week prior to CTL019 infusion.
- 11 patients had relapsed ALL after previously undergoing allogeneic stem cell transplantation (allo-SCT).
- T cells were collected from patients regardless of prior SCT status.

Grupp SA et al. *Proc ASH* 2013;Abstract 67 (abstract only).

Study Methods (Continued)

- No graft-versus-host disease (GVHD) or GVHD treatment in the 6 months after allo-SCT was allowed.
- The targeted T-cell dose ranged from 10^7 to 10^8 cells/kg with a transduction efficiency (TE) of 11% to 45%.
- On the adult protocol, the target dose was 5 x 10^9 total cells split over 3 days with a TE of 6% to 31%.
- A median of 3.7 x 10^6 CTL019 cells/kg (0.7 to 18 x 10^6/kg) were infused over 1 to 3 days.
- Lymphodepleting chemotherapy varied, with most patients receiving a cyclophosphamide-containing regimen the week prior to CTL019 infusion.

Grupp SA et al. *Proc ASH* 2013;Abstract 67 (abstract only).

Baseline Characteristics

- 16 children of median age 9.5 years (5-22) and 4 adults of median age 50 years (26-60) with CD19-positive ALL received treatment.
- 1 child had T-cell ALL aberrantly expressing CD19.
- 14 of 16 pediatric patients had active disease or minimal residual disease (MRD) after chemotherapy on the day prior to CTL019 cell infusion, and 2 were MRD-negative.
- 3 of the 4 adults had active disease prior to lymphodepleting chemotherapy, and 1 was in morphologic CR.

Grupp SA et al. *Proc ASH* 2013;Abstract 67 (abstract only).

Response Rates

Response	n = 17*
CR	82%†
With ongoing bone marrow (BM) CR	64.7%
With no response	17.6%

* Evaluable patients

† Includes the patient with CD19-positive T-cell ALL

- Median follow-up: 2.6 months
- Patients pending evaluation (n = 3)
- Three patients who achieved a CR at 1 month have experienced relapse
  - One had CD19-negative ALL

Grupp SA et al. *Proc ASH* 2013;Abstract 67 (abstract only).

Clinical Outcomes

- Although T cells collected from the 11 patients who experienced relapse after allo-SCT were generally 100% of donor origin, no GVHD has been seen.
- Persistence of CTL019 cells detected by flow cytometry and/or quantitative PCR in patients with ongoing responses continued for 1 to 15 months after infusion, resulting in complete B-cell aplasia during the period of CTL019 persistence.
- Patients have been treated with IVIg without any unusual infectious complications.
- One child who achieved a CR subsequently developed myelodysplastic syndrome with a new trisomy 8 abnormality and has undergone SCT.
- One child who developed a single leukemia cutis lesion at 6 months still has BM MRD-negative status.

Grupp SA et al. *Proc ASH* 2013;Abstract 67 (abstract only).

Treatment-Related Adverse Events

- All patients with responsive disease developed some degree of delayed cytokine release syndrome (CRS), concurrent with peak T-cell expansion.
  - Manifested by fever, myalgia, nausea and anorexia
- Some patients experienced hypotension and hypoxia.
- Treatment for CRS was required for hemodynamic or respiratory instability in 7 of 20 patients.
- CRS was rapidly reversed in all cases with the IL-6 receptor antagonist tocilizumab (7 patients), which was combined with corticosteroids in 4 cases.

Grupp SA et al. *Proc ASH* 2013;Abstract 67 (abstract only).

Treatment-Related Adverse Events (Continued)

- Cytokine analysis showed marked increases from baseline values of IL-6 and interferon gamma (both up to 1,000 times) and the IL-2 receptor, with mild or no significant elevation in systemic levels of tumor necrosis factor alpha or IL-2.
- There were no infusion-related toxicities of Grade >2 intensity.
- However, 5 patients developed fevers within 24 hours of infusion and did not receive the planned subsequent infusions of CTL019 cells.

Grupp SA et al. *Proc ASH* 2013;Abstract 67 (abstract only).

Author Conclusions

- CTL019 cells are T cells genetically engineered to express an anti-CD19 scFv coupled to CD3ζ signaling and 4-1BB costimulatory domains.
- These cells can undergo robust in vivo expansion and can persist for ≥15 months in patients with relapsed ALL.
- CTL019 therapy is associated with a significant CRS that responds rapidly to IL-6-targeted anticytokine treatment.
- This approach has promise as salvage therapy for patients with relapsed disease after allo-SCT, and the collection of tolerized cells from the recipient appears to have a low risk of GVHD.
- CTL019 cells can induce potent and durable responses for patients with relapsed/refractory ALL.
- Multicenter trials are being developed to test this therapy in ALL in the Phase II setting.

Grupp SA et al. *Proc ASH* 2013;Abstract 67 (abstract only).

Investigator Commentary: T Cells Engineered with CAR Targeting CD19 in Patients with Relapsed/Refractory ALL

This is an exciting new field of reengineering T cells. Several studies of CAR T cells have reported that most patients — particularly those with ALL — achieve complete remissions, some of which are durable. In this study, of the 17 evaluable patients with ALL who received CAR T cells, 14 (82%) achieved a CR. However, the delayed CRS experienced by patients who respond is of concern. This phenomenon is believed to result from a reaction to the T cells killing the leukemia cells. Within a week or 2 of receiving treatment, patients develop fever, bone aches, muscle aches, nausea/vomiting and anorexia. With appropriate management and early intervention, particularly with the use of steroids and anti-IL-6 receptor antibody, CRS symptoms can be alleviated.

This approach is exciting, but it needs to be further evaluated in terms of applicability and side effects.

Interview with Hagop M Kantarjian, MD, January 29, 2014

These investigators previously published results on the potent activity of CAR T cells in 2 patients with relapsed or refractory ALL (*NEJM* 2013;368(16):1509). The current study is the largest experience so far with genetically modified T cells in ALL. The most important aspect of the study is the high, remarkable response rate observed in children and adults — 82% achieved a CR. Most of the CRs had no evidence of MRD, indicating that they were deep and significant. Of note, many of the patients on the study had experienced relapse after allo-SCT. In all cases, either before or after SCT, the patients essentially had no effective treatment options.

Another important aspect of this study is that it detailed CRS extremely well. Many investigators using CAR T cells have identified CRS as an issue. Dr Grupp's group was the first to recognize that IL-6 levels are high in these patients and that intervention with an anti-IL-6 receptor antagonist, tocilizumab, works in a matter of hours to reverse critical and life-threatening side effects. Finally, a unique finding is the description of long-term persistence of the CAR T cells. This is crucial due to the possibility that persistence is necessary for ongoing disease control. I believe that therapy with CAR T cells has the potential to revolutionize treatment of relapsed ALL after the failure of allo-SCT.

Interview with David L Porter, MD, March 3, 2014

Chimeric Antigen Receptor Modified T Cells Directed Against CD19 (CTL019 Cells) Have Long-Term Persistence and Induce Durable Responses in Relapsed, Refractory CLL[1]

Randomized, Phase II Dose Optimization Study of Chimeric Antigen Receptor Modified T Cells Directed Against CD19 (CTL019) in Patients with Relapsed, Refractory CLL[2]

[1]Porter DL et al.

Proc ASH 2013;Abstract 4162.

[2]Porter DL et al.

Proc ASH 2013;Abstract 873.

Chimeric Antigen Receptor Modified T Cells Directed Against CD19 (CTL019 Cells) Have Long-Term Persistence and Induce Durable Responses in Relapsed, Refractory CLL

Porter DL et al.

Proc ASH 2013;Abstract 4162.

Background

- Patients with relapsed or refractory chronic lymphocytic leukemia (CLL) have poor prognoses.
- CD19, an antigen expressed on normal and malignant B cells, can be targeted with chimeric antigen receptors (CARs).
- CARs combine the antigen recognition domain of an antibody and intracellular signaling domains that mediate T-cell activation into a single chimeric protein.
- Inclusion of the CD137 (4-1BB) signaling domain results in potent antitumor activity and in vivo persistence of CAR-modified T cells in mice.
- Antitumor activity of CAR-modified autologous T cells targeted to CD19 (CTL019 cells) was observed in 3 patients with CLL with relatively short follow-up (*Sci Transl Med* 2011;3(95):95ra73).
- **Study objective:** To report on safety, feasibility and efficacy after longer follow-up of the pilot study with CTL019 cells in relapsed/ refractory CLL.

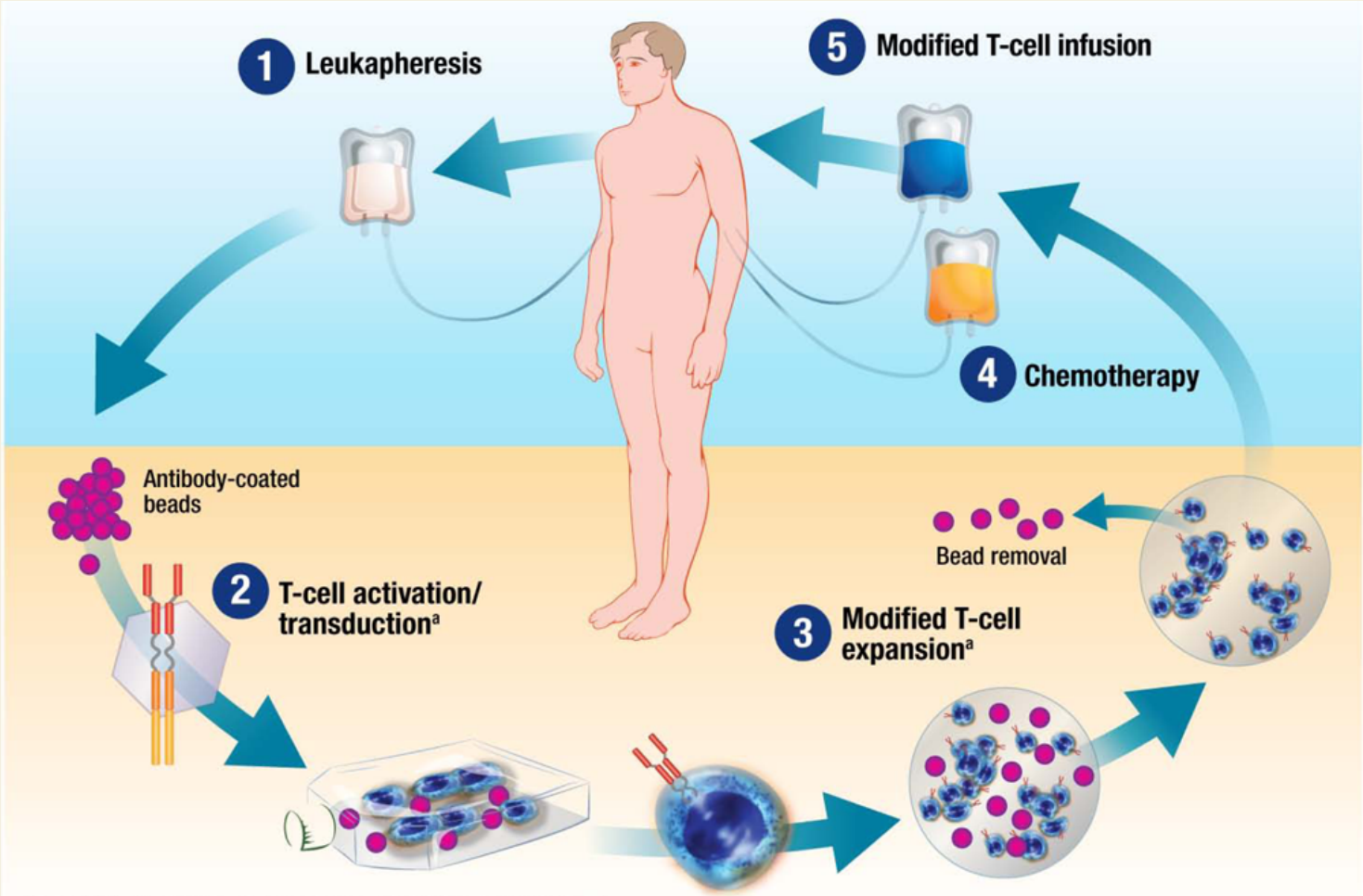
Porter DL et al. *Proc ASH* 2013;Abstract 4162.

Study Methods

- Patients with relapsed/refractory, CD19+ CLL (n = 14)
  - Failed ≥2 therapies; progressed within 2 y of last therapy
- Autologous T cells were collected by leukapheresis
- The cells were transduced with a lentivirus encoding anti-CD19 single-chain variable fragment (scFv) linked to 4-1BB and CD3-ζ signaling domains
- Gene-modified T cells were expanded and activated ex vivo
- All patients received lymphocyte-depleting chemotherapy ending 3 to 5 days before cell infusion
- Target CTL019 cell dose 5×10^9 mononuclear cells
  - Transfection efficiency 10% to 40% (total dose 5×10^8 to 2×10^9)
  - Cell infusions over 3 days (10% d 1, 30% d 2, 60% d 3)

Porter DL et al. *Proc ASH* 2013;Abstract 4162.

Overview of CTL019 Therapy



[a] Transduced ex vivo with a lentivirus encoding anti-CD19 scFv linked to 4-1BB and CD3-ζ signaling domains

With permission from Porter DL et al. *Proc ASH* 2013;Abstract 4162.

Response

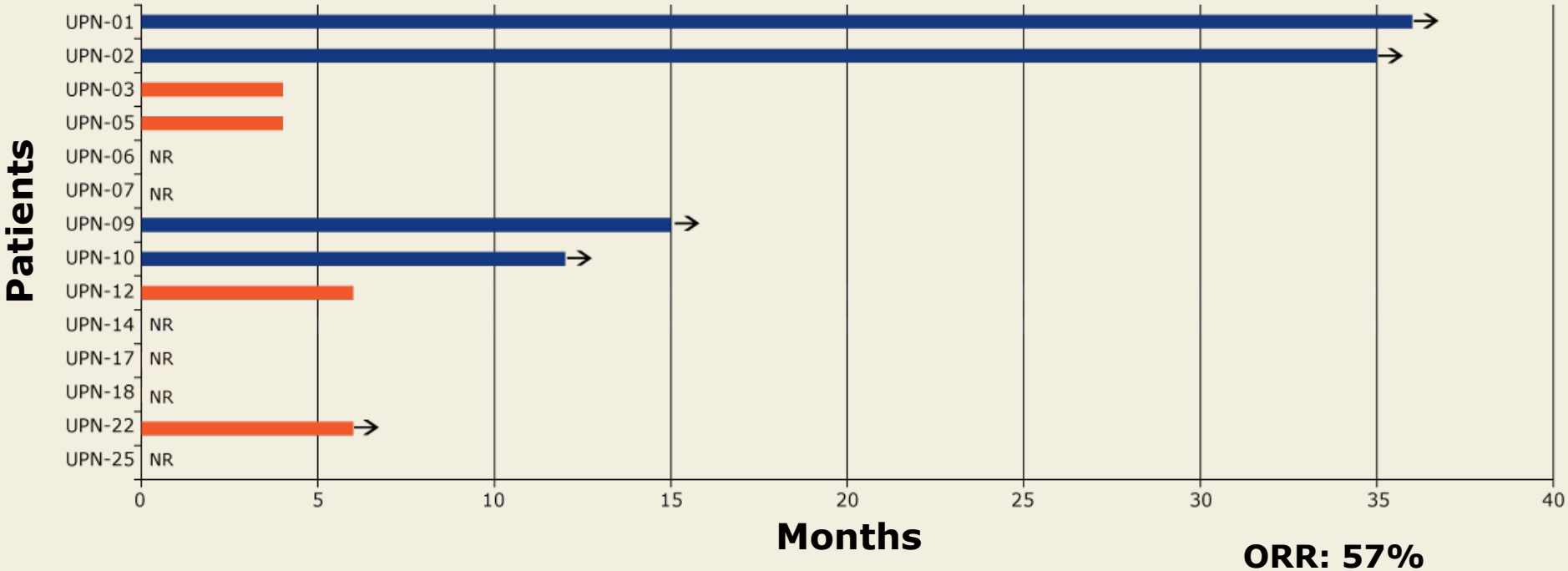
Best response	n = 14
Overall response rate Complete response (CR)* Partial response (PR)	8 (57%) 4 (29%) 4 (29%)
No response	6 (43%)

* Minimal residual disease-negative

- All 4 patients who achieved CR had no evidence of disease (NED) in blood, bone marrow (BM) and lymph nodes (LN).
- Two patients who achieved PR experienced PR in blood, BM and LN.
- Two patients who achieved PR had NED in blood and BM but PR in LN.
- Cell expansion: Responders (n = 8): ≥2-3 logs
  Nonresponders (n = 6): none/minimal/<2 log

Porter DL et al. *Proc ASH* 2013;Abstract 4162.

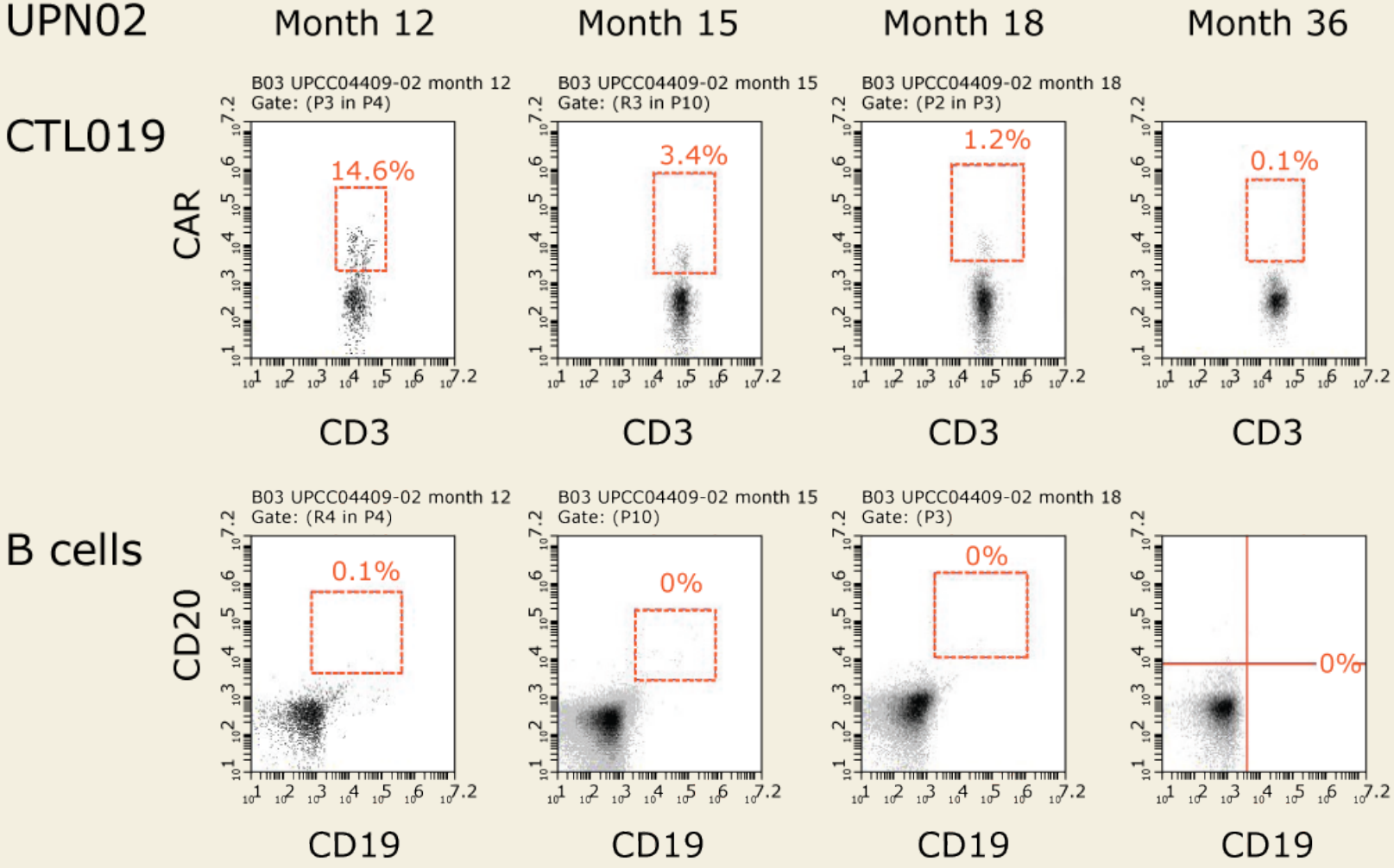
Duration of Response



- Median follow-up:
  - All patients: 12 mo (range 6-39)
  - Responding patients: 20 mo (range 6-39)

With permission from Porter DL et al. *Proc ASH* 2013;Abstract 4162.

CTL019 Persistence and B-Cell Aplasia in a Patient with Relapsed/Refractory CLL



With permission from Porter DL et al. *Proc ASH* 2013;Abstract 4162.

Adverse Events

- Tumor lysis syndrome (TLS) (delayed, coincident with T-cell expansion)
- Hepatotoxicity (reversible, Grade 3/4 in 4 responding patients)
- Renal toxicity (Grade 3/4 in 4 patients)
  - Related to TLS, acute tubular necrosis from hypotension, reversible
- B-cell aplasia and hypogammaglobulinemia in all patients achieving CR, supported with intravenous immunoglobulin
  - No excessive or frequent infections
- Cytokine release syndrome (CRS) in all responding patients
  - High fever, myalgia, nausea, hypotension, hypoxia
  - High levels of interleukin-6 (IL-6), marked increase in interferon-gamma
  - Modest levels of TNF-alpha, mild increase in IL-2 levels
  - CRS rapidly reversed with steroids (n = 1) or tocilizumab (n = 4)
  - CRS associated with hemophagocytic lymphohistiocytosis/ macrophage-activating syndrome

Porter DL et al. *Proc ASH* 2013;Abstract 4162.

Author Conclusions

- Robust in vivo 1,000-fold to 10,000-fold expansion in CTL019 T cells
- Persistence for >36 months
  - Persisting cells are functional in vitro (data not shown)
  - Ongoing responses over many months and B-cell aplasia imply that persisting cells are functional in vivo
- Overall response rate in heavily pretreated CLL: 8/14 (57%) — 4 CR, 4 PR
- Eradication of bulky tumor
- Responses are durable: No patient in CR has relapsed; some patients with PRs cleared blood and BM with ongoing lymph node responses
- Responding patients developed B-cell aplasia and CRS
  - CRS treated effectively with anticytokine therapy
- No obvious dose-response or dose-toxicity effects as yet
- CAR therapy holds great promise for patients with hematologic cancers

Porter DL et al. *Proc ASH* 2013;Abstract 4162.

Randomized, Phase II Dose Optimization Study of Chimeric Antigen Receptor Modified T Cells Directed Against CD19 (CTL019) in Patients with Relapsed, Refractory CLL

Porter DL et al.

Proc ASH 2013;Abstract 873.

Ongoing Phase II Study Design

NCT01747486

Stage I

Eligibility (target accrual = 32)

CD19+ relapsed/ refractory CLL or SLL
Failed ≥2 prior therapies
Progressed within 2 y of last treatment

R

Arm A (n = 12)
1-5 x 10^7 CTL019 cells

Arm B (n = 12)
1-5 x 10^8 CTL019 cells

SLL = small lymphocytic lymphoma

- **Primary endpoint:** CR at 3 months
- Stage II will enroll an additional 8 patients to the selected dose cohort when safety, tolerability and clinical responses have been evaluated to determine the optimal dose cohort.

Porter DL et al. *Proc ASH* 2013;Abstract 873.

Response

Response rate	n = 18
Overall response rate	7 (39%)
CR	3 (17%)
PR	4 (22%)

- CRS in 6 of 7 responding patients
  - 5 of 11 patients with no response had CRS (p = 0.15 compared to responders)

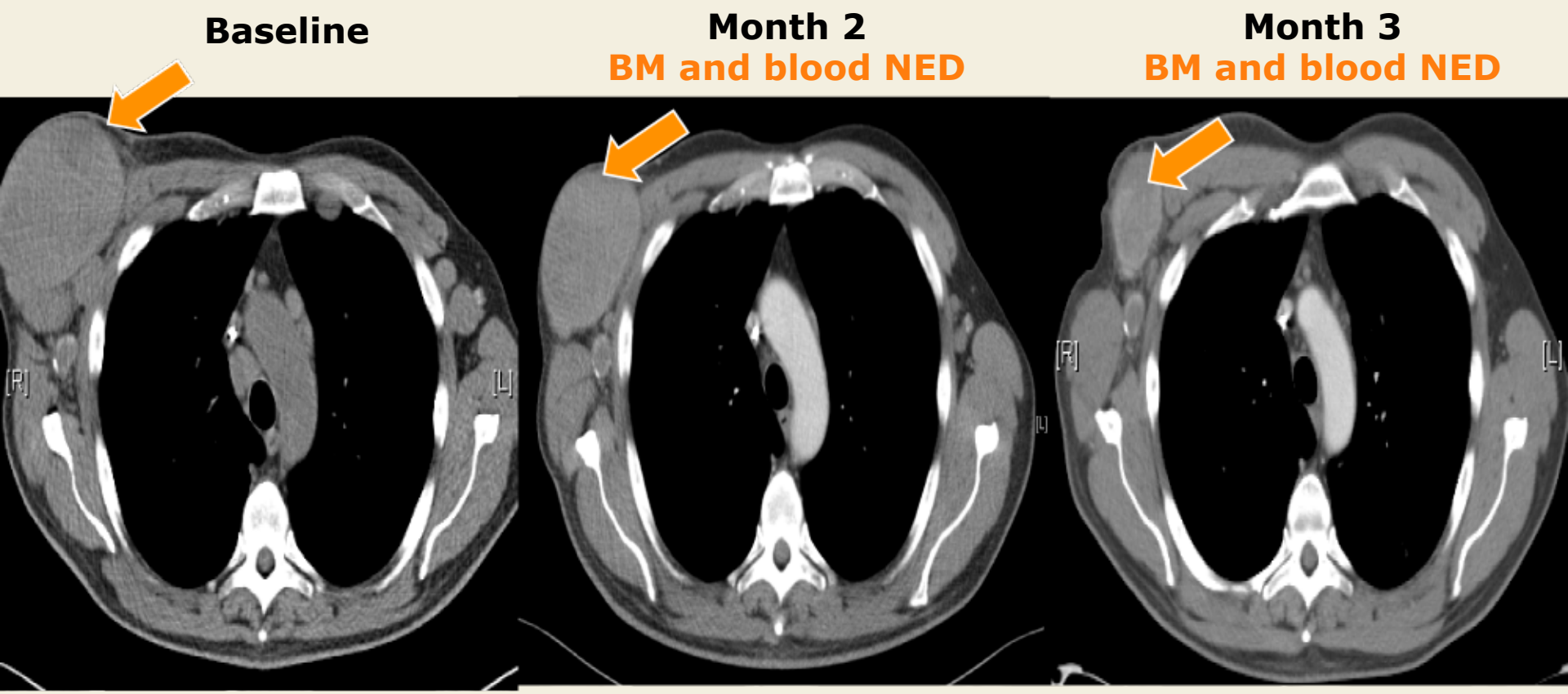
Porter DL et al. *Proc ASH* 2013;Abstract 873.

No Correlation between CTL019 Dose and Response or Toxicity

Response (n)	High dose (5×10^8)	Low dose (5×10^7)
Major response (CR + PR)	4	3
No response	5	6
Toxicity (n)	**High dose (5×10^8)**	**Low dose (5×10^7)**
CRS	5	6
No CRS	4	3

Porter DL et al. *Proc ASH* 2013;Abstract 873.

Ongoing Response in a Patient with Transformed CLL



10 prior therapies, transformed CLL, del(17p), ibrutinib resistant, XRT resistant

With permission from Porter DL et al. *Proc ASH* 2013;Abstract 873.

Adverse Events

- No significant acute infusional toxicity
- Hepatotoxicity (1 reversible, Grade 3)
- TLS (3 Grade 3/4), reversible and manageable
- B-cell aplasia and hypogammaglobulinemia in responding patients, supported with intravenous immunoglobulin (IVIG)
  - No excessive or frequent infections
- CRS in 6 of 7 responding patients
  - High fever, myalgia, nausea, hypotension, hypoxia
  - High levels of IL-6, mild increase in IL-2
  - Modest levels of interferon-gamma and TNF-alpha
  - CRS rapidly reversed with tocilizumab
  - CRS associated with hemophagocytic lymphohistiocytosis/macrophage-activating syndrome

Porter DL et al. *Proc ASH* 2013;Abstract 873.

Author Conclusions

- Massive CTL019 expansion (1,000-fold to 10,000-fold in vivo*)*
- Overall response rate of 7 out of 18 (39%) in heavily pretreated CLL
  - 3 CR, 4 PR; several showing clearance of blood and bone marrow with ongoing lymph node responses
  - Eradication of bulky tumor
- Most responding patients develop CRS, which is treated effectively with supportive care and anti-IL-6 receptor antagonist therapy when needed
- No obvious dose-response or dose-toxicity effects as yet
- Responding patients develop B-cell aplasia
  - Hypogammaglobulinemia was treated with IVIG
- CAR therapy is promising for patients with advanced, relapsed/refractory CLL

Porter DL et al. *Proc ASH* 2013;Abstract 873.

Investigator Commentary: CAR-Modified T Cells Against CD19 in Relapsed/Refractory CLL

The first study, a pilot trial with 14 patients with relapsed/refractory CLL, aimed to assess the feasibility, safety and efficacy of CAR therapy. We reported an overall response rate of >50% with CAR therapy, even in patients with bulky disease and high-risk features.

One of the unique aspects of this study is that we were able to provide long-term follow-up, and 2 of the patients have been in remission for more than 3 years. In addition to long-term remission, we observed long-term persistence of the CAR T cells for more than 3 years. No correlation between dose and response or toxicity was seen. It was important to identify the optimal cell dose to establish consistency, and that led to the subsequent Phase II dose-optimization study.

That study randomly assigned patients to 2 doses of CTL019 cells. Both doses were used previously in patients who had responded. Again, no correlation between dose and toxicity or response was noted. The overall response rate was about 40%. Robust expansion of the CTL019 cells in the body occurred. This confirms our hypothesis that if T cells proliferate in vivo a lower dose can be administered. It may be useful to use a lower starting dose in certain situations if the activity is the same. The importance of this study will be to help define a therapeutic dose moving forward.

(Continued)

The CAR construct we use is unique in that it includes the 4-1BB costimulatory domain. This not only provides signals for T-cell activation through the costimulatory pathway but also provides survival signals to T cells. We believe that the 4-1BB domain may account for the long-term persistence of the modified T cells.

CRS was noted in responding patients and could be reversed with steroids and anti-IL-6 therapy. Responding patients also develop B-cell aplasia and hypogammaglobulinemia. We believe this to be an on-target effect because CD19 is present on normal B cells. To date, we have not observed any unexpected side effects from hypogammaglobulinemia, but these patients are being maintained with intravenous immunoglobulin repletion. B-cell aplasia is thought to be a marker of ongoing CAR T-cell activity.

Interview with David L Porter, MD, March 3, 2014

Phase I Trial of Autologous CD19-Targeted CAR-Modified T Cells as Consolidation After Purine Analog-Based First-Line Therapy in Patients with Previously Untreated CLL

Park JH et al.

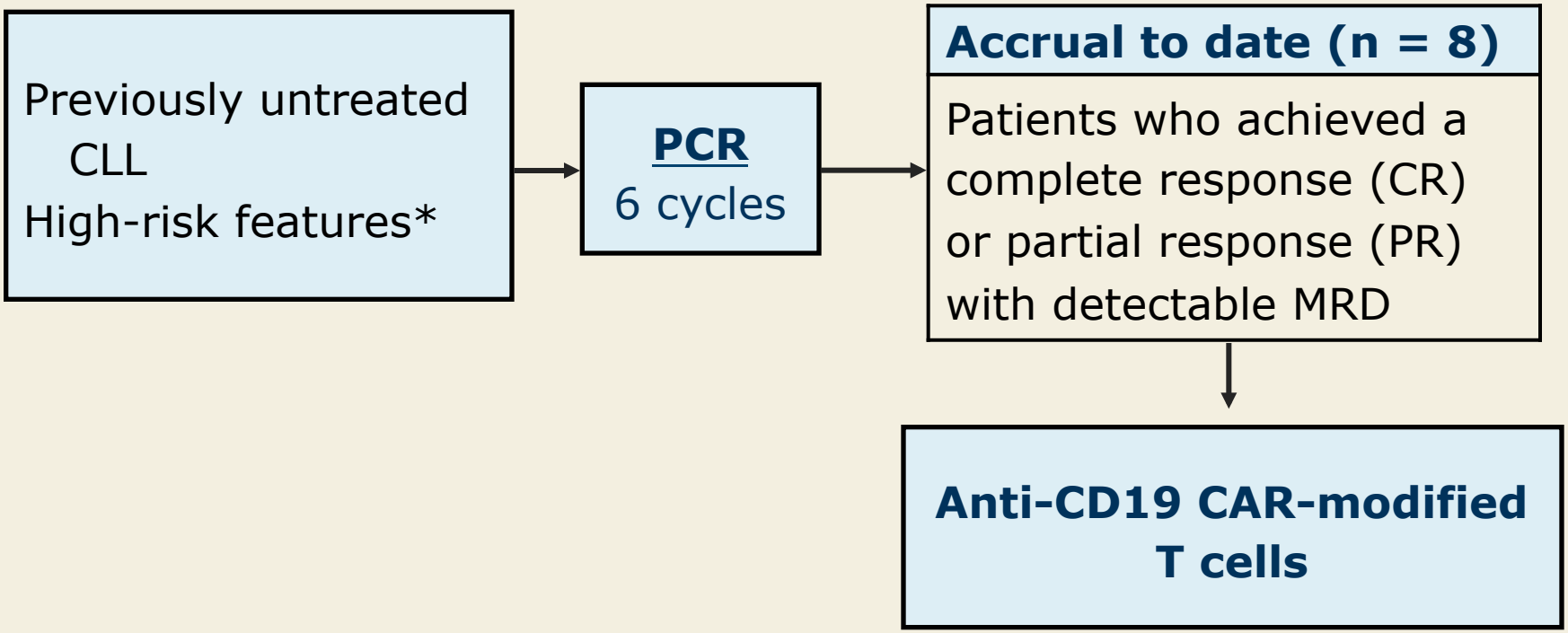
Proc ASH 2013;Abstract 874.

Background

- T cells may be genetically modified to express chimeric antigen receptors (CARs) targeted to antigens expressed on tumor cells.
- Initial results from a Phase I trial in chemotherapy-refractory/relapsed CLL treated with autologous T cells modified to express CAR targeted to the CD19 antigen showed promise (*Blood* 2011;118:4817).
- Treatment with CD19-targeted CAR-modified T cells resulted in significantly increased rates of durable responses in CLL with reduced disease burden and chemotherapy-sensitive disease.
- **Study objective:** To determine the efficacy and safety of anti-CD19 CAR-modified T cells as consolidation therapy for patients with minimal residual disease (MRD) after first-line chemotherapy.

Park JH et al. *Proc ASH* 2013;Abstract 874.

Ongoing Phase I Trial Design



P = pentostatin; C = cyclophosphamide; R = rituximab

* Defined by the presence of unmutated IgHV, del(11q) or del(17p)

Park JH et al. *Proc ASH* 2013;Abstract 874 (abstract only).

Study Methods

- Autologous T cells collected by leukapheresis were transduced with a retroviral vector encoding the anti-CD19 single-chain variable fragment linked to CD28 costimulatory and CD3zeta signaling domains.
- Patients received cyclophosphamide conditioning therapy followed 2 days later by the infusion of the CAR-modified T cells in 3 dose-escalating cohorts.
- Response assessment was performed according to the criteria established by the National Cancer Institute-sponsored working group.
- Serial bone marrow aspirate and blood samples were assessed for modified T-cell persistence.
  - This was assessed by flow cytometry, real-time polymerase chain reaction and cytokine profile analysis.

Park JH et al. *Proc ASH* 2013;Abstract 874 (abstract only).

Patient Characteristics

Characteristic	n = 8*
Median age (range)	61.5 years (45-68)
Unmutated IgHV	75%
Del(11q)	25%
Median follow-up from the time of T-cell infusion (range)	7 mo (2-12)
Median time from completion of up-front PCR chemotherapy to T-cell infusion (range)	6.5 mo (4-12)

* To date, 8 patients have been enrolled
  - 6 patients have received CAR-modified T cells, completing the 2 dose cohorts of 3 x 10^6 and 1 x 10^7 CAR-modified T cells/kg (3 in each cohort)

Park JH et al. *Proc ASH* 2013;Abstract 874 (abstract only).

Response Rates

- Patients who experienced a PR after first-line PCR chemotherapy and achieved a CR after T-cell infusion: n = 2
- Patients who achieved and maintained a PR: n = 2
- Patients with progressive disease (PD): n = 2
  - Of these 2 patients, 1 achieved a PR after 6 cycles of PCR but had progressive lymphocytosis with an absolute lymphocyte count doubling time of 1 month at the time of T-cell infusion.
  - The other patient experienced lymph node-only relapse while the bone marrow remained MRD-negative.

Park JH et al. *Proc ASH* 2013;Abstract 874 (abstract only).

Treatment-Related Adverse Events

- No dose-limiting toxicity was observed.
- Cytokine release syndrome (CRS), manifested by fever, nausea, anorexia and transient hypotension, was observed (n = 2).
  - None required treatment with steroids or other anti-inflammatory agents.
- There was a positive correlation between the development of CRS and modified T-cell persistence.

Park JH et al. *Proc ASH* 2013;Abstract 874 (abstract only).

Author Conclusions

- The infusion of autologous CD19-targeted CAR-modified T cells appears to be safe and has demonstrated promising antitumor efficacy to further improve CR rates in patients with high-risk CLL undergoing first-line purine analog-based chemotherapy.
- Although the number of treated patients in this study is too small to draw a definitive conclusion, the findings suggest that:
  - The second-generation CD19-targeted CAR-modified T cells elicit enhanced antitumor activity in patients with reduced disease burden.
  - Treatment with CD19-targeted CAR-modified T cells is more effective in eradicating disease in the bone marrow than in the lymph nodes.
- Enrollment to this study is ongoing.

Park JH et al. *Proc ASH* 2013;Abstract 874 (abstract only).

Investigator Commentary: Ongoing Phase I Trial of Anti-CD19 CAR-Modified T Cells in CLL

In this study, patients with previously untreated CLL with high-risk features other than persistent disease received standard induction PCR chemotherapy. Then, patients who achieved CR/PR with detectable MRD received genetically modified T cells. This is an interesting study that attempts to move the use of CAR-modified T cells to earlier settings as consolidation therapy for patients who one can predict will not fare well with conventional chemotherapy. It is known that patients who achieve a CR and have no MRD after conventional induction therapy have a long progression-free survival (PFS). However, patients with MRD after initial induction have a shorter PFS and overall survival. Therefore, the presence of MRD is a marker for subsequent poor outcome.

Only a small number of patients had received treatment at the time of the ASH presentation. It was a relatively limited study with a short follow-up. Of interest, the incidence of CRS was limited. This adds to other findings that seem to show that the degree of disease burden may predict the severity of CRS. All of the patients in this study had limited disease and did not experience severe CRS.

(Continued)

Two of the patients with persistent disease achieved a CR after T-cell infusion, 2 achieved a PR and 2 experienced PD. Although this study involved a small number of patients, it shows that one can potentially move this treatment strategy up front as consolidation after first-line therapy. This is the direction the field is and should be moving — trying to use this approach earlier in the course of the disease to prevent the disease from becoming refractory or extensive.

I believe that enough data will soon be generated to be able to move this strategy up as initial up-front treatment instead of consolidation therapy. It's early yet and we need more information about whether this approach will be effective for patients with low-burden disease. That would lend support to trying to use it as initial therapy in a chemotherapy-free regimen. An advantage over some of the newer therapies would be that this approach has the potential to be a "once-and-done" treatment if the CAR-modified T cells persist and are able to eradicate CLL. The patient may not need ongoing lifelong therapy as he or she would with some of the newer agents.

Interview with David L Porter, MD, March 3, 2014

Safe and Effective Re-Induction of Complete Remissions in Adults with Relapsed B-ALL Using 19-28z CAR CD19-Targeted T Cell Therapy

Davila ML et al.

Proc ASH 2013;Abstract 69.

Background

- A majority of adults with B-cell acute lymphoblastic leukemia (B-ALL) relapse and develop chemorefractory disease.
- Novel, nonchemotherapy-based treatments are needed for this group of patients.
- Minimal residual disease (MRD)-negative remissions were observed in small numbers of patients with B-ALL treated with adoptive immunotherapy using T cells genetically engineered to express chimeric antigen receptors (CARs) targeting CD19, a molecule expressed on normal and malignant B cells (*Sci Transl Med* 2013;5(177):177ra38).
- **Study objective:** To determine the safety, appropriate dose and efficacy of CARs in adults with relapsed/ refractory (RR) B-ALL.

Davila ML et al. *Proc ASH* 2013;Abstract 69 (abstract only).

Phase I Study Methods (NCT01044069)

- Adults with RR CD19+ B-ALL (target enrollment = 40) were enrolled.
- Patients were leukapheresed and reinduced with salvage chemotherapy.
- Patients received an infusion of the genetically modified T cells (3×10^6 19-28z CAR T cells/kg) after conditioning chemotherapy.
  - 19-28z is composed of a CD19 binding domain fused to the signaling domains of the CD28 costimulatory receptor and the ζ chain of the CD3 complex.
- 13 patients enrolled to date: 11 had RR disease, 2 were enrolled and leukapheresed during CR1 but were not treated until relapse.

Davila ML et al. *Proc ASH* 2013;Abstract 69 (abstract only).

Baseline Characteristics

- Median patient age was 42 (range: 23 to 74).
- 3 of the 13 patients had Philadelphia chromosome-positive (Ph+) B-ALL.
- The required T-cell dose was achieved in all but 1 patient.
- 7 of the 13 patients were infused with 19-28z CAR T cells while they had gross residual disease (>5% to 70% blasts in the bone marrow).
- The remaining patients had MRD, detected by flow cytometry or deep sequencing, at the time of 19-28z CAR T-cell infusion.

Davila ML et al. *Proc ASH* 2013;Abstract 69 (abstract only).

Clinical Outcomes

- 10 of the 12 patients with detectable disease before T-cell infusion developed MRD-negative responses.
  - 5 patients with gross residual disease (blasts >5% in bone marrow) became MRD-negative.
  - 5 MRD-positive patients became MRD-negative.
- The rapidity of the responses was remarkable, with MRD-negative results obtained as early as 7 to 14 days after T-cell infusion.
- Patients with relapsed Ph+ B-ALL achieved MRD-negative status as determined by both deep sequencing for the IgH rearrangement and qPCR for the BCR-ABL transcript.

Davila ML et al. *Proc ASH* 2013;Abstract 69 (abstract only).

Adverse Events

- 6 of 13 patients developed toxicities, including high-grade fevers, hypotension, hypoxia, mental status changes and seizures.
- These episodes occurred for approximately 1 week before they were halted by treatment with steroids or tocilizumab.
- All patients completely recovered and were able to leave the hospital.
- The occurrence of toxicities correlated with tumor burden such that patients with gross residual disease (>5% blasts in bone marrow) developed toxicities, whereas those with MRD had no evidence of toxicities.
- No toxicities were experienced by the other 7 patients.

Davila ML et al. *Proc ASH* 2013;Abstract 69 (abstract only).

Author Conclusions

- The potent induction of MRD-negative responses and the reversibility of toxicities occurring in a subset of patients strongly support integration of this therapy into the modern paradigm for B-ALL therapy.
- Of the 13 patients treated, 4 underwent an allogeneic stem cell transplant (allo-SCT) and 5 are being prepared for an allo-SCT. Of the remaining 4 patients, 1 is a nonresponder, 1 relapsed, 1 is in CR2 (medically not eligible for allo-SCT) and 1 has not been evaluated for response yet.
- Toxicities associated with this therapy are predictable and manageable.
- The remarkable MRD-negative reinduction rate and facilitation for allo-SCT reported warrant further evaluation of this therapy in a Phase II protocol.

Davila ML et al. *Proc ASH* 2013;Abstract 69 (abstract only).

Investigator Commentary: Reinduction of Complete Remissions in Relapsed B-ALL Using 19-28z CAR T-Cell Therapy

In this study 10 of the 12 patients with relapsed/refractory B-ALL treated with CAR therapy achieved MRD-negative status. In terms of toxicity, most patients develop delayed cytokine release syndrome after a week or 2. It manifests as fever, bone and muscle aches, nausea and vomiting and anorexia. These side effects can be effectively managed with steroids and anti-IL-6.

This is an exciting approach that needs further study. I believe that it is possible to develop this therapy for use in the front-line setting. Patients with ALL who have MRD could be treated with CAR therapy, potentially increasing the chance of a cure and obviating the need for additional chemotherapy.

Interview with Hagop M Kantarjian, MD, January 29, 2014

Investigator Commentary: Reinduction of Complete Remissions in Relapsed B-ALL Using 19-28z CAR T-Cell Therapy (Continued)

This study used a CAR construct that is slightly different than the one used in our studies, but it appears to have extremely potent activity in ALL. The authors reported that 10 of 12 patients with detectable disease prior to T-cell infusion subsequently achieved MRD-negative status. The highlight of the study was that the therapy was effective even in patients with Ph+ B-ALL, which is important and unique. Four of the 13 patients who underwent treatment were able to undergo allo-SCT and another 5 patients were being prepared for transplant. The investigators were using this approach as a bridge to transplant for patients who would have been otherwise ineligible so that they might have a chance of being cured.

Interview with David L Porter, MD, March 3, 2014

Effective Treatment of Chemotherapy-Refractory Diffuse Large B-Cell Lymphoma with Autologous T Cells Genetically-Engineered to Express an Anti-CD19 Chimeric Antigen Receptor

Kochenderfer JN et al.

Proc ASH 2013;Abstract 168.

Background

- Chimeric antigen receptors (CARs) are fusion proteins incorporating an antigen recognition moiety and T-cell activation domains.
- T cells can be genetically modified to express CARs and transferred to patients.
- Previous reports of the first 9 patients on a clinical trial who received CAR T-cell treatment showed that this is a promising new approach for treating B-cell cancers because of a potent ability to eradicate CD19-positive cells in vivo (*Blood* 2010;116:4099; *Blood* 2012;119:2709).
- **Study objective:** To report results from 14 patients on this clinical trial who received anti-CD19 CAR T cells with a new 10-day culture process.

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168.

Study Methods

- The CAR used in this study was encoded by a gammaretrovirus and incorporates the variable regions of an anti-CD19 antibody, part of CD28 and part of CD3-zeta.
- A total of 14 patients received anti-CD19 CAR T cells that were produced with a new 10-day culture process.
  - Eight evaluable patients with primary mediastinal B-cell lymphoma or diffuse large B-cell lymphoma not otherwise specified had chemotherapy-refractory disease defined as progression or no response 1 month after the end of the most recent chemotherapy.
  - No patients received exogenous interleukin-2.

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Study Methods (Continued)

- A mean of 70.5% of the infused T cells expressed the CAR, and the infused cells produced cytokines and degranulated in a CD19-specific manner.
- Because prior chemotherapy has been shown to enhance the activity of adoptively transferred T cells, patients received:
  - Cyclophosphamide (different doses) in combination with fludarabine (25 mg/m^2 daily for 5 days) before a single infusion of anti-CD19 CAR-transduced T cells.

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Patient Characteristics

Pt	Age (years)/ gender	Cancer	No. of prior therapies	Total cyclo dose
1	56/M	SMZL	4	120 mg/kg
2	43/F	PMBCL	4	60 mg/kg
3	61/M	CLL	2	60 mg/kg
4	30/F	PMBCL	3	120 mg/kg
5	63/M	CLL	4	120 mg/kg
6	48/M	CLL	1	60 mg/kg
7	42/M	DLBCL	5	60 mg/kg

cyclo = cyclophosphamide; SMZL = splenic marginal zone lymphoma; PMBCL = primary mediastinal B-cell lymphoma; CLL = chronic lymphocytic leukemia; DLBCL = diffuse large B-cell lymphoma not otherwise specified

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Patient Characteristics (Continued)

Pt	Age (years)/ gender	Cancer	No. of prior therapies	Total cyclo dose
8	44/F	PMBCL	10	60 mg/kg
9	38/M	PMBCL	3	120 mg/kg
10	57/F	Low-grade NHL	4	60 mg/kg
11	58/F	DLBCL from CLL	13	60 mg/kg
12	60/F	DLBCL	3	60 mg/kg
13	68/M	CLL	4	60 mg/kg
14	43/M	DLBCL	2	60 mg/kg

NHL = non-Hodgkin lymphoma

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Responses

Pt	No. of CAR T cells infused (x 10^6/kg)	Response type	Time to response after infusion
1	5	PR	20+ months
2	5	CR	19+ months
3	4	CR	16+ months
4	2.5	NE	—
5	2.5	CR	10+ months
6	2.5	CR	7+ months
7	2.5	CR	4+ months

PR = partial remission; CR = complete remission; NE = not evaluable;
+ indicates ongoing response

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Responses (Continued)

Pt	No. of CAR T cells infused (x 10^6/kg)	Response type	Time to response after infusion
8	2.5	PR	6+ months
9	2.5	SD	1 month
10	1	PR	4+ months
11	1	PR	2 months
12	1	SD	1+ month
13	1	PR	2+ months
14	1	PR	1+ month

SD = stable disease

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Clinical Outcomes

- All of the 8 treated patients with either PMBCL or DLBCL were chemotherapy refractory.
  - Five of these 8 patients obtained either a CR or PR on this trial.
- Blood B-cell depletion lasting for more than 3 months occurred in 3 of 3 evaluable patients.
- Most patients were not evaluable for B-cell depletion due to B-cell depletion by prior treatments.
- One patient died suddenly of unknown etiology 16 days after infusion of CAR T cells.
- Peak blood levels of cells containing the CAR gene ranged from 2.3% to 66.5% of blood mononuclear cells.

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Adverse Events

- The acute toxic effects observed included:
  - Fever, hypotension and delirium, which occurred after the infusion of anti-CD19 CAR T cells.
- The toxicities resolved in less than 3 weeks after the cell infusion and were temporally associated with elevated serum interleukin-6 and interferon gamma levels in most patients.

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Author Conclusions

- These results demonstrate the feasibility of treating patients with chemotherapy-refractory B-cell cancers with autologous anti-CD19 CAR T cells.
- The numerous remissions obtained should encourage further development of this treatment approach.

Kochenderfer JN et al. *Proc ASH* 2013;Abstract 168 (abstract only).

Investigator Commentary: Treatment of Chemotherapy-Refractory CD19-Positive DLBCL with CAR T Cells

This study expands on the use of CAR T cells and includes patients with CD19-positive cancers such as DLBCL and PMBCL. The investigators use a technique of retroviral transduction and CD28 costimulation instead of lentiviral transduction with a CAR composed of 4-1BB. This is important because it shows that activity is possible not only in acute lymphocytic leukemia (ALL) and CLL but also in more nodal CD19-positive diseases, including NHL.

Out of 8 patients with PMBCL or DLBCL, 5 achieved a CR or a PR. Unlike PRs observed with chemotherapy, many of the partial responses achieved with CAR T cells are significant and ongoing. These responses can continue over time as long as the T cells have survived and remain active. So it's like having continuous exposure to treatment, which makes the achievement of a PR important. A couple of patients achieved a CR, and this is a significant observation.

The toxicities observed were similar to what other investigators have reported in ALL and CLL. In this study, a significant expansion of CAR T cells similar to that seen in other trials was observed. It will be important to be able to use this treatment approach in NHL. Of note, this is currently being studied by this group and others.

Interview with David L Porter, MD, March 3, 2014

Results of a Phase 2 Randomised, Open-Label, Study of Lower Doses of Quizartinib (AC220; ASP2689) in Subjects with FLT3-ITD Positive Relapsed or Refractory Acute Myeloid Leukemia[1]

First Clinical Results of a Randomized Phase 2 Study of SGI-110, a Novel Subcutaneous (SQ) Hypomethylating Agent (HMA), in Adult Patients with Acute Myeloid Leukemia[2]

[1]Cortes JE et al.
Proc ASH 2013;Abstract 494.

[2]Kantarjian HM et al.
Proc ASH 2013;Abstract 497.

Adding the KIT Inhibitor Dasatinib (DAS) to Standard Induction and Consolidation Therapy for Newly Diagnosed Patients (pts) with Core Binding Factor (CBF) Acute Myeloid Leukemia (AML): Initial Results of the CALGB 10801 (Alliance Study)[3]

Low-Dose Lenalidomide plus Low-Dose Cytarabine Induce Complete Remission That Can Be Predicted by Genetic Profiling in Very Elderly Acute Myeloid Leukemia Patients[4]

[3]Marucci G et al.
Proc ASH 2013;Abstract 357.

[4]Visani G et al.
Proc ASH 2013;Abstract 496.

Results of a Phase 2 Randomised, Open-Label, Study of Lower Doses of Quizartinib (AC220; ASP2689) in Subjects with FLT3-ITD Positive Relapsed or Refractory Acute Myeloid Leukemia

Cortes JE et al.

Proc ASH 2013;Abstract 494.

Comparison of Lower-Dose Quizartinib to Higher Doses

	2689-CL-2004		AC220-002 (cohort 2)		
	30 mg/ day (n = 38)	**60 mg/ day** (n = 38)	**90 mg/ day** (n = 57)	**135 mg/ day** (n = 67)	**200 mg/ day** (n = 12)
Best response					
CRc rate	47%	47%	47%	45%	42%
PR rate	13%	24%	25%	28%	50%
Maximum change in QTcF from baseline (msec)					
≤30	50%	44%	9%	9%	0%
>30 to ≤60	47%	36%	46%	51%	8%
>60	3%	19%	46%	39%	92%

CRc rate = complete remission (CR) + CR with incomplete platelet recovery + CR with incomplete hematologic recovery

Cortes JE et al. *Proc ASH* 2013;Abstract 494.

Results Summary

- Sustained efficacy and decreased QT signal with lower doses of quizartinib
  - Efficacy:
    - Substantial activity at both doses
  - Safety:
    - Similar safety profile at 30- and 60-mg doses
    - QTcF prolongation is dose-dependent. QTcF at both doses was decreased compared to prior Phase II study at 90 mg/day and 135 mg/day.
- Next step:
  - Global Phase III randomized trial of quizartinib in patients with FLT3-ITD-positive disease in first relapse is under way (NCT02039726).

Cortes JE et al. *Proc ASH* 2013;Abstract 494.

First Clinical Results of a Randomized Phase 2 Study of SGI-110, a Novel Subcutaneous (SQ) Hypomethylating Agent (HMA), in Adult Patients with Acute Myeloid Leukemia

Kantarjian HM et al.
Proc ASH 2013;Abstract 497.

Results Summary

- SQ SGI-110 is a new HMA that is well tolerated and clinically active in the treatment of AML.
- Complete remissions and potent demethylation of ≥10% were equally observed at the doses of 60 and 90 mg/m^2.
- These data support further Phase III investigation of this agent in the treatment of AML.
- Preliminary overall remission rate of 53% in treatment-naïve elderly AML seems to compare favorably to previous results reported for HMA treatment, but this result needs confirmation in a larger number of patients and randomized studies.

Kantarjian HM et al. *Proc ASH* 2013;Abstract 497.

Investigator Commentary: First Clinical Results of a Phase II Study of the Novel Hypomethylating Agent SGI-110

SGI-110 is a molecule that combines decitabine with guanosine, so this drug can produce higher areas under the curve for the release of decitabine and has a longer half-life. This was an update of a study with SGI-110. From my own impression and from the studies reported, SGI-110 is a drug to be reckoned with in terms of its further evaluation with pivotal trials that compare SGI-110 to either azacitidine or decitabine in the setting of myelodysplastic syndrome or AML. This is a drug to keep in mind, and I do believe it is going to be a significant drug in the future based on the published data so far.

Interview with Hagop M Kantarjian, MD, January 29, 2014

Adding the KIT Inhibitor Dasatinib (DAS) to Standard Induction and Consolidation Therapy for Newly Diagnosed Patients (pts) with Core Binding Factor (CBF) Acute Myeloid Leukemia (AML): Initial Results of the CALGB 10801 (Alliance Study)

Marucci G et al.

Proc ASH 2013;Abstract 357.

Results Summary

- Early results from this study show:
  - Rapid diagnostic screening for CBF AML is feasible within a cooperative group
  - DAS plus chemotherapy in patients with CBF AML is tolerable, including in older patients
  - Initial clinical outcomes are at least comparable to those historically observed in this patient population
    - CR rate = 92%
    - 1-year OS rates: 95% (younger patients) and 62% (older patients)
- Patient follow-up and molecular characterization are ongoing and will be correlated with toxicity and clinical outcome.

Marucci G et al. *Proc ASH* 2013;Abstract 357.

Low-Dose Lenalidomide plus Low-Dose Cytarabine Induce Complete Remission That Can Be Predicted by Genetic Profiling in Very Elderly Acute Myeloid Leukemia Patients

Visani G et al.
Proc ASH 2013;Abstract 496.

Results Summary

- Low-dose lenalidomide (10 mg/day) plus low-dose cytarabine has high clinical activity in elderly patients with AML, with an overall CR rate of 43%.
  - 9 of 16 responding patients are still in CR after median follow-up of 12 months
  - Responding patients had a longer median overall survival than nonresponders (428 versus 74 days)
- A molecular signature including 114 genes and 18 microRNA was identified as being associated with clinical response (CR versus no CR).
- Based on the expression of 5 genes, an algorithm was developed to predict treatment response that was validated by showing 87% overall accuracy.

Visani G et al. *Proc ASH* 2013;Abstract 496.

Gemtuzumab Ozogamicin (GO) in Children with *De Novo* Acute Myeloid Leukemia (AML) Improves Event-Free Survival (EFS) by Reducing Relapse Risk — Results from the Randomized Phase III Children's Oncology Group (COG) Trial, AAML0531[1]

The Addition of Gemtuzumab Ozogamicin (GO) to Induction Chemotherapy Reduces Relapse and Improves Survival in Patients without Adverse Risk Karyotype: Results of an Individual Patient Meta-Analysis of the Five Randomized Trials[2]

Reasons for Survival Improvement in Core Binding Factor AML: A 23 Year Analysis of the UK MRC/NCRI AML Trials[3]

[1]Gamis AS et al.
Proc ASH 2013;Abstract 355.

[2]Hills RK et al.
Proc ASH 2013;Abstract 356.

[3]Burnett AK et al.
Proc ASH 2013;Abstract 358.

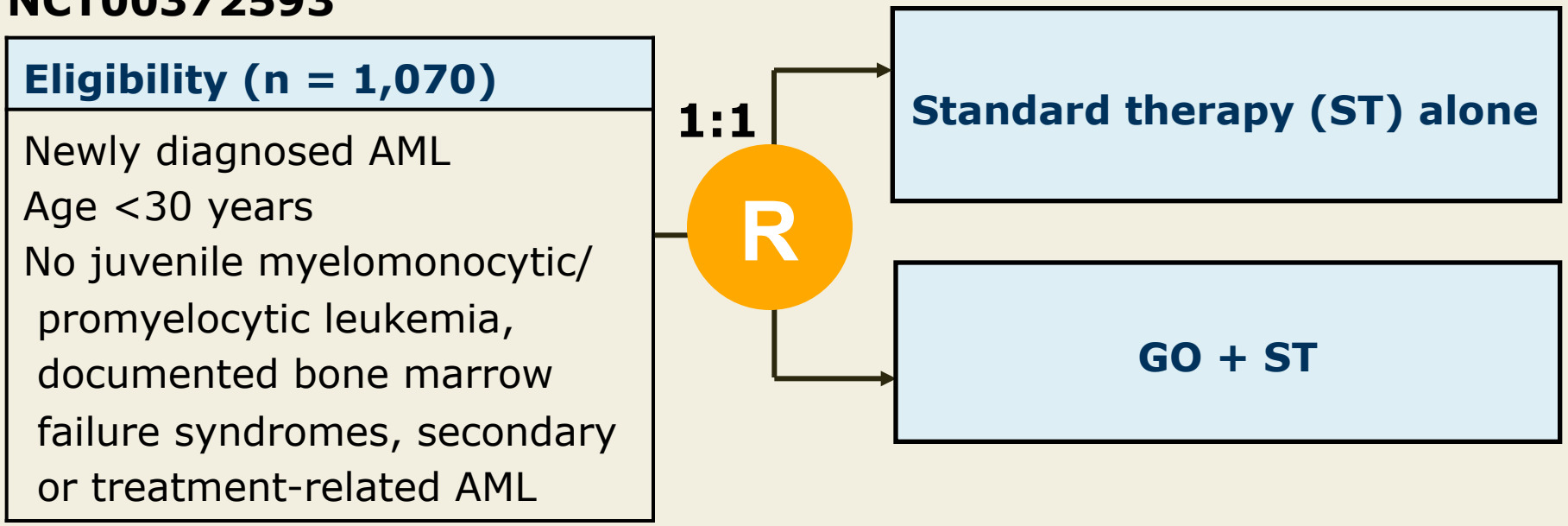
Gemtuzumab Ozogamicin (GO) in Children with *De Novo* Acute Myeloid Leukemia (AML) Improves Event-Free Survival (EFS) by Reducing Relapse Risk — Results from the Randomized Phase III Children's Oncology Group (COG) Trial, AAML0531

Gamis AS et al.

Proc ASH 2013;Abstract 355.

Phase III COG-AAML0531 Trial Design

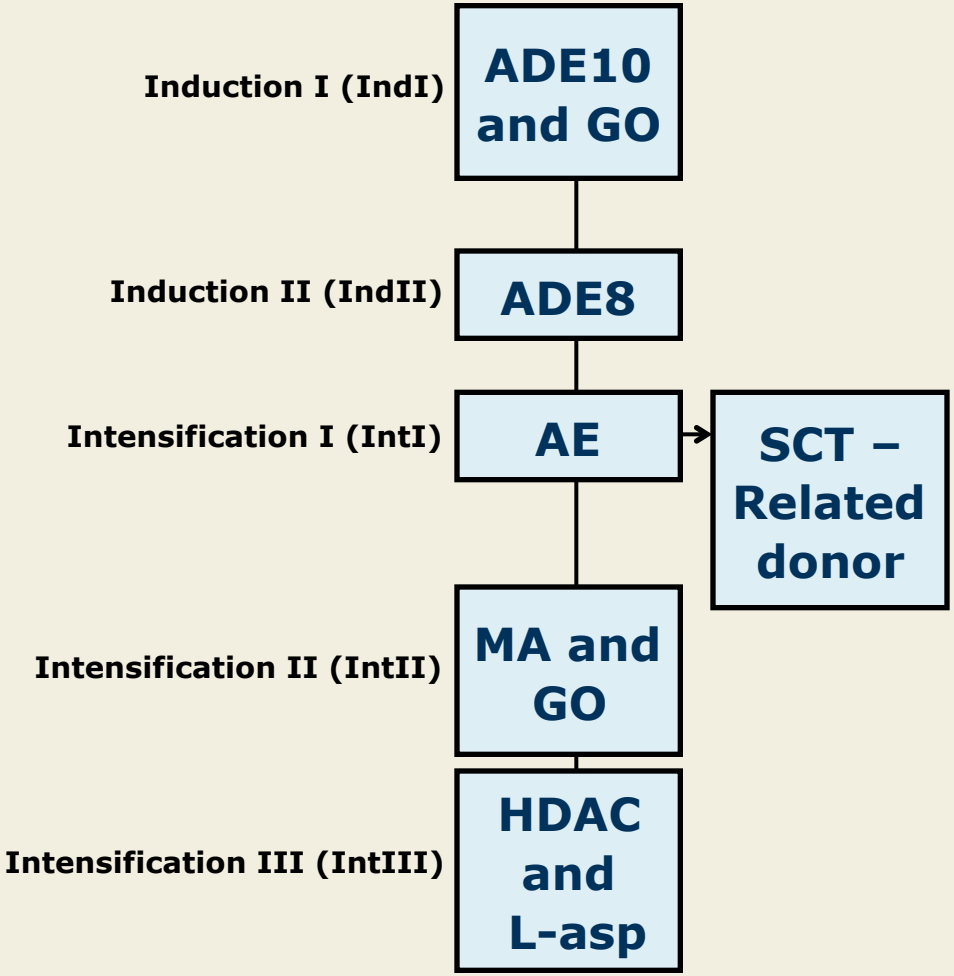
NCT00372593



- **Primary endpoint:** EFS and overall survival (OS)
- Use of allogeneic hematopoietic stem cell transplant (SCT) was stratified by overall risk group assignment in which patients were classified as:
  - High risk (HR) allocated to best allogeneic donor SCT after the first intensification phase of the study
  - Low risk (LR) received ST only
  - Intermediate risk (IR) assigned to SCT if there was a matched family donor

Gamis AS et al. *Proc ASH* 2013;Abstract 355.

Treatment Plan



- **IndI:** Cytarabine (A), 100 mg/m^2/dose every 12 h on d1-10; daunorubicin (D), 50 mg/m^2/dose on d1, 3, 5; etoposide (E), 100 mg/m^2/dose on d1-5; GO, 3 mg/m^2/dose on d6
- **IndII:** A, 100 mg/m^2/dose every 12 h on d1-8; D, 50 mg/m^2/dose on d1, 3, 5; E, 100 mg/m^2/dose on d1-5
- **IntI:** A, 1 g/m^2/dose every 12 h on d1-5; E, 150 mg/m^2/dose on d1-5
- **IntII:** Mitoxantrone, 12 mg/m^2/dose on d3-6; A, 1 g/m^2/dose every 12 h on d1-4; GO, 3 mg/m^2/dose on d7
- **IntIII:** A, 3 g/m^2/dose twice daily on d1, 2, 8, 9; *Escherichia coli* L-asparaginase 6,000 U/m^2 (IM) on d2, 9
- **CNS prophylaxis:** Intrathecal A on d1 of IndI and II and IntI

Cooper TM et al. *Cancer* 2012;118(3):761-9.

Response

Response rate	ST alone	GO + ST	*p*-value
Complete response (CR)	88%	85%	NSD

NSD = no significant difference

Gamis AS et al. *Proc ASH* 2013;Abstract 355 (abstract only).

Results Summary

At 3 years from time of CR	OS		Relapse risk		DFS	
	ST	GO + ST	ST	GO + ST	ST	GO + ST
All	70%	74%	41%	33%*	55%	61%†
By ORG						
LR	86%	85%	30%	20%†	68%	73%
IR	67%	70%	46%	40%	51%	56%
HR	49%	68%†	45%	27%†	40%	56%

DFS = disease-free survival; ORG = overall risk group

* p-value ≤0.01; † p-value ≤0.1

- At 3 years from time of enrollment, GO was significantly associated with better overall EFS (53% vs 47% , p = 0.05). OS was not significantly improved (69% vs 65%, p = 0.18).
- GO improves EFS in children, adolescents and young adults with AML by reducing the risk of relapse among those achieving remission.

Gamis AS et al. *Proc ASH* 2013;Abstract 355 (abstract only).

Investigator Commentary: AAML0531 — A Phase III Trial of GO in Children with AML

This study reports updated results from a pediatric leukemia study of about 1,000 patients with AML in which GO was administered at a dose of 3 mg/m^2 during the induction course and during the intensification phase. The results demonstrated that 3-year EFS was significantly better among children who received GO (53% versus 47%). Among patients who achieved a CR, the incidence of relapse among those who received GO was significantly lower compared to those who did not.

I believe enough data are available to suggest that we need to give gemtuzumab a second look and perhaps make it available again in community practice not only for patients with favorable leukemias but also for those with IR disease. It is important to make the right treatment decision for these patients. Now that we have enough data suggesting that gemtuzumab could be effective in AML and in several subsets, we have to allow this agent to get back on the market to help the patients who need it the most.

Interview with Hagop M Kantarjian, MD, January 29, 2014

The Addition of Gemtuzumab Ozogamicin (GO) to Induction Chemotherapy Reduces Relapse and Improves Survival in Patients without Adverse Risk Karyotype: Results of an Individual Patient Meta-Analysis of the Five Randomized Trials

Hills RK et al.

Proc ASH 2013;Abstract 356.

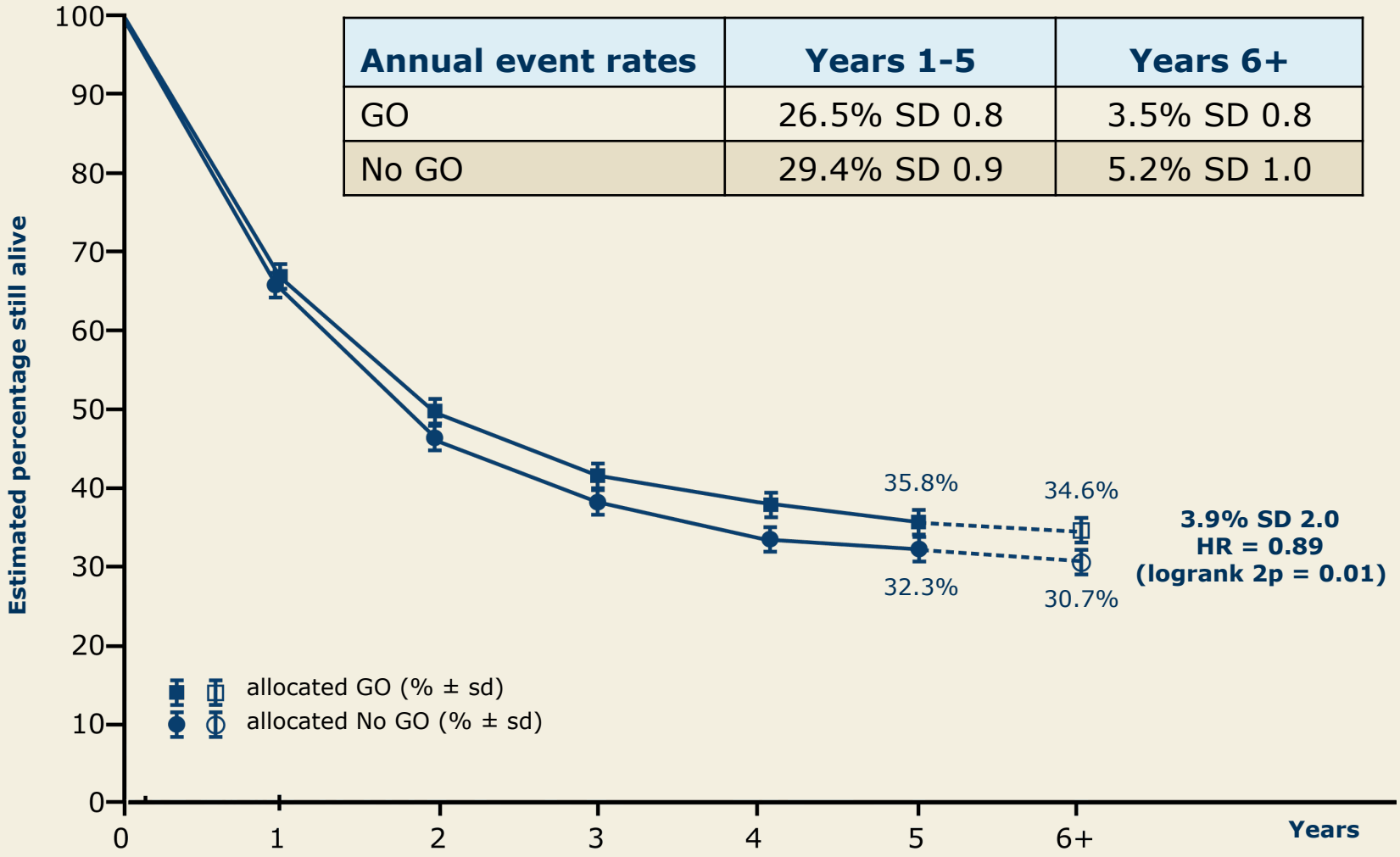
Patient and Trial Details

Trial	GO schedule	Induction chemo	No. of patients in meta-analysis	Median age
ALFA-0701	3 mg/m^2 d1, 4, 7	DA (3 + 7)	278	62
GOELAMS AML2006IR	6 mg/m^2	DA (3 + 7)	238	50.5
MRC AML15	3 mg/m^2 d1	ADE, DA, FLAG-Ida	1,113	49
NCRI AML16	3 mg/m^2 d1	DA, DClo	1,115	67
SWOG-0106	6 mg/m^2 d4	DA (3 + 7)	595	47

Hills RK et al. *Proc ASH* 2013;Abstract 356.

Overall Survival

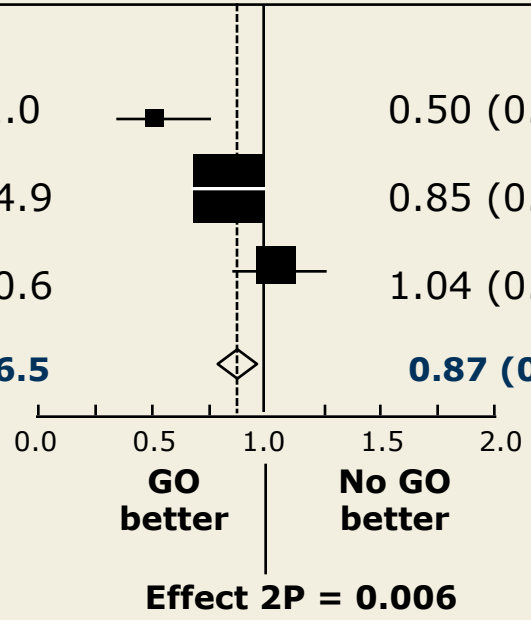
Annual event rates	Years 1-5	Years 6+
GO	26.5% SD 0.8	3.5% SD 0.8
No GO	29.4% SD 0.9	5.2% SD 1.0



With permission from Hills RK et al. *Proc ASH* 2013;Abstract 356.

Survival: Stratified by Self-Reported Cytogenetics

	Events/patients		Statistics		HR and 95% CI
Trial	**GO**	**No GO**	**(O-E)**	**Var.**	**(GO : No GO)**
Self-reported cytogenetics:					
Favorable	32/128	54/127	-14.6	21.0	0.50 (0.33, 0.77)
Intermediate	549/966	598/968	-45.7	284.9	0.85 (0.76, 0.96)
Adverse	223/261	227/257	4.4	110.6	1.04 (0.86, 1.25)
Total:	**804/1,355**	**879/1,352**	**-55.9**	**416.5**	**0.87 (0.79, 0.96)**



Test for heterogeneity (3 groups): $\chi^2_2 = 10.2$; $p = 0.006$
Overall test for trend: $\chi^2_1 = 8.6$; $p = 0.003$

With permission from Hills RK et al. *Proc ASH* 2013;Abstract 356.

Results Summary

- GO produces a significant improvement in OS
  - No interactions with age, sex, mutation status or chemotherapy administered
- Improved survival is a result of reducing relapse (data not shown)
  - HR 0.80, $p = 0.00006$
- Benefit restricted to those with favorable and intermediate cytogenetics
- No excess mortality with a dose of 3 mg/m^2 (data not shown)
- Fractionated GO may further reduce relapse risk

Hills RK et al. *Proc ASH* 2013;Abstract 356.

Reasons for Survival Improvement in Core Binding Factor AML: A 25 Year Analysis of the UK MRC/NCRI AML Trials

Burnett AK et al.

Proc ASH 2013;Abstract 358.

Results Summary

- Multivariate analysis of survival showed the following variables to be significant:
  - Use of GO in induction (HR 0.40, $p < 0.0001$)
  - Performance status (HR 1.20, $p = 0.001$)
  - Age (HR per decade 1.18, $p = 0.001$)
  - Log WBC (HR per unit increase 1.38, $p = 0.002$)
- For survival from remission, GO was the most significant factor (HR 0.50, $p < 0.0001$).
- Core binding factor leukemia is now highly curable, with the most important contribution being the introduction of GO in induction. When combined with the adoption of high-dose Ara-C in consolidation, we observe an OS of 89%.

Burnett AK et al. *Proc ASH* 2013;Abstract 358.

Investigator Commentary: 25-Year Analysis of Improved Survival in the UK MRC/NCRI AML Trials

This study was focused on a subtype of AML with core binding factor mutations that also typically contain translocations between chromosome 8 and 21 or an inversion in chromosome 16. It has been demonstrated that patients with these cytogenetic abnormalities were more likely to be cured with standard chemotherapy, and other studies have shown that high-dose Ara-C consolidation was useful in increasing the cure rate in these patients.

In this abstract, the outcomes of 896 patients with these cytogenetic abnormalities randomly assigned to treatment with GO or not were analyzed, and multivariate analysis demonstrated that GO increased the probability of survival to a likelihood of being cured. Their analysis demonstrated that this effect, which was demonstrated in patients with either type of cytogenetic abnormality, was due to the drug. Some additional toxicity occurred with GO, but that was outweighed by the efficacy. Using this approach, about 90% of patients seemed to have been cured, which is astounding for AML. It begs the question whether the FDA decision to withdraw this drug from the market should be revisited.

Interview with David L Porter, MD, March 3, 2014

Anti-CD19 Chimeric Antigen Receptor (CAR) T Cells Produce Complete Responses with Acceptable Toxicity but without Chronic B-Cell Aplasia in Children with Relapsed or Refractory Acute Lymphoblastic Leukemia (ALL) Even After Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

Lee DW III et al.

Proc ASH 2013;Abstract 68.

Background

- Survival with relapsed/refractory pediatric pre-B-cell ALL and non-Hodgkin lymphoma (NHL) is poor despite intensive chemotherapy and HSCT.
- CAR T cells combine the specificity and major histocompatibility complex independence of antibodies with the cytotoxic capacity of T cells.
- Through genetic engineering of T cells with a CAR that links a single-chain variable fragment for CD19, present on most NHL and ALL blasts, cytotoxicity can be specifically and fully activated with a single interaction.
- **Study objective:** To determine the efficacy and safety of anti-CD19 CAR T cells in pediatric patients with relapsed/refractory ALL and NHL.

Lee DW III et al. *Proc ASH* 2013;Abstract 68.

Ongoing Phase I Trial Design

NCT01593696

Target accrual (n = 48)

Relapsed/refractory B-cell NHL or ALL
Age 1-30 years
CD19 positivity
Ineligible or refused allogeneic stem cell transplant (SCT)

→ **Anti-CD19 CAR T cells**

Endpoints include:

- Feasibility of generating adequate numbers of CAR T cells from a population of patients at high risk, including after HSCT
- Response rate
- CAR T cell persistence and trafficking to extramedullary sites
- Safety

Lee DW III et al. *Proc ASH* 2013;Abstract 68; www.clinicaltrials.gov. Accessed April 2014.

Study Methods

- Peripheral blood mononuclear cells were collected from patients by apheresis and immediately enriched for T cells using activating anti-CD3/CD28 beads.
- These cells underwent retroviral transduction of the CAR gene.
- After an 11-day process, patients who had received fludarabine (25 mg/m^2/day on days -4, -3, -2) and cyclophosphamide (900 mg/m^2/day on day -2) received a fresh infusion of CAR T cells.
- At data cutoff, 8 patients were enrolled and had received treatment.

Lee DW III et al. *Proc ASH* 2013;Abstract 68 (abstract only).

Patient Characteristics

Characteristic	n = 8
Age range	10-23 years
ALL NHL	7 (87.5%) 1 (12.5%)
Pre-HSCT Post-HSCT	4 (50%) 4 (50%)
With successful expansion of CAR T cells meeting the assigned dose level (transduction efficiencies of 18%-87%)	6 (75%)
Insufficient expansion to meet target dose	2 (25%)*

* These patients received 3% and 14% of the target dose.

Lee DW III et al. *Proc ASH* 2013;Abstract 68 (abstract only).

Response Rates

	n = 8
Complete response (CR)	5/8 (62.5%)
ALL	5/7 (71.4%)
NHL	0/1 (0%)
Minimal residual disease (MRD)-negative CR	3/8 (37.5%)
ALL	3/7 (42.9%)*
NHL	0/1 (0%)

* Includes 1 patient with primarily refractory disease to chemotherapy who proceeded to HSCT after CD19 CAR therapy

- Both patients who received cells below the target dose experienced antileukemia effects:
  - One experienced a transient CR
  - One experienced MRD-negative CR

Lee DW III et al. *Proc ASH* 2013;Abstract 68 (abstract only).

CAR T-Cell Expansion, Persistence and Trafficking

- CAR T cells have been identified in blood (0.1%-38%) and bone marrow (0.1%-5%) in all responding patients.
- CAR T cells have been found in:
  - Cerebrospinal fluid (CSF) of 3 patients (0.3%-17%)
  - Pleural fluid (13%) of a patient with NHL with pre-existing malignant pleural effusions
- CAR T cells are suspected to have caused Grade 1 scrotal edema in a patient with a remote history of testicular disease.
- One patient with CNS2 disease (<5 white blood cells/uL in the CSF and cytospin-positive for blasts) at the time of enrollment cleared all CSF blasts without additional intrathecal chemotherapy after CAR T cells were administered (max: 17% CAR T cells in CSF).
- The mean time to undetectable CAR T cells in any tissue in responding patients was 55 days.

Lee DW III et al. *Proc ASH* 2013;Abstract 68 (abstract only).

Treatment-Related Adverse Events

- Treatment was well tolerated.
- Two patients had Grade 2 cytokine release syndrome (CRS): Grade 3 fever, Grade 2 hypotension
  - These toxic effects resolved with IV fluids and correlated with high IL-6, GM-CSF, IFNgamma, TNFa and C-reactive protein.
- One dose-limiting toxicity (Grade 4 CRS) occurred and required vasopressors for hypotension.
- After identification of high plasma IL-6, the anti-IL-6 receptor antibody tocilizumab was administered and quickly reversed most toxicity from CRS.

Lee DW III et al. *Proc ASH* 2013;Abstract 68 (abstract only).

Treatment-Related Adverse Events (Continued)

- At the time of day 28 restaging, CD19-positive hematogones were detected in 4 of 5 patients with responsive disease (mean 81.4% of all CD19-positive cells).
  - This indicates that significant antileukemic effects can be induced by CD19 CAR T cells without chronic depletion of B-cell precursors.
- None of the patients with prior HSCT developed graft-versus-host disease despite the administration of donor-derived activated T cells harvested from the recipient.

Lee DW III et al. *Proc ASH* 2013;Abstract 68 (abstract only).

Author Conclusions

- Anti-CD19-CD28-zeta CAR T cells that mediate potent antileukemic effects can be reliably generated, even from patients with advanced disease with or without a history of allogeneic HSCT.
- Using intent-to-treat reporting, CR rates are high (62.5%) in this population of patients with refractory disease.
- CD19 CAR T cells traffic to extramedullary sites and can mediate antitumor effects in the CSF.
- Acute toxicity is manageable, and because the anti-CD19-CD28-zeta CAR T cells do not persist at high levels for prolonged periods, rapid resumption of B-cell lymphopoiesis occurs after therapy.

Lee DW III et al. *Proc ASH* 2013;Abstract 68 (abstract only).

Investigator Commentary: Ongoing Phase I Study of Anti-CD19 CAR T Cells in Pediatric Patients with Relapsed/Refractory NHL or ALL

This study focuses on a small number of patients with NHL or ALL treated with anti-CD19 CAR T cells. The results show remarkable complete remission rates — 5 out of 7 patients with ALL achieved a CR. Also, the study showed that this treatment strategy can be effective after allogeneic SCT. A unique and interesting finding from this study is that the modified CAR T cells were found in other compartments of the body. In 3 patients, the genetically modified cells were found in the CSF, and the cells were even found in the pleural fluid in 1 patient with NHL who had preexisting malignant pleural effusions.

This study describes a patient with active CNS2 disease and leukemia at the time of enrollment that were effectively eradicated by the CAR T cells. So the fact that these cells have the potential to traffic beyond the blood — into the bone marrow, CSF and pleural fluid — is remarkable. This suggests that it may also be possible to treat extramedullary disease with these cells and, in particular, leukemic meningitis, which is extremely complicated and difficult to eradicate with more conventional therapies. These results are unique, exciting and promising.

Interview with David L Porter, MD, March 3, 2014