IOR Institute of Oncology Research

Abstract 272

OTX015 (MK-8628) targets genes with high levels of promoter H3K4me3 involved in key signalling pathways in splenic marginal zone lymphoma (SMZL) and mantle cell lymphoma (MCL)

Chiara Tarantelli [1], Afua A. Mensah [1], Luciano Cascione [1,2], Elena Bernasconi [1], Andrea Rinaldi [1], Eugenio Gaudio [1], Anastasios Stathis [2], Maria E. Riveiro [3], Emanuele Zucca [2], Ivo Kwee [1,4,5], Francesco Bertoni [1,2]

[1] Lymphoma and Genomics Research Program, IOR Institute of Oncology Research, Bellinzona, Switzerland; [2] IOSI Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [3] Oncology Therapeutic Development, Clichy, France; [4] IDSIA Dalle Molle Institute for Artificial Intelligence, Manno, Switzerland; [5] SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland.

INTRODUCTION AND RESULTS

INTRODUCTION: BET bromodomain inhibitors have shown preclinical activity in several lymphoma models, including SMZL and MCL. Objective clinical responses in diffuse large B cell lymphoma (DLBCL) patients have been observed in a phase I trial with OTX015 (NCT01713582). Downregulation of NFKB and JAK/STAT signaling and the E2F1 proliferation/apoptosis program have been proposed as mechanisms of action in DLBCL. We evaluated candidate genes and/or pathways impacted by OTX015 in SMZL and MCL.

RESULTS: We first analyzed the effect of OTX015 on genes known to play a role in MCL and SMZL proliferation. There was no downregulation of *CCND1* in 4/4 MCL cell lines nor of *NOTCH2* in 3/3 SMZL cell lines (500 nM OTX015; 4h, 8h and 24h). We thus performed GEP in sensitive cell lines (3 MCL, 3 SMZL) exposed to DMSO or 500 nM OTX015 for 2, 4, 8, 12 and 24h. OTX015 downregulated transcripts were enriched of genes involved in NFKB/BCR signaling and RNA metabolism, as well as MYC and NOTCH target genes, and genes downregulated by HDAC inhibitors and BET bromodomain inhibitors in other tumor models (Figure 1). Conversely, upregulated transcripts were enriched of genes involved in chromosome formation and maintenance, genes involved in the cell cycle and response to UV, genes upregulated by HDAC and BET bromodomain inhibitors (Figure 2). The five most downregulated genes were *NAPSB*, *SLC2A5*, *TNFRSF17*, *TLR10* and *FCRL2* in MCL and *TNFRSF17*, *MYC*, *TLR10*, *LRRC33*, *PLD6* in SMZL. Cluster 1 and 2 histones were the most upregulated genes in both lymphoma types. Figure 3 represents average profiles for two gene groups ordered by mRNA expression levels by GEP after OTX015 treatment (500 nM), defined as “downregulated” or “not differentially expressed” and illustrates increasing levels of this histone mark with decreasing gene expression after treatment. In addition, genes downregulated by OTX015 had significantly more H3K4me3 binding to the promoter regions than expected for random sets of genes (Figure 4).

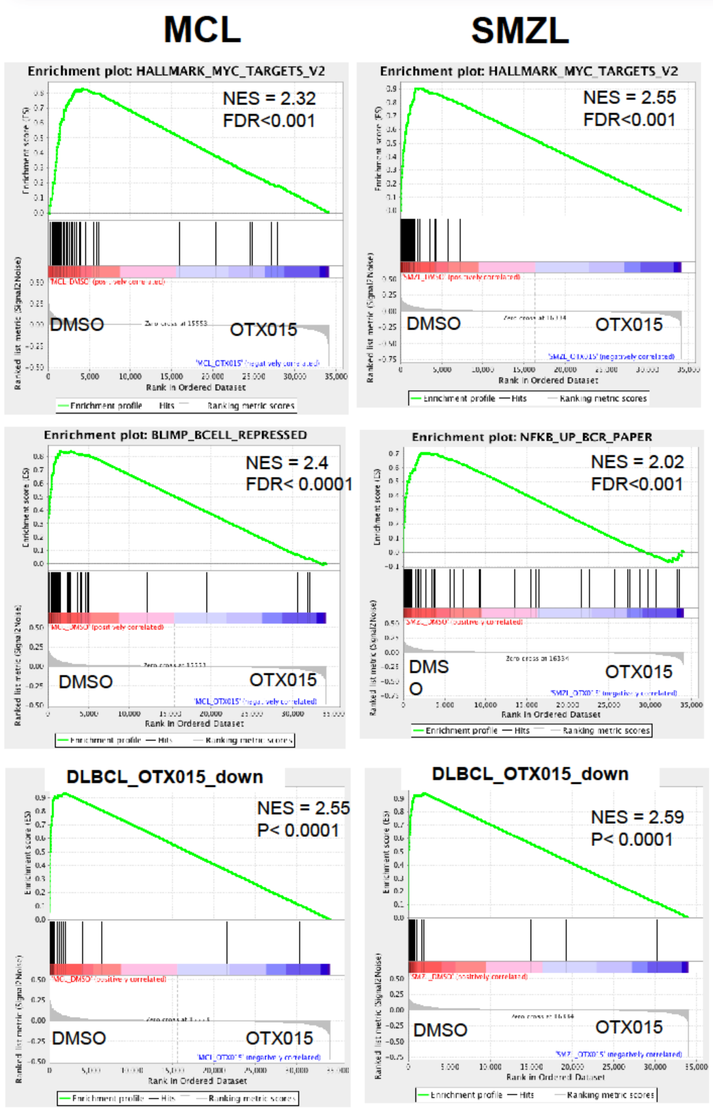

Figure 1. GSEA plots illustrating the enrichment of representative gene-sets in MCL and SMZL cell lines.
FDR, false discovery rate; NES, normalized enrichment score

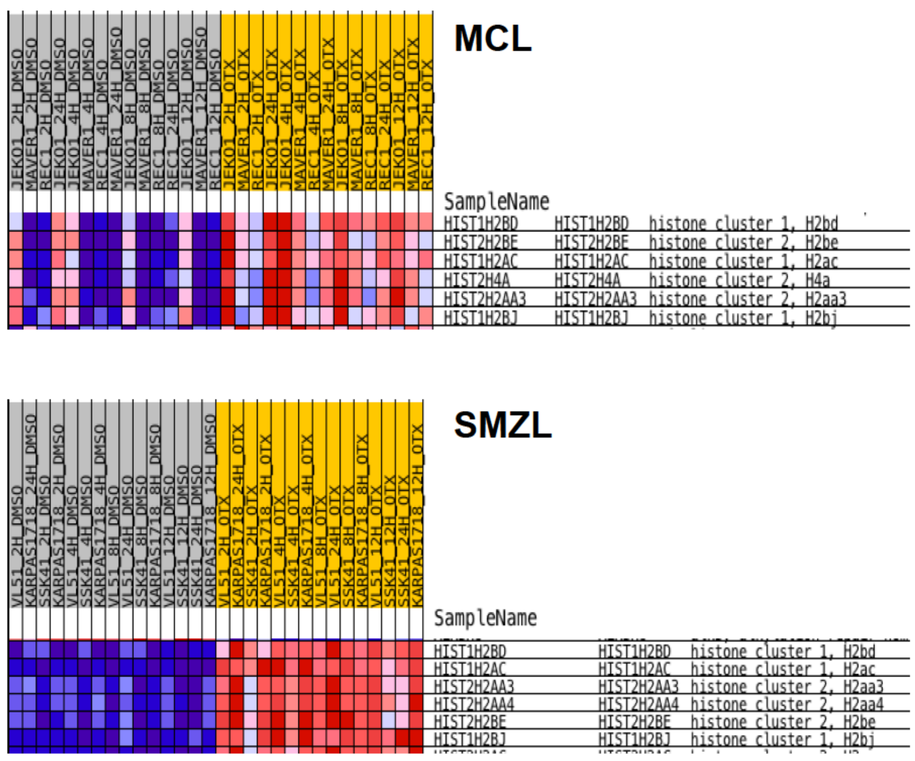

Figure 2. GSEA most upregulated genes in MCL and SMZL cell lines treated with 500 nM OTX015 for 2, 4, 8, 12 and 24h compared to DMSO.

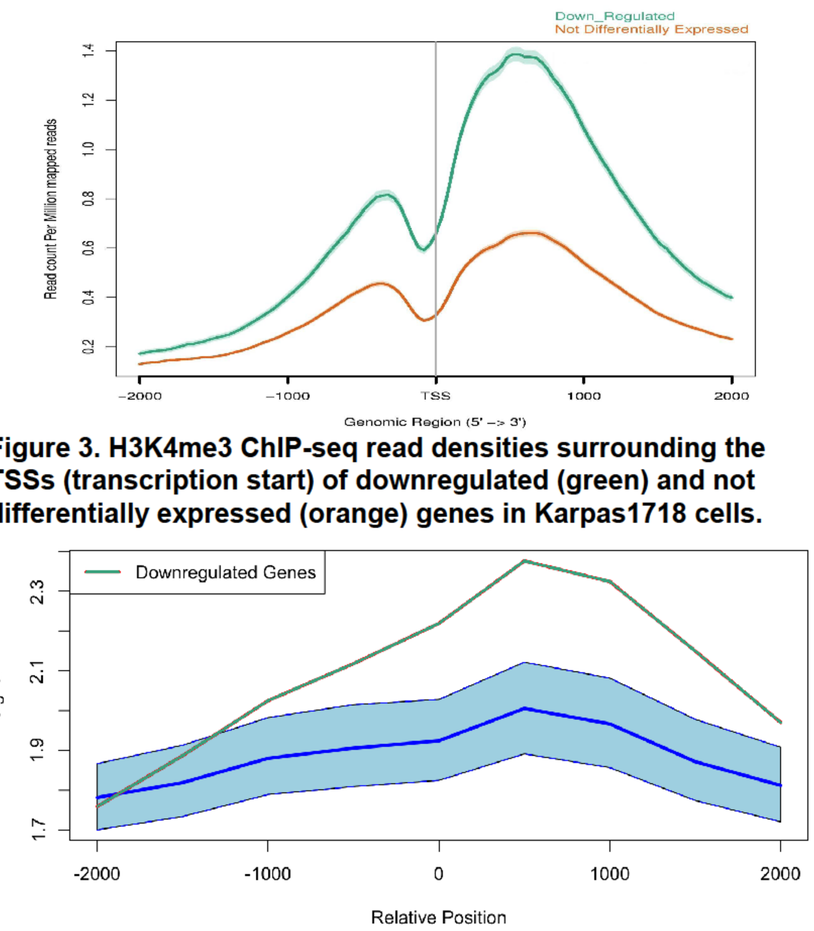

Figure 3. H3K4me3 ChIP-seq read densities surrounding the TSSs (transcription start) of downregulated (green) and not differentially expressed (orange) genes in Karpas1718 cells.

Figure 4. H3K4me3 ChIP-seq read density profiles in Karpas1718. The blue region is the “null” distribution created by repeatedly sampling random gene lists of the same size as the downregulated gene list. The null region is between the 0.025 and 0.975 quantiles of the null distribution. Genes silenced by OTX015 have a significant gain of H3K4me3 binding (green curve) 2000 bases either side of the TSSs, compared to random sets of other genes.

MATERIALS AND METHODS

Gene expression profiling (GEP) was performed with Illumina HumanHT12 Expression BeadChips. ChIP-Seq was performed in SMZL cell lines (Karpas1718, VL51) using antibodies against H3K4me3 and, as controls, anti-IgG and input DNA. Libraries were prepared with the ChIP-Seq Sample Preparation kit (Illumina), according to the manufacturer’s instructions. Sequencing was performed on an Illumina HiScanSQ, with 50-bp single reads. Sequence tags were aligned to human genome build hg19 with Bowtie software using default settings. Redundant reads were removed and reads uniquely mapping to the reference genome were used for further analysis. A maximum of one mismatch was allowed per read. To perform “peak calling” we used the Homer Genomic Suite. Promoter peaks were defined as peaks whose apex was located within a ±2 kb window from a representative transcription start site (TSS). ChIP-seq data were normalized by total tags count.

CONCLUSIONS

In MCL and SMZL, OTX015 affects the NFKB and TLR pathway, as has been observed in DLBCL models. Genes with a high degree of H3K4me3 binding to their promoters were more sensitive to OTX015 activity.

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

Acknowledgments

This work was partially supported with research funds the Nelia et Amadeo Barletta Foundation and the Gelu Foundation.

13icml 272--P

Pre-clinical studies

Chiara Tarantelli

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

Poster presented at: 13-ICML

Printing supported by: Takeda ONCOLOGY

PosterSessionOnline.com