Table 1. ETP Subtype Does Not Affect EFS or OS Outcome

| | n | Frequency, % | MRD D29 < 0.01%*, % | Induction Failure*, % | 4-Year EFS, % ± SE | 4-Year OS, % ± SE |
|---|---|---|---|---|---|---|
| ETP | 130 | 11.3 | 18.6 | 7.8 | 82.9 ± 6.2 | 91.0 ± 4.8 |
| Near ETP | 195 | 17.0 | 35.2 | 6.7 | 84.7 ± 6.2 | 92.6 ± 4.4 |
| Not ETP | 819 | 71.6 | 69.5 | 1.1 | 86.9 ± 2.5 | 91.5 ± 2.0 |

*$P$ < .0001 for MRD day 29 and induction failure values.

D, day; EFS, event-free survival; ETP, early thymic precursor; MRD, minimal residual disease; OS, overall survival.

probability of EFS and OS compared with an MRD D29 ≥ 10%. EFS and OS were similar regardless of the ETP subtype.

Patients with MRD ≥ 0.1% at the end of consolidation fared poorly ($P$ < .0001) compared with those with MRD < 0.1%. Early (day 8) blast clearance from peripheral blood was associated with significantly better outcomes ($P$ < .02). However, it did not identify poor risk among the < 0.01% MRD D29 subset. In addition, a white blood cell count ≥ 200 000 was associated with worse outcome with near ETP ($P$ = .003) and not ETP ($P$ = .012), whereas frequency was low with ETP. Off-protocol therapy events at the end of induction and during consolidation appeared similar among the subtypes. No preferential attrition (15% to 20% on Children's Oncology Group ALL trials) by MRD stratification at the end of induction was seen among ETP subtypes.

## Clonal Mapping of Hematopoiesis In Vivo Provides Direction for New Clinical Protocols

Written by Maria Vinall

Luca Biasco, PhD, San Raffaele Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, Milan, Italy, presented the results of the first molecular tracking of individual hematopoietic clones in humans [Biasco L et al. *Blood*. 2014]. Data from these studies demonstrated that retroviral vector insertional barcoding will likely be an essential element in the design of therapeutic approaches for hematological disorders and cancers.

The use of viral vectors for gene therapy (GT) in which the patient's own hematopoietic stem cells (HSCs) are harvested, exposed in the laboratory to a viral vector carrying the corrected gene sequences, and then re-infused into the patient, may provide curative therapy for several monogenic diseases [Kaufmann KB et al. *EMBO Mol Med.* 2013].

Prof Biasco reported the results of a phase 1/2 clinical trial [Aiuti A et al. *Science.* 2013] in which 3 patients with Wiskott-Aldrich syndrome (WAS) were treated with gene-corrected HSCs after pretreatment with a reduced-intensity myeloablative regimen. Administration of autologous HSCs transduced with lentiviral vectors at > 90% was associated with the following: robust gene transfer in unfused CD34+cells; persistent multilineage engraftment; restoration of WAS expression to near-physiological levels; immunologic and hematologic improvement; and clinical benefit. Clonal tracking of stem-cell dynamics by vector insertions showed details of hematopoietic reconstitution after GT. Seven patients have been treated so far using this protocol with similar positive results and no severe adverse events or evidence of leukemia.

Another study has found that GT, combined with reduced-intensity conditioning, is safe and effective in the treatment of severe combined immunodeficiency due to the lack of adenosine deaminase [Aiuti A et al. *N Engl J Med*. 2009].

Linear amplification-mediated polymerase chain reaction is a powerful tool for analyzing integration sites and enriching for specific vector gene function. When combined with Illumina-Miseq sequencing, it becomes a tool to identify the clonal engraftment of the cells that have been barcoded and re-infused into the patient. To date, Prof Biasco's group has used this technology to record the clonal diversity of gene-corrected cells from > 89 000 clones and to mark the recapture of identical clones over time. These clones belong to 13 different cell types purified from the bone marrow and the peripheral blood of 4 patients with WAS up to 4 years after GT.

The technique also made it possible to identify 3 major waves of clonal reconstitution. Clones that were detected 9 months after GT showed the highest relationship and were stable over time. This protocol has also helped to identify the detection of multipotent progenitors, the clonal output of CD43+progenitors, and the relationship between HSPC and mature lineages.

Prof Biasco concluded that in vitro activated hematopoietic stem and progenitor cells (HSPC) could sustain long-term hematopoiesis; multipotency could be exerted long-term with fluctuating outputs; and a few thousand HSPC clones are responsible for the maintenance of steady-state hematopoiesis. In addition, there is evidence of a defined switch between short- and long-term engrafting of HSPC, while takeover of hematopoiesis by HSPC could occur between 6 and 12 months after transplant, and clonal diversity stabilizes at 12 months after GT.