Introduction

In our previous report we were able to show that the hedgehog pathway is ectopically activated in a subgroup of T-ALL patients. Furthermore we demonstrated that hedgehog inhibition results in lower proliferation in several T-ALL cell lines *in vitro*. Finally, we showed that ectopic activation of the pathway by the leukemic cells results in increased leukemic growth and in manipulation of the microenvironment *in vivo*. Overall, these data clearly stated that hedgehog pathway is important for leukemic growth and therefore a suitable pharmaceutical target.

Transcriptional deregulation can explain hedgehog pathway expression in T-ALL.

Several mechanisms could be responsible for hedgehog pathway activation in T-ALL. We have not found any evidence for rearrangements in any of the hedgehog pathway genes in cases with increased expression. Furthermore, activating mutations of hedgehog genes are extremely rare in T-ALL cases (~4%).

Based on the gene expression data, it is plausible to expect that the general transcriptional deregulation present in T-ALL could lead to increased expression of hedgehog pathway genes and/or ectopic SHH expression. For this reason we performed a computational analysis by using iRegulon in order to identify transcription factors that potentially could bind the promoters of the hedgehog genes. Moreover, we exclude from our analysis transcriptional factors that do not share a similar expression profile with most hedgehog genes in the T-ALL cohorts. This combined analysis revealed 32 candidates; by performing CHIP we were able to show that GATA1, which is not expressed by normal
T-cell progenitors but is present in T-ALL cases, is able to bind to both GLI1 and SHH promoters in T-ALL cell lines. These data provide a rationale that ectopic activation of transcriptional factors (like GATA1) might be responsible for hedgehog activation.

**Pharmacological inhibition of the hedgehog pathway lowers the chemoresistance of T-ALL cell lines in vitro.**

Several T-ALL cell lines were treated with ARA-C or doxorubicin, two chemotherapeutic agents that are used for the treatment of ALL. As expected, all T-ALL cell lines were sensitive to both drugs. Interestingly, T-ALL cell lines sensitive to hedgehog inhibitors, were further sensitized to both chemotherapeutic agents. On the contrary, T-ALL cell lines insensitive to GDC-0449 or GANT61 were not further sensitized to chemotherapy upon hedgehog inhibitor addition. In conclusion, these data show that hedgehog pharmacological inhibition lowers the chemoresistance of T-ALL cells both *in vitro.*

**Hedgehog ectopic activation and BCL2 upregulation co-occur in T-ALL patients and combined treatment could be beneficial.**

We identified several genes that play an important role to T-ALL and share a similar expression pattern with hedgehog activity. A potent candidate is BCL2 as i) it is up/downregulated in several T-ALL cell lines upon activation/inhibition of the hedgehog pathway respectively and ii) it is upregulated in both TECs and lymphocytes in our *in vivo* mouse models.

For these reasons, several T-ALL cell lines were treated with ABT-199 (BCL2 inhibitor), GANT61 (GLI1/2 inhibitor) or both inhibitors. Once more, combined treatment was significantly more effective in those T-ALL cell lines that were sensitive to GANT61 inhibitor and irrespective to sensitivity to BCL2 inhibition alone. In a subsequent experiment, simultaneous expression of Shh with BCL2 in the JAK3(M511I) leukemic model was performed. Additional expression of BCL2 results in a more severe phenotype, with a clear disease acceleration. In this model, the Shh-BCL2-JAK3(M511I) transduced cells, is the only leukemic population in all organs in all mice. BCL2 ectopic expression had no impact in T-cell/leukemia development when combined with the Shh or the JAK3(M511I) genes alone.

Overall, these data demonstrate that BCL2 levels are partially but not entirely dependent upon hedgehog activation. Therefore, hedgehog downregulation combined with BCL2 inhibition could be significantly beneficial to T-ALL patients.

**Pharmacological inhibition of the hedgehog pathway affects the survival rate of a subset of T-ALL xenograft samples ex vivo and in vivo.**

Seventeen primary T-ALL samples were collected, RNA was extracted in order to measure the expression levels of the hedgehog genes. Six of the T-ALL samples (35%) had a detectable GLI1 expression and expressed in significant levels most hedgehog components verifying our previous bioinformatics results (“hedgehog high”). On the other hand, the expression levels of GLI1 as well as
most hedgehog genes in 11 samples were below the detection level and therefore were considered as “hedgehog low”. 

Next, 4 “hedgehog high” and three “hedgehog low” samples were cultured ex vivo and treated with GDC-0449 (SMO inhibitor), GANT61 (GLI1/2 inhibitor) or DMSO. Cell numbers were measured on a daily base for up to 5 days. As expected, all “hedgehog high” samples were sensitive to both hedgehog inhibitors compared to “hedgehog low” samples. In a parallel experiment, two “hedgehog high” samples were treated with ARA-C, which is a known chemotherapeutic agent together with/without the GANT61 inhibitor. Treatment of both PDX samples with the GLI1/2 inhibitor sensitized leukemic cells against ARA-C verifying our in vitro data.

Next, we injected three “hedgehog high” and two “hedgehog low” samples into immunodeficient NSG mice, to investigate the in vivo response of the samples to the hedgehog inhibitors. Mice were treated for 2-3 weeks by oral gavage with GDC-0449, GANT61 or vehicle, starting when the leukemic clone was detectable in the blood (>1%). Only “Hedgehog high” samples were sensitive to both hedgehog inhibitors; as a result the percentage of the leukemic clone was lower in the PB, BM and spleen compared to controls. No significant induction of apoptosis was noticed but a 50% reduction in proliferative cells was observed.

Taken together, these data clearly show that “Hedgehog high” PDX samples are sensitive to hedgehog inhibition both ex vivo and in vivo. Sensitivity to hedgehog inhibition seems to correlate with GLI1 expression which is the main transcriptional factor and one of the most important target genes of the hedgehog pathway.
**FINANCIAL REPORT**
- Overview of the expenses related to the fellowship.

*Will be sent by KU Leuven.*

**LIST OF SCIENTIFIC OUTPUT:**
- Scientific publications which were realized with the fellowship (only published or accepted for publication publications).


- Other initiatives taken for wider disclosure of the investigation and any valorisation of research results (conferences, presentations, ..).


**SHORT NON-CONFIDENTIAL SUMMARY OF THE ACHIEVED RESULTS (IN DUTCH, FOR PUBLICATION ON THE WEBSITE AS A WAY TO COMMUNICATE THE RESULTS TO THE GENERAL PUBLIC, DONORS, SPONSORS, ..) (MAX. ½ PAGE)**

T-cel acute lymfatische leukemie (T-ALL) is een zeldzame kanker van de witte bloedcellen die vooral voorkomt bij kinderen, maar ook bij volwassenen kan deze leukemie nog voorkomen. Dankzij sterke verbetering van de chemotherapie is de overleving bij kinderen nu meer dan 80%, maar bij volwassenen komt veel herval van de ziekte voor en is de overleving slechts 50%. Om de overleving verder te verbeteren en om de therapie minder toxisch te maken met minder nevenwerkingen zijn wij nog steeds op zoek naar nieuwe doelwitten voor therapie. Eén van de mogelijke nieuwe doelwitten voor therapie is de ‘hedgehog’ signaalweg. Deze signaalweg is belangrijk bij normale T-cel ontwikkeling, en met dit project tonen we aan dat deze signaalweg ook nog steeds belangrijk is bij de ontwikkeling van T-ALL. We hebben gevonden dat T-ALL cellen van patiënten met hoge GLI1 expressie (een merker voor hedgehog signaalweg activatie) een goede respons vertonen op behandeling met hedgehog signaalweg inhibiteurs, zowel in vitro als in vivo in muis xenograft modellen voor T-ALL. Deze resultaten tonen aan dat hedgehog signaalweg inhibiteurs verder geëvalueerd moeten worden voor de behandeling van T-ALL. Dergelijke inhibiteurs zijn reeds beschikbaar voor de behandeling van andere vormen van kanker.

Jan Cools

Signature Researcher
Antonis Dagklis