the control group (P=0.003).[12] It is plausible that at least some of the benefit was achieved by dosing in consolidation, but also the fractionation of the total GO dose during induction may have contributed to the most prominent effect in this French ALFA group study. This benefit was also apparent in patients with unfavorable cytogenetic characteristics, but the impact of complex karyotype has not been analyzed separately.[12] In addition, early mortality seems to be reduced when a dose of 3 g/m^2 is used either as a single dose or in a fractionated schedule.[6] There was some hematologic toxicity, particularly to platelets. So, while a 3 mg/m^2 dose appears adequate, it is still not certain what is the optimal schedule.

However, the MRC AML15 trial did not show any additional benefit of adding GO to consolidation irrespective of whether it had been given with the first induction course;[7] therefore the urgent issue to be resolved is whether a single dose or a fractionated schedule is to become the standard approach. In an attempt to do this, the NCRI have initiated a direct comparison of a 3 mg/m^2 dose on day 1 *versus* days 1 and 4 in their ongoing trials.

In conclusion, data from this study and from studies published after the withdrawal of GO from the market support the need for a re-appraisal of the regulatory approval of GO by the responsible authorities, at least for certain subtypes of AML.

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HOXA-activated early T-cell progenitor acute lymphoblastic leukemia: predictor of poor outcome?

Jules PP. Meijerink,[1]* Kirsten Canté-Barrett,[1] Eric Vroegindeweij[1] and Rob Pieters[2]

[1]Department of Pediatric Oncology/Hematology, Sophia Children's Hospital, Rotterdam and [2]The Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands.

E-mail: j.meijerink@erasmusmc.nl

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The outcome for T-cell acute lymphoblastic leukemia (T-ALL) has strongly improved over the last decades using high-intensity treatment protocols approaching cure rates of 80% for pediatric patients and 60% for adult patients. Fifteen percent of pediatric ALL patients present with T-ALL, and they represent nearly half of the ALL patients who require the most intensive treatment. Intensive chemotherapy increases the risk for treatment related morbidity and mortality. For relapsed patients, the outcome is poor, as T-ALL cells in those patients are highly resistant to further treatment. Therefore, patient-tailored treatment and the introduction of high precision medicines remain important. Molecular cytogenetic characterization of T-ALL has greatly increased our understanding of the pathogenic events that drive this disease. In contrast to precursor B-ALL, this improved insight into T-ALL has not yet yielded prognostic factors that allow for the identification of patients at high-risk of relapse and who may be eligible to receive alternative treatment, including allogeneic stem cell transplantation.

One cytogenetic entity in pediatric and adult T-ALL patients that has been suspected to cause poor outcome include patients bearing a *CALM-AF10* (*PICALM-MLLT10*) fusion as a consequence of a t(10;11)(p13.14;q14-21) chromosomal translocation.[1] A first systematic study comprising unselected pediatric and adult T-ALL patients treated on FRALLE-93, FRALLE 2000 or LALA-94 protocols identified the *CALM-AF10* fusion in approximately 9% of patients. This fusion is associated with early and late T-cell developmental arrest in the γδ lineage. In this study, late *CALM-AF10*$^+$ T-ALL patients responded well to therapy, but 2 out of 12 *CALM-AF10*$^+$ patients with an immature phenotype did not respond to therapy, and another 8 patients with an immature phenotype

relapsed.[2] Therefore, *CALM-AF10* may be associated with poor outcome in T-ALL. The 3 *CALM-AF10*$^+$ patients treated in the Dutch Childhood Oncology Group (DCOG) ALL-9 protocol demonstrated early relapses during therapy.[3] Another study comprising 187 children treated on the AIEOP-BFM ALL 2000 or AIEOP R-2006 protocols identified *CALM-AF10* fusions in 14 children, 8 of whom presented with high-risk features, including high white blood cell counts and prednisone poor responses. However, event-free survival and cumulative incidence of relapse were comparable for *CALM-AF10*$^+$ and *CALM-AF10*$^-$ patients.[4] In contrast to the earlier study in which immature *CALM-AF10*$^+$ patients were associated with poor outcome,[2] only 1 out of the 14 *CALM-AF10*$^+$ patients in these protocol studies had an immature T-cell immunophenotype.[4]

In 2005, two independent studies led to the discovery of a chromosomal inversion on chromosome 7 (inv(7)(p15;q34)) in T-ALL patients.[5,6] This fusion leads to ectopic activation of *HOXA* genes (*HOXA5-10* genes in particular) by a *cis*-acting mechanism due to the close proximity of the *TCRB* enhancer region.[5,6] A high activation of *HOXA* genes was observed previously in *MLL*-rearranged T- and B-cell neoplasms,[7] but gene expression profiling studies revealed that *HOXA*-deregulation was a more common feature among T-ALL patients bearing inv(7), *MLL*-rearrangements, *CALM-AF10* or *SET-NUP214* gene fusions.[5,8,9] The activation of *HOXA* genes therefore seems to play an important role in the cellular transformation of thymocytes. Various other *HOXA*-activating events have nowadays been identified in T-ALL including a *TCRD-HOXA* translocation[10] and novel *MLLT10* gene fusions. *MLLT10* was identified fused to *XPO1/CRM1*, which encodes for a nuclear export protein,[11] to *NAP1L1* that encodes for a nucleosome assembly protein,[12] and to *HNRNPH1* and *DDX3X* genes that are both involved in RNA processing.[13] In the latter study by Brandimarte *et al.*,[13] all *MLLT10*-translocated *HOXA*$^+$ cases clustered separately from other *HOXA*$^+$ T-ALL cases including *MLL*-rearranged, *SET-NUP214* and inv(7) cases. These *MLLT10*-translocated cases highly expressed the hematopoietic stem cell homeobox *HHEX* and *MEF2C* genes, two genes that are commonly expressed in immature T-ALL denoted as early T-cell precursor ALL (ETP-ALL).[12,14]

Regarding this issue, Bond and colleagues further delineate the ambiguous nature of *HOXA*-activated T-ALL patients with respect to T-cell developmental arrest and outcome.[15] They extended their previous observations regarding *CALM-AF10*$^+$ cases in a cohort of 190 adult T-ALL patients that were treated in LALA-94/GRAALL03-05 protocols. In that study, 42 T-ALL patients expressed an ETP-ALL immunophenotypic profile (CD5^{low}, CD1a$^-$, CD8$^-$ and expression of CD34, CD13, CD33 and/or CD117) whereas 148 patients had arrested at later stages of maturation.[16] ETP-ALL patients fared equally well compared to non-ETP-ALL patients. The ETP-ALL group comprised 9 *CALM-AF10*$^+$ patients who were TCR negative due to absent or incomplete TCRδ or TCRγ recombinations (i.e. IM, IMδ or IMγ). In the non-ETP-ALL group, 2 out of 5 *CALM-AF10*$^+$ patients had a similar TCR-negative genotype while the remaining 3 had the more mature sCD3$^+$/TCR$^+$ or cortical/pre-αβ TCR-genotype. ETP-ALL patients who were positive for *CALM-AF10*$^+$ (and were TCR-) had a significantly higher risk for adverse events and a trend toward reduced overall survival compared to *CALM-AF10*$^-$ ETP-ALL patients. *CALM-AF10*$^-$ ETP-ALL patients fared equally well compared to non-ETP-ALL patients regardless of their CALM-AF10 status.[16]

In the study, Bond and colleagues extended their observations by analyzing the prognostic impact of all *HOXA*-positive cases in relation to ETP-ALL and outcome in a cohort of 209 adult T-ALL treated in the GRAALL-2003/2005 protocol.[15] Fifty-five T-ALL cases (26%) expressed *HOXA9* at levels similar to those in *CALM-AF10*$^+$ patients as defined by RT-QPCR. Apart from 8 patients with *CALM-AF10* translocations (1 patient also had an inv(7)), 10 patients had inv(7), 9 were positive for *SET-NUP214*, and 6 patients had *MLL*-rearrangements. Further screening for alternative *HOXA*-activating events revealed *XPO1-MLLT10*, *DDX3X-MLLT10* or *NAP1L1-MLLT10* fusions in 1 patient each, whereas 1 additional patient had an unresolved *MLLT10* rearrangement. Two patients were identified with *NUP98-RAP1GDS1* fusions, whereas no *HOXA*-activating events were found in the remaining 16 cases. *HOXA* cluster activation in T-ALL patients by these oncogenic fusion products (denoted as *trans*-acting mechanism) is mostly linked with the presence of an immature TCR-genotype (IM0, IMδ or IMγ) and an ETP-ALL immunophenotype. In contrast, inv(7)-positive (*cis-HOXA*-acting) patients almost exclusively present with a sCD3$^+$/TCR$^+$ or cortical/pre-αβ TCR-genotype. In line with previous observations,[4] *HOXA*$^+$ patients demonstrated increased resistance to corticosteroid treatment and chemotherapy and frequently remained MRD positive (>10^{-4}) after induction therapy.[15] Surprisingly, overall outcome (OS, EFS and DFS) for *HOXA*$^+$ patients was identical to *HOXA*$^-$ T-ALL patients. Further discriminating patients based on ETP-ALL immunophenotype revealed that *HOXA*$^+$/ETP-ALL patients had a significantly poor outcome as compared to *HOXA*$^-$/ETP-ALL patients (OS: 31.2% *vs.* 74.2%; EFS: 25% *vs.* 60.8%; DFS: 28.6% *vs.* 64.7% and CIR: 53.7% *vs.* 29.2%, respectively). The outcome for *HOXA*$^-$/ETP-ALL patients was as favorable as for non-ETP-ALL patients regardless of the presence of *HOXA*-activating rearrangements.[15]

The Children's Oncology Group (COG) has now reported similar findings for pediatric T-ALL patients.[17] This study investigated 100 children with T-ALL who were treated in the COG AALL0434 protocol, including 17 patients for whom initial treatment failed. Evidence for *MLL*-rearrangements were found in 12 patients in addition to 6 *CALM-AF10*$^+$ patients, 3 *DDX3X-AF10*$^+$ patients (1 case had a complex *CASK-DDX3X-AF10* translocation), 2 patients with *NUP98*-rearrangements and 3 inv(7)+ patients. *MLL*- but not *AF10*-rearrangements were strongly associated with induction failure and inferior EFS in uni- and multivariate analyses. Expression of an ETP-ALL expression signature also predicted for inferior EFS, and trended towards enrichment of *MLL*-rearranged cases. *MLL*-rearrangements combined with an ETP-ALL expression profile most strongly associated with induction failure, refractory disease and relapse.[17] Both studies therefore point to *HOXA*-activated ETP-ALL cases that are at a higher risk to fail on induction therapy or have inferior survival

rates. Further studies are needed to investigate whether this can be attributed to specific *HOXA*-activating events: 13 *HOXA*-activated adult T-ALL patients that relapsed included 3 patients with *SET-NUP214* fusions, 2 patients with *MLLT10*-rearrangements, 1 *MLL*-rearranged case and 1 patient with an inv(7),[15] while in the pediatric study the *MLL-AF6* or *Del3'MLL* rearranged patients were at the highest risk to fail on therapy.[17]

Based on these important findings in both studies, routine screening for *HOXA*-activation events and ETP-ALL profiles in future T-ALL patients may help to identify patients at risk for induction failure or relapse. For this to happen, several issues need to be resolved: What would be the best detection method to identify *HOXA*-activated ETP-ALL patients? Gene expression profiling to identify *HOXA*-activated T-ALL patients failed to classify 3 patients carrying *HOXA*-activating events in the COG series.[17] On the other hand, not all *HOXA*-activating genetic events have been resolved.[15] Also, what is the best method to identify ETP-ALL patients? Will this rely on ETP-ALL immunophenotypic markers[18] or on the expression of ETP-ALL signature genes? Several retrospective studies on historical T-ALL samples did not consistently identify an ETP-ALL-specific immunophenotype for cases that expressed an ETP-ALL gene signature.[19,20] Finally, what alternative treatment should be given? *HOXA*-activated ETP-ALL patients may receive allogeneic stem cell transplantations if suitable donors are available, or receive precision medicine like the DOT1L inhibitor EPZ-5676 compound that is currently being tested in clinical trials.[21] An additional important question that needs to be resolved in the future is which other genetic factors may cause *HOXA*-activated cases to arrest at the ETP-stage and define poor outcome, while other *HOXA*-activated T-ALL cases with seemingly identical chromosomal rearrangements arrest at late stages and have a better prognosis? The answer may reveal the true determinant that defines ETP-arrest and the high-risk of treatment failure for *HOXA*-activated ETP-ALLs.

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