Epigenomic and Transcriptional Profiling of CRC Cell Lines with Distinct Response Patterns to the BET Inhibitor BI 894999

Daniel Gerlach*, Larissa Koller*, Susa Strasburger, Paula Träxler, Onur Kaya, Norbert Schweifer, Ulrike Tontsch-Grunt, Norbert Kraut and Fabio Savarese

Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria

* Equal contribution, corresponding author email address: fabio.savarese@boehringer-ingelheim.com

INTRODUCTION

The bromodomain and extra-terminal (BET) protein BRD4 is a ‘reader’ of histone acetylation and plays a central role in transcriptional elongation by interacting with transcription factors and the transcriptional elongation factor P-TEFb. It acetylates the amino-terminal tails of histones by recruiting the bromodomains of the BET family members. Despite lack of direct transcriptional effect of BET inhibition, BRD4 has been linked to invasive behavior of multiple cancers. BET inhibition has proven to be a novel treatment option in hematological indications, while solid tumors show much less responses. Our pre-clinical studies show that BET inhibitor BI 894999 is highly active in a fraction of cell lines tested in ongoing clinical trials for both hematological as well as solid cancer patients [Jun et al., 2016]. While BET inhibition showed promising pre-clinical models of hematological indications, solid tumors show more heterogeneous responses in pre-clinical models. Consequently, BET inhibition is of high interest. To identify potential biomarkers predicting response to BET inhibitor treatment, we performed transcriptomic and epigenomic analyses of clinical models of hematological indications, solid tumors show much more heterogeneous responses in pre-clinical models.

METHODS

Cell culture and proliferation assay

Tumor cell lines were obtained from ATCC or DMSZ and STR profiled. Proliferation assays were performed on 96-well flat-bottom microtiter plates and incubated with or without BET inhibitor BI 894999 and PrestoBlue® dye assay. Viability assays were performed on 96-well flat-bottom microtiter plates and incubated with or without BET inhibitor BI 894999.

Transcriptome profiling (RNA-seq & Quant-seq)

Tumor cell lines were obtained from ATTC or DMSZ and STR profiled. RNA isolation, library preparation, and sequencing was performed as in ref. [Gerlach et al., 2018]. Differential gene expression was analyzed via CLIFF, Wernitznig A) and plots were computed in R with the Bioconductor packages limma/voom, pheatmap, and tidyverse.

RESULTS

CRC cell lines show a heterogenous response to BI 894999 treatment. Baseline RNA-seq reveals a putative expression signature predictive of response. This includes the A31-1 transcription factor A31-1 which is overexpressed in the majority of resistant cell lines. The transcription factor A31-1 shows consistent gene induction across all cell lines, this includes the BI 894999-resistant cell line SW620. A sub-cluster (top) shows consistent gene induction per cell line and kmeans clustering using 7 groups. Some clusters show cell lines enriched in the A31-1 gene signature and separable from resistant cell lines. Selection of regions that show specific enrichment in the BETi resistant cell line cluster.

Fig. 1: CRC cell line sensitivity panel & gene mutation plot. The order of cell lines is based on BI 894999 50nM treatment with the lowest resistance against BET inhibition. Baseline gene expression changes are associated with resistance or sensitivity (not significant after multiple testing correction).

Fig. 2b: BATF shows higher expression in A31-1 resistant cell lines. The enhancer-template RNA CCAT1 does not predict sensitivity in colorectal cancer [Krauw et al., 2016].

Fig. 2a: BI 894999 induced gene expression changes confirm HDACi as a pharmacodynamic (PD) biomarker. HDACi shows dose-dependent induction on both resistant and susceptible cell lines. MYC is down-regulated in 3/5 BETi sensitive cell lines, and in 24 resistant cell lines. Bubblemap represent log2 fold-change expression.Comparisons.

Fig. 3b: Gene expression changes upon BI 894999 treatment in 9 CRC cell lines. A total of 174 genes with a significant change of ≥ 4-fold in at least one cell line are identified. A common gene signature reflects the high-growth potential of the cell lines under study and is only partially comparable. A sub-cluster (top) shows consistent gene induction across all cell lines, this includes the PD biomarker gene HDAC1. No cluster of currently down-regulated genes with a full overlap of the BETi sensitive/resistant status can be detected.

Fig. 3a: Baseline HDACi3 CHIP-seq coverage profiles across 5 CRC cell lines based on SE overlapping peaks summary. Normalized read coverage across all cell lines is presented. Some cell lines show a clear border between high specific HDACi3 enrichment (e.g. cluster 5, cluster 7), however even with some subclusters very few regions emerge that show an association with HDACi3 signature and response to BETi. The SE signal for SW620 reflects the HDACi3 signal for the same cell line.

Fig. 4b: TF motifs enriched within CCAT1 (0.01) vs. CCAT2 (0.00) regions in SW623 & association with TFs and oncogenes.

Fig. 5: Only a few regions show HDAC7a signatures associated with BETi responses. Selection of regions that show specific enrichment in the BETi resistant panel (yellow) vs. the sensitive one (blue).

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REFERENCES


SUMMARY & CONCLUSIONS

A subset of CRC cell lines is sensitive to BETi inhibition. Baseline gene expression separates sensitive from resistant cell lines. CCAT1 expression is not a predictive biomarker [Nicoli et al., 2016]. BI 894999 mediated BET inhibition in 9 CRC cell lines reveals HDACi3 as a valid PD biomarker for CRC and BETi treatment induced gene expression changes that are mostly modified the individual cell lines. Epigenomic profiling of CRC cell lines reveals few HDACi3 enriched loci that can be associated with BETi sensitivity. FCGBP is a marker for CIMP+ in colorectal cancer [Ishihara et al., 2016]. The CCAT1/CCAT2 locus is predictive for the CCIP status, however this does not correlate with efficacy of BETi inhibitor treatment. RNA- and chromatin-based BETi biomarkers are currently in solid cancer remain elusive and warrant retrospective analysis in clinical trials.