The small molecule YK-4-279 shows anti-lymphoma activity in pre-clinical models

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Introduction

Up to 20% of diffuse large B cell lymphomas (DLBCLs) present recurrent 11q23 gains leading to upregulation of ETS1 and FLI1, two transcription factors of the ETS family and FLI1 silencing leads to cell death in 6/6 DLBCL cell lines (*Blood* 2013). Friend leukemia integration1 (FLI1) was initially identified as a proto-oncogene in Friend virus-induced erythroleukemia and is expressed in endothelial and hematopoietic cells. The EWS1-FLI1 translocation is a hallmark of Ewing Sarcoma (ES) and the fusion product (**Fig1a**) functions as an aberrant transcription factor with the ETS-DNA Binding domain of FLI1 recognizing GGAA microsatellites upstream of its target genes (**Fig1b**). In pre-clinical models of ES, YK-4-279 inhibits binding of the transcriptional co-activator, RNase Helicase A (RHA), to EWS1-FLI1 leading to growth arrest and apoptosis (*Nat Med* 2009). With the core canonical binding motifs of EWS1-FLI1 (GGAA) shared by FLI1 and other ETS family members (*Pediatric Research 2012*), perhaps YK-4-279 could exert an anti-proliferative effect in lymphomas with deregulated expression of FLI1 or other ETS family members. Here, we assessed the anti-proliferative effects of YK-4-279 in a panel of human B/T-cell lymphomas cell lines.

Results

YK-4-279 showed potent dose-dependent anti-proliferative activity in most cell lines with the majority of IC50 values below 1uM (**Fig2**). The median IC50 value was 393 nM (95% C.I., 272 - 3622 nM). There were no apparent differences among the different lymphoma subtypes: DLBCL (median IC50=386 nM; 95% C.I., 223-497), MCL (596 nM; 95% C.I., 230-1305), SMZL (217 nM; 95% C.I., 183-524) and ALCL (403 nM; 95% C.I., 170-3387). Results were confirmed by performing further MTT experiments using the R-YK-4-279 and S-YK-4-279, showing activity only when cells were exposed to the latter compound. GEP signature of the highly sensitive group was enriched for E2F1 and PAX5 targets (including FLI1), and genes of the germinal center (GC) B cells while the low sensitivity group was associated with NFKB-related genes. When we treat a panel of 5 YK-4-279 sensitive DLBCLs cells lines (3 ABC, and 2 GCB) with the compound, cells undergo apoptosis (**Fig3**) and cell cycle impairment (**Fig4**).

Fig1

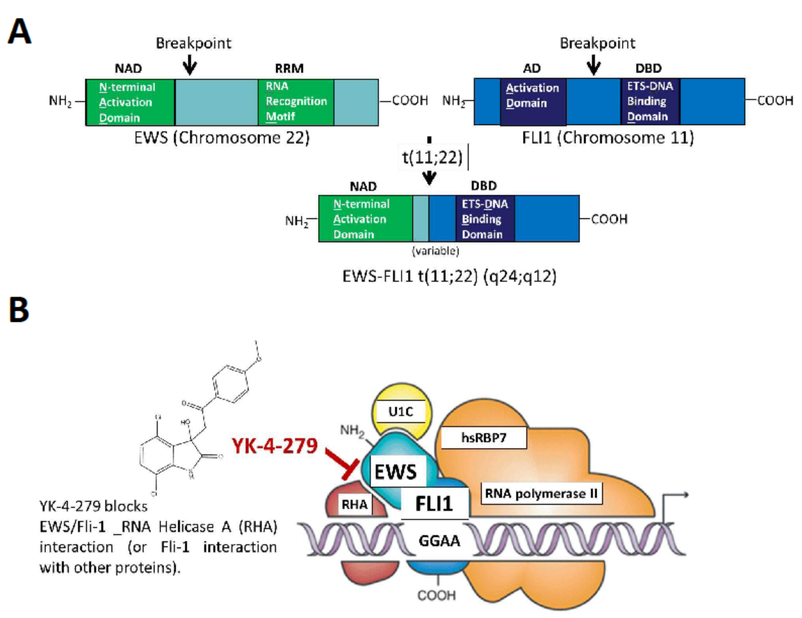


Fig2

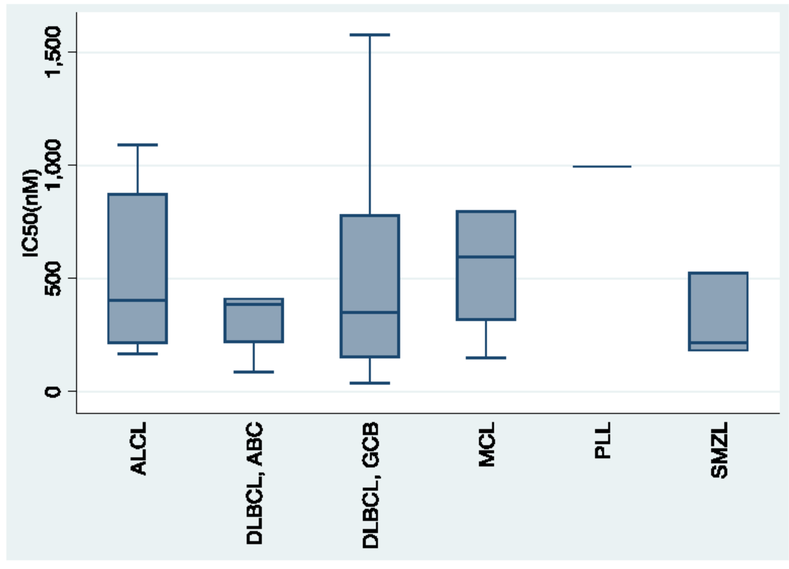


Fig1. Mechanism of YK-4-279 function in Ewing's (EW) sarcoma. (**a**) The EWS-FLI1 fusion includes the N-terminal activation domain of EWS, and the C-terminal ETS DNA-binding domain (DBD) of FLI1. (**b**) As an aberrant transcription factor, EWS-FLI1 regulates genes in part by binding to GGAA microsatellites upstream of target genes. EWS-FLI1 has been shown to interact with the splicing factor U1C (also known as SNRPC, small nuclear ribonucleoprotein polypeptide c), RNA helicase A (RHA), and the hRBP7 subunit of RNA polymerase II (Pol II), leading to transcription initiation.

Fig2. IC50 values for YK-4-279 distribution in different lymphoma pre-clinical models. In each box-plot, the line in the middle of the box represents the median and the box extends from the 25th to the 75th percentile (interquartile range, IQ); the whiskers extend to the upper and lower adjacent values (i.e., ±1.5 IQ); ALCL, anaplastic large cell lymphoma; ABC-DLBCL, Activated B-cell like, diffuse large B cell lymphoma; GCB-DLBCL, Germinal Center B-cell like, diffuse large B cell lymphoma; MCL, mantle cell lymphoma; PLL, prolymphocytic leukemia; SMZL, splenic marginal zone lymphoma.

Fig3

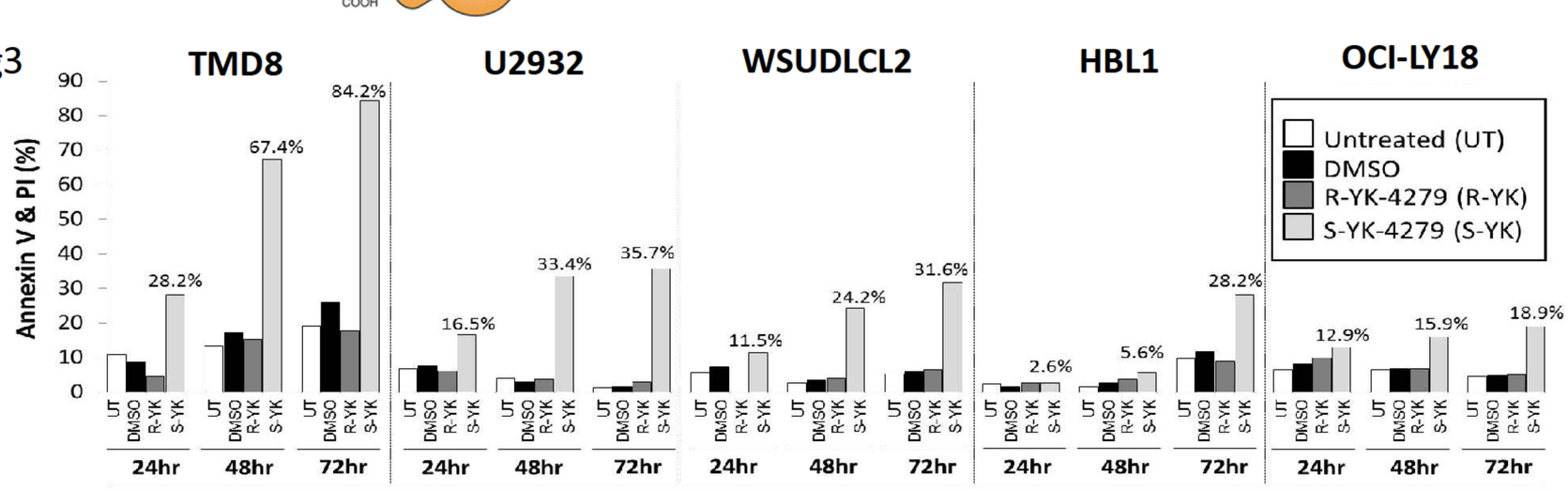
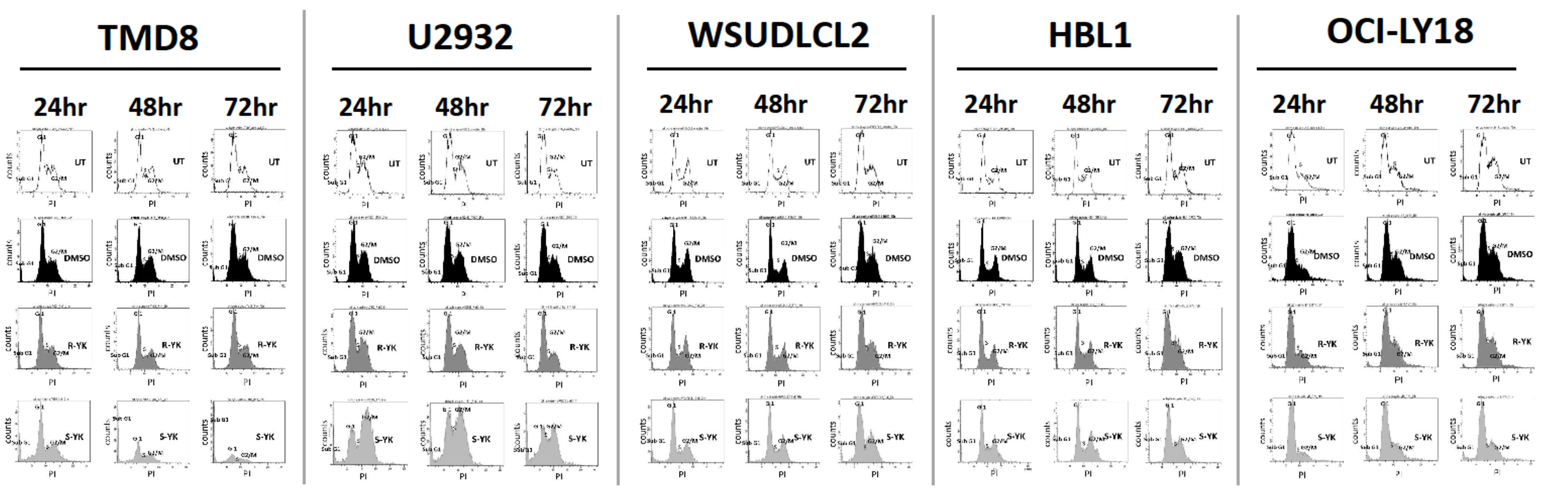


Fig4



S-YK-4-279 induces apoptosis rather than cell cycle arrest in a panel of YK-4-279 sensitive-DLBCL cell lines. Cells were either untreated (UT) or treated with 500nM of YK-4-279 with DMSO serving as the solvent control. **Fig3** Flow cytometry was performed 24hr, 48hr and 72hr post treatment to determine the levels of apoptosis. Results were graphed according to the percentage annexin V (AV) and propidium (PI) positivity. **Fig4.** Cell cycle analysis was also performed by staining the cells with propidium iodide followed by flow cytometry.

Materials and Methods

IC50s were obtained from MTT assays after 72 hrs post treatment with increasing doses of YK-4-279. Baseline Gene expression profiling (GEP) was performed on 48 lymphomas cell lines to determine which gene profiles might render the cells either more or less sensitive to YK-4-279. Levels of apoptosis cell cycle arrest were also quantified upon treatment with the compound.

Literature Citations

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Conclusion

YK-4-279 demonstrates impressive anti-proliferative activity in lymphomas which underscores the need for further investigations as an anti-lymphoma compound.

Acknowledgments

We are grateful for the funding we received from both the Barletta Foundazion and the Gelu Foundazion to drive this research project forward.

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13icml 269--P

Pre-clinical studies

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DOI: 10.3252/pso.eu.13icml.2015

Poster presented at: 13-ICML

Printing supported by: Takeda ONCOLOGY

PosterSessionOnline.com