Title
Genetic characterization and therapeutic targeting of MYC-rearranged T cell acute lymphoblastic leukaemia.

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T-cell receptor (TCR) driven MYC translocations characterize a rare but aggressive subtype of T cell acute lymphoblastic leukaemia (T-ALL). In these tumours, the proto-oncogene MYC is juxtaposed to enhancer elements of the TCR α/δ (TRA/TRD) locus by the translocation, t(8;14)(q24;q11), eventually resulting in its constitutive activation (Erikson et al, 1986). Given that MYC regulates the leukaemia-initiating capacity of malignant T cells (King et al, 2013), elevated MYC levels might have a severe impact on the clinical behaviour of this rare T-ALL subtype. Indeed, TRA/TRD-MYC positive T-ALLs have been associated with an unfavourable prognosis, rapid disease progression and poor response to conventional therapy (Parolini et al, 2014). Here, we performed a detailed molecular genetic characterization of an extensive series of t(8;14)(q24;q11) positive paediatric T-ALL patients (n = 26, Table S1) and evaluated a new therapeutic strategy for the treatment of this poor prognostic subtype of human leukaemia. See the Supplemental Methods for methodological details.

TRA/TRD-MYC positive T-ALLs were characterized by frequent loss of the T-ALL tumour suppressor genes PTEN (23%), CDKN2A/B (73%) and LEF1 (8%), and often displayed genomic deletions that cause aberrant activation of the STIL-TAL1 or LMO2 oncogenes (30%) [Fig 1A, frequency for a general T-ALL group is reported in brackets (Liu et al, 2017)]. Sequence analysis revealed lack of NOTCH1 or FBXW7 mutations, but a high number of loss-of-function mutations targeting PTEN (34%). Therefore, t(8;14)(q24;q11) positive leukaemias represent a NOTCH1-independent subtype of T-ALL that often depends on activated PI3K/AKT signalling [PTENmut/del in 12 out of 26 (46%)] (La Starza et al, 2014). In line with this notion, the t(8;14)(q24;q11) positive T-ALL cell lines, KE-37 and MOLT16, lack NOTCH1/FBXW7 mutations and present with genomic loss of PTEN, displaying aberrant phosphorylated AKT activation in the absence of activated NOTCH1 (Figure S1).

Although the TRA/TRD-MYC-rearranged T-ALL patients analysed in this study were treated according to different protocols, the available clinical information confirmed the aggressive nature of this specific genetic subtype of paediatric leukaemia. Indeed, most cases [19 out of 22 (86%)] presented with high white blood cell counts at diagnosis (>100 × 10⁹/L), poor response to glucocorticoid therapy and largely unfavourable outcomes. More specifically, the leukaemia was fatal in 13 of 26 (50%) of TRA/TRD-MYC positive T-ALLs due to progressive disease, the development of a secondary malignancy, specific toxicities or infections. Moreover, relapse of leukaemia occurred in 8 out of 23 cases (Table SII). Although the prognostic significance of PTEN alterations in T-ALL remains highly debated (Zuurbier et al, 2012; Jenkinson et al, 2016), some studies have suggested that this particular genetic subtype (PTEN loss in the absence of NOTCH1/FBXW7 mutations) identifies a subset of highly aggressive human T-ALLs (Petit et al, 2018).

Previous studies have also shown that TRA/TRD-MYC-rearranged T-ALLs cluster with TAL1/LMO2-rearranged mature leukaemias based on their gene expression signature (Hommenga et al, 2011; La Starza et al, 2014). To further characterize the transcriptional differences between TAL1/LMO2-rearranged T-ALLs with and without MYC translocations, 13 TAL1/LMO2-rearranged T-ALLs (5 TRA/TRD-MYC positive and 8 TRA/TRD-MYC negative leukaemias) were RNA-sequenced. Unsupervised clustering of RNA sequencing data revealed the presence of two clusters, including one group that consisted of all 5 TRA/TRD-MYC positive T-ALLs and 2 additional TRA/TRD-MYC negative cases (Figure S2). Notably, copy number profiling and mutational analysis revealed that both of these TRA/TRD-MYC negative T-ALLs also displayed PTEN alterations without NOTCH1 abnormalities, resembling the characteristic genetic landscape of TRA/TRD-MYC T-ALLs. Therefore, we grouped these leukaemias together and termed them TRA/TRD-MYC-like T-ALL. Differential expression analysis revealed a common transcriptional signature of these TRA/TRD-MYC-like T-ALLs compared to the 6 other non-MYC rearranged TAL1/LMO2 T-ALLs (Fig 1B), with 1856 transcripts differentially expressed between both tumour entities (adjusted P-value <0.05; 852 up- and 1004 down-regulated in TRA/TRD-MYC like). Interestingly, several canonical NOTCH1 target genes, including NOTCH3, HEY1, HEY4, PTCRA, IIL7 and DTX1, were significantly downregulated in the TRA/TRD-MYC like group, in line with the lack of NOTCH1 or FBXW7 mutations in this genetic subtype (Fig 1B). Differential expression analysis of NOTCH1 target genes was confirmed by reverse transcription quantitative polymerase chain reaction (qRT-PCR) analyses using a larger series of TRA/TRD-MYC rearranged cases and an independent cohort of non-MYC rearranged TAL1/LMO2 T-ALLs (Fig 1C, Figure S3). Nevertheless, and as expected,
Fig 1. Genetic characterization of TRA/TRD-MYC translocated T-ALLs. (A) Copy number and targeted mutation screening of 26 TRA/TRD-MYC rearranged T-ALLs. Graphical representation of deletions (dark blue), mutations (light blue) and amplifications (grey) present in a set of T-ALL oncopgenes and tumour suppressor genes. Male and female T-ALL cases are indicated as green and pink rectangles, respectively. All studied T-ALLs are paediatric cases (age <18 years). The frequency of described aberrations is reported for this cohort and in brackets for a general T-ALL group. (B) The top 75 most differentially expressed genes between TRA/TRD-MYC like T-ALLs (n = 7) and non-MYC rearranged TAL1-LMO2 T-ALLs (n = 6) based on RNA sequencing. NOTCH1 target genes are indicated by red arrows. M = TRA/TRD-MYC positive T-ALL; T = TAL1-LMO2 T-ALL. Value shown as colour scale are mean centred regularized log counts. (C) Validation of NOTCH1 target expression in an independent set of TRA/TRD-MYC translocated T-ALLs (n = 16) and non-MYC translocated TAL1-LMO2 T-ALLs (n = 7). HES4, PTCRA, IL7R, NOTCH3 mRNA expression was assessed by reverse transcription quantitative polymerase chain reaction. Mann–Whitney test was performed to compare the different groups (**P < 0.01, ***P < 0.001). Horizontal lines represent the median for each group. (D) MYC expression in TRA/TRD-MYC translocated T-ALLs (n = 13) and in TAL1-LMO2 T-ALLs (n = 7). Mann–Whitney test was performed to compare the different groups (*P < 0.05). Horizontal lines represent the median for each group.

Fig 2. BET bromodomain inhibition in t(8;14)(q24q11) positive T-ALL. (A) Hockey-stick plot representing the normalized rank and signal of H3K27ac peaks in t(8;14)(q24q11) positive MOLT16 cells. TRA/TRD enhancer elements (in red) showed the highest level of H3K27ac. (B) Cell viability in a panel of human T-ALL cell lines after 72 h of JQ1 treatment, relative to control cells treated with dimethylsulfoxide. TRA/TRD-MYC rearranged T-ALL cell lines are represented in red. Average and standard deviation of 3 independent experiments are plotted. 50% inhibitory concentration (IC50) values (nmol/l) are reported for each cell line. (C) Schematic representation of the t(8;14)(q24q11) translocation, H3K27ac chromatin immunoprecipitation (ChIP) sequencing tracks at TRA/TRD locus in MOLT16 cell line and H3K27ac levels after JQ1 treatment (7 h, 2 μmol/l) as evaluated by ChIP quantitative polymerase chain reaction (qPCR) analysis. Primers used were designed on putative TRA/TRD enhancer regions (H3K27ac positive targets, red bar). Signal enrichment at target regions is reported in H3K27ac and IgG ChIP vs. relative inputs. Negative regions downstream of the positive target were analysed as control (chr14:22,626,300–22,626,420). Means were calculated on 4 replicates with standard deviation represented by the error bars (**P < 0.01). (D) JQ1 in vivo treatment experimental design. NSG mice were retro-orbital injected with TRA/TRD-MYC translocated cells from a T-ALL patient (Case 4, see Table S1) to generate primary xenografts. After leukaemia engraftment, blasts were isolated from primary models and injected in other NSG mice to obtain a larger cohort of secondary xenografts for treatment. hCD45 positivity was checked from peripheral blood after 3 weeks. Upon engraftment, JQ1 (50 mg/kg bodyweight) was intraperitoneally administered twice/day for 14 days. Vehicle was administered to the control group following the same schedule. At the end of the experiment, animals were sacrificed and tissues analysed. (E) Percentage of hCD45 leukaemic cells in peripheral blood of NSG mice xenotransplanted with TRA/TRD-MYC T-ALL cells after 14 days of JQ1 treatment vs. control (dimethyl sulphoxide, DMSO). (F) Percentage of hCD45 leukaemic cells in the bone marrow at the end of the experiment (day 15). (G) Xenografts spleen weight (mg) after 14 days of JQ1 treatment versus DMSO. Mann–Whitney test was used to compare the treatment groups (**P < 0.01). Horizontal lines on the graph indicate the median for each group.
TRA/TRD-MYC leukaemias displayed higher MYC expression as compared to their TAL1/LMO2 rearranged counterparts (Fig 1D).

BET bromodomain inhibitors, such as JQ1, exploit the transcriptional addiction of cancer cells. At low concentrations, it has been shown that JQ1 preferentially targets t(8;14)(q24;q11) MYC chr8 TRA/TRD locus chr14 (A) (B) (C) (D) (E) (F) (G)

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enhancer elements with the highest levels of H3K27ac (Hnisz et al., 2013). Here, we performed H3K27ac chromatin immunoprecipitation (ChIP) sequencing analysis on t(8;14)(q24;q11) positive MOLT16 cells and identified the highest levels of H3K27ac in the enhancer elements of the TRA/TRD locus (Fig 2A, Figure S4). Therefore, and given that these strong TRA/TRD locus control regions drive MYC expression in these tumours, we anticipated that BET bromodomain inhibition could serve as a valuable therapeutic strategy for this aggressive T-ALL subtype.

In vitro drug sensitivity screening, using a panel of 7 human T-ALL cell lines, revealed that the TRA/TRD-MYC positive cell lines, MOLT16 and KE-37, showed the highest sensitivity towards JQ1 treatment (MOLT16 IC$_{50}$ = 199 nmol/l; KE-37 IC$_{50}$ = 497 nmol/l) (Fig 2B, Figure S5). In addition, using ChIP qPCR, we confirmed that loss of MYC expression upon JQ1 treatment was accompanied by decreased levels of H3K27ac at the rearranged enhancer region of the TRA/TRD locus (Fig 2C).

Finally, we established patient-derived xenograft (PDX) models from t(8;14)(q24;q11) positive primary T-ALLs to study JQ1 drug efficacy in vivo. A primary xenograft was first treated for 14 days with one single administration a day of JQ1 (50 mg/kg), revealing a decrease of leukemic blasts in the peripheral blood and a reduction in splenomegaly, although a limited effect was observed in the bone marrow (Figure S6). Therefore, the therapeutic schedule was reset and a second PDX was treated with JQ1 double dosage (50 mg/kg, twice/day) (Fig 2D). Notably, the intense treatment resulted in a marked reduction of human leukemic blasts both in peripheral blood (Fig 2E) and bone marrow (Fig 2F) and produced a significant decrease in splenomegaly (Fig 2G). The in vivo effect of JQ1 was further confirmed by treating an additional xenograft model established from a different TRA/TRD-MYC translocated T-ALL patient, following the same treatment schedule (Figure S7).

Altogether, our study reveals that TRA/TRD-MYC rearranged T-ALL is an aggressive and NOTCH1-independent high-risk subtype of human leukaemia that displays therapeutic sensitivity towards BET bromodomain inhibition.

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Authorship contributions

GM, FM, SP, TP, LR, BL, NV, NVR, SG and GB performed experiments. GM, PVV, FM, KD, MT, JR, and WVL performed analyses. LR and BL provided technical assistance. FVN and DD performed RNA sequencing experiments. TL, CS, SR, LDP, AC, BDM, YB, CJH, GB, HC, RS, VA, JM, CM and ML collected and provided primary T-ALL patient material. PVV and GM designed research and wrote the paper, with help from the other authors. All authors have seen, reviewed and approved the final version.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Biological and clinical characteristics of 26 studied T-ALLs carrying t(8;14)(q24;q11) translocation.

Table SII. Primers used for mutation screening, qRT-PCR and ChIP qPCR analyses.

Fig S1. T-ALL cell lines screening for cleaved NOTCH1, AKT and PTEN protein expression or activation. Among the cell line panel, MOLT16 and KE-37 are TRA/TRD-MYC translocated cells (*) presenting NOTCH1 and FBXW7 wild-type, PTEN deletion or mutation and pAKT activation. Deletion and mutation are reported in dark and light blue, respectively.

Fig S2. Transcriptomic profiling by RNA-seq analysis of TRA/TRD-MYC translocated TALLs (n = 5) and TALI-MO2 T-ALLs (n = 8). Unsupervised clustering of the two T-ALL subsets revealed the presence of two clusters of leukemias, including one group that consisted of all 5 TRA/TRD-MYC positive T-ALLs and 2 additional TRA/TRD-MYC negative leukemias, named TRA/TRD-MYC like.

Fig S3. NOTCH1 target genes validation in an independent set of TRA/TRD-MYC translocated T-ALLs (n = 16) and TALI-MO2 T-ALLs (n = 7). HES1 and DTX1 expression was assessed by qRT-PCR in the studied T-ALL subgroups. Mann–Whitney test was performed to compare the different groups (**P < 0.01, ***P < 0.001). Horizontal lines represent the median for each group.

Fig S4. Schematic representation of TRA/TRD locus in MOLT16 cell line detected by H3K27ac CHIP sequencing analyses (chr14:22 275–25 597 kb, GRCh38). In pink, TRA/TRD enhancer elements (chr14:22 507 600–22 570 848 bp) identified by high level of H3K27ac and reported in the Hockey-stick plot (Fig 2A, red dot).

Fig S5. MYC mRNA and protein downregulation after JQ1 treatment (1 μmol/l) in MOLT16 and KE-37 cell lines. Control cells were treated with dimethylsulfoxide. MYC mRNA expression was analyzed after JQ1 treatment at 6 and 12 h. MYC protein expression was studied after 72 h upon JQ1 treatment for MOLT16, KE37, JURKAT, ALL-SIL and HPB-ALL cell lines. Protein downregulation was confirmed after JQ1 treatment in both t(8;14)(q24;q11) positive cell lines (*). Minor effects at protein level were observed in less sensitive cell lines, such as ALL-SIL and HPB-ALL.

Fig S6. NSG mice xenotransplanted with primary TRA/TRD-MYC T-ALL cells and treated with JQ1 (50 mg/kg) once/day for 14 days. Percentage of hCD45 leukemic cells in peripheral blood and in bone marrow. Xenografts spleen weight (mg). Mann–Whitney test was used to compare the treatment groups (**P < 0.05, ***P < 0.01).

Fig S7. NSG mice xenotransplanted with primary TRA/TRD-MYC T-ALL cells (from patient 17, see Table SI) and treated with JQ1 (50 mg/kg) twice/day for 14 days. Percentage of hCD45 leukemic cells in peripheral blood and in bone marrow. Mann–Whitney test was used to compare the treatment groups (**P < 0.05).

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