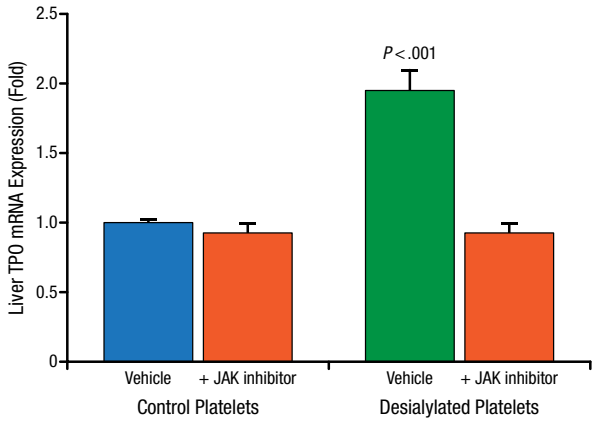
Figure 2. JAK Inhibitors Block TPO mRNA Increase in Wild Type Mice




JAK, Janus kinase; TPO, thrombopoietin.

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In the in vivo treatment, AMR-deficient mice had increased platelet count, survival, and loss of sialic acid compared with wild type mice showing that removal of desialylated platelets by the AMR regulated in vivo platelet survival.

In livers isolated from Asgr2-null mice, TPO mRNA expression decreased by 40%, while TPO mRNA expression in livers from mice lacking sialyltransferase (St3gal4-null) was increased by 30%, compared with wild type mice. In St3gal4-null mice, desialylated platelet clearance is increased and specifically mediated by the AMR. This indicates that desialylated platelet uptake by the AMR regulates liver TPO levels.

When desialylated platelets isolated from St3gal4-null or Asgr2-null mice are infused into wild type mice, hepatic TPO mRNA levels increase as early as 12 hours after infusion, while desialylated platelets infused into Asgr2-null mice have no effect on TPO mRNA synthesis. Further, treatment with JAK inhibitors block the desialylated platelet stimulated TPO mRNA increase in wild type mice (Figure 2).

The accumulated data show that circulating desialylated platelets by way of the AMR and JAK2-STAT3 signaling stimulated TPO production, providing a physiological feedback mechanism to regulate plasma TPO levels and platelet production in vivo and in vitro. The complete understanding of this feedback mechanism illuminates the pathophysiology of platelet diseases, such as thrombocythemia and immune thrombocytopenia. This feedback has clinical implications, however, as the administration of JAK1/2 inhibitors can cause thrombocytopenia in some groups of patients.

# AALL0434 Treatment Therapy Has Excellent Outcomes in Children With T-ALL

Written by Maria Vinall

Children with T-cell acute lymphoblastic leukemia (T-ALL) have excellent outcomes after treatment with AALL0434, a standard 4-drug induction therapy. End-induction minimal residual disease (MRD) was more important in predicting treatment response than the early thymic precursor (ETP) immunophenotype, according to the results from the Combination Chemotherapy in Treating Young Patients With Newly Diagnosed T-Cell Acute Lymphoblastic Leukemia or T-Cell Lymphoblastic Lymphoma [NCT00408005] trial presented by Brent L. Wood, MD, PhD, University of Washington, Seattle, Washington, USA.

The phase 3 trial enrolled 1895 patients (aged 1 to 30 years) with T-ALL. The prognostic impact of MRD was measured by 8- and 9-color flow cytometry in the peripheral blood at day 8 and in bone marrow at end of induction (day 29), and end of consolidation was compared with prognostic ability of ETP status assessed at diagnosis. AALL0434 therapy consisted of prednisone for 28 days, vincristine weekly for 4 weeks, pegaspargase on day 4, and daunorubicin weekly for 4 weeks. An initial subset of cases confirmed that near ETP meets ETP criteria except for elevated CD5 B-cells.

An initial subset of thymocytes, ETP, retains stem-cell-like features, as reviewed by Coustan-Smith and colleagues [*Lancet Oncol.* 2009], who helped to define criteria for standardization in the AALL0434 study. The presence of ETP cells in patients with T-ALL confers a poor prognosis with use of standard intensive chemotherapy. Thus, early recognition is believed essential for the development of an effective clinical management strategy.

In the AALL0434 study, ETP, near ETP, and not ETP were noted in 11.3%, 17.0%, and 71.6% of patients, respectively. At day 29, MRD < 0.01% was significantly more evident in the group without ETP compared with those with ETP or near ETP ($P < .0001$). Induction failure, defined as > 35% blasts by morphology in bone marrow at day 29, was proportionally more evident in the group with ETP (45.5%) and near ETP (48.1%) compared with not ETP (11.2%). However, percentage outcomes for 4-year event-free survival (EFS) and overall survival (OS) were similar regardless of the subtype (Table 1).

MRD is an important predictor of relapse in children with T-ALL. An MRD day 29 risk < 0.1% was considered low risk, < 1.0% intermediate, and ≥ 1.0% high. An MRD D29 < 0.01% to < 10.0% was associated with a higher

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.