The ETS inhibitors YK-4-279 and TK-216 interfere with SPIB and synergize with lenalidomide in diffuse large B cell lymphoma of the activated B cell-like type (ABC DLBCL)

Abstract #2810

Elaine Chung 1, Filippo Spiriano 1, Ivo Kwee 1, Sara Napoli 1, Chiara Tarantelli 1, Eugenio Gaudio 1, Luciano Cascione 1, Andrea Cavalli 2, Alberto Arribas 1, Andrea Rinaldi 1, Davide Rossi 1,3, Emanuele Zucca 1,3, Anastasios Statthis 1, Katti Jessen 4, Brian Lannuti 4, Jeffrey Toretsky 4, Francesco Bertoni 1,3

1 Institute of Oncology Research (IOR) and 2 Institute for Research in Biomedicine (IRB), Università della Svizzera Italiana (USI), Bellinzona, Switzerland; 3 Oncology Institute of Southern Switzerland (IOSI), Bellinzona, Switzerland; 4 Oncterra Therapeutics, San Diego, CA, USA; 5 Georgetown University, Washington D.C., USA

BACKGROUND

The two main DLBCL subtypes are the germinal center B (GCB) and the ABC types, characterized by individual biologic and clinical features. Since up to 40% DLBCL patients are still not cured with the standard treatments there is the need of novel therapies. YK-4-279 is a small molecule that inhibits binding of the EWS1-FL1 fusion protein to RHA resulting in growth arrest and apoptosis in Ewing sarcoma cells. Its derivative TK-216 is the first in class inhibitor of the ETS-family of transcription factors in phase I (NCT02657005 for relapsed or refractory Ewing sarcoma). Both compounds have shown promising preclinical anti-lymphoma activity. Here, we present novel data on their mechanisms of action in DLBCL.

Material and Methods

Cell lines were exposed to YK-4-279 or TK-216 alone or in combination with other compounds for 72h using Tecan D300e Digital Dispenser and 96 well plates; cell proliferation was measured with MIT; synergy with Chou-Talalay combination index. Gene expression profiling (GEP) was performed with the Illumina HumanHT 12 Expression BeadChips. In vivo studies were done in NOD-SCID mice and treatments started with 60%3 tumor volumes sc.

RESULTS

While TK-216 has shown strong in vitro and in vivo anti-lymphoma activity, only in vitro data are available for YK-4-279 in lymphomas. Here, we first confirmed the anti-tumor activity also of the latter compound in the same ABC-DLBCL model (TMD8 xenograft) (Figure 1). Compared with control group (n=10), mice treated with YK-4-279 (100 mg/kg, BID; n=9) clearly presented a reduction in tumor volume at D8 and D11 (P<0.01) and D13 (P not available since control group had to be stopped due to tumor volume). In accordance with our previous combination data showing a specific synergism of TK-216 when combined with the immunomodulatory drug (IMD) lenalidomide in ABC DLBCL, when YK-4-279 was combined with the 8TX-inhibitor irbritinib, the P3K-delta inhibitor idelisib, the BET inhibitor OTX-015 and lenalidomide in four DLBCL cell lines (2 ABC, 2 GCB), the biggest benefit was achieved with the combination of YK-4-279 plus lenalidomide with synergism in both ABC DLBCL (Figure 2). Finally, since lenalidomide is active in mantle cell lymphoma (MCL), the synergism of the combination of TK-216 and lenalidomide was confirmed also in two MCL cell lines. With the aim to understand the mechanism of action of the two small molecules, we correlated the baseline RNA expression levels of the different ETS factors with sensitivity to the drugs. SPIB was the gene presenting the most significant negative correlation with both YK-4-279 and TK-216, especially among the ABC DLBCL cell lines (P<0.05). Interestingly, SPIB is aknown oncogene for ABC DLBCL (Lenz et al, PNAS 2008) and is involved in the response to lenalidomide in ABC DLBCL (Yang et al, Cancer Cell 2012). YK-4-279 inhibits the binding of EWS1-FL1 to the helicases RHA and DDX5 (Selvanathan et al, PNAS 2012). Thus, we assessed whether YK-4-279 and TK-216 can have a similar effect on SPIB and whether they induce cellular effects similar to lenalidomide. Protein modelling demonstrated that the 3D structure of FL1 and SPIB are highly overlapping. Co-IP experiments showed the binding of SPIB to RHA and DDX5 in two ABC DLBCL cell lines (Figure 3). The binding to RHA and, at lesser extent, to DDX5 was decreased exposing the cells to TK216 or YK-4-279 (500 nM, 4h). Similarly to lenalidomide (Yang et al, Cancer Cell 2012), TK-216 decreased IRF4 and upregulated IRF7 protein in cells (Figure 4). Finally, GEP of two ABC DLBCL cell lines exposed to the active (S)- or to the inactive (R)-enantiomer (500 nM, 4-8h) showed that (S)-YK-4-279 caused an important upregulation of multiple snoRNAs, an effect compatible with an interference of the compound on helicases and RNA editing.

CONCLUSIONS

In ABC DLBCL, the ETS inhibitor YK-4-279 and its clinical derivative TK-216 interfere with SPIB and helicases involved in RNA editing. Moreover, both compounds act similarly to lenalidomide inhibiting IRF4 and upregulating IRF7 and synergize with the IMD in both ABC DLBCL and MCL.

Acknowledgments

Supported by Leukemia and Lymphoma Society and from Oncosuisse

Contact Francesco Bertoni, MD, Lymphoma & Genomics Research Program, IOR Institute of Oncology Research, via Vela 6, 6500 Bellinzona, Switzerland; phone: +41 91 8200 367; fax: +41 91 8200 305; e-mail: fbertoni@mac.com

Figure 1. Effects of TK-216 and YK-4-279 in a xenograft model of ABC-DLBCL. NOD-SCID mice subcutaneously inoculated with TMD8 cells (15 x 10⁶) were split in two groups respectively treated with TK-216 or YK-4-279 (100 mg/kg, BID, po, n=9) and control vehicle (n=10). 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).

Figure 2. TK216 and YK-4-279 combinations. Box-plots of the CI values obtained in individual cell lines. Y-axis: CI values. 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). Outside values have been omitted from the figure. CI values > 3. Numbers colored in red, synergism; blue, additive, black, no benefit effects.

Figure 3. CD-IP after YK-4-279 or TK-216 treatment. Cells were exposed to 500 nM of YK-4-279 or TK-216 for 4 hours, proteins were extracted and Immunoprecipitation was performed using SPIB antibody followed by immunoblotting for RHA and DDX5.

Figure 4. SPIB, IRF4 and IRF7 Immunoblotting after TK-216 treatment. Cell line were treated with 1 μM of DMSO or TK-216. Proteins extraction was performed after 10 and 24h of exposure to the drug. GAPDH was used as a loading control.