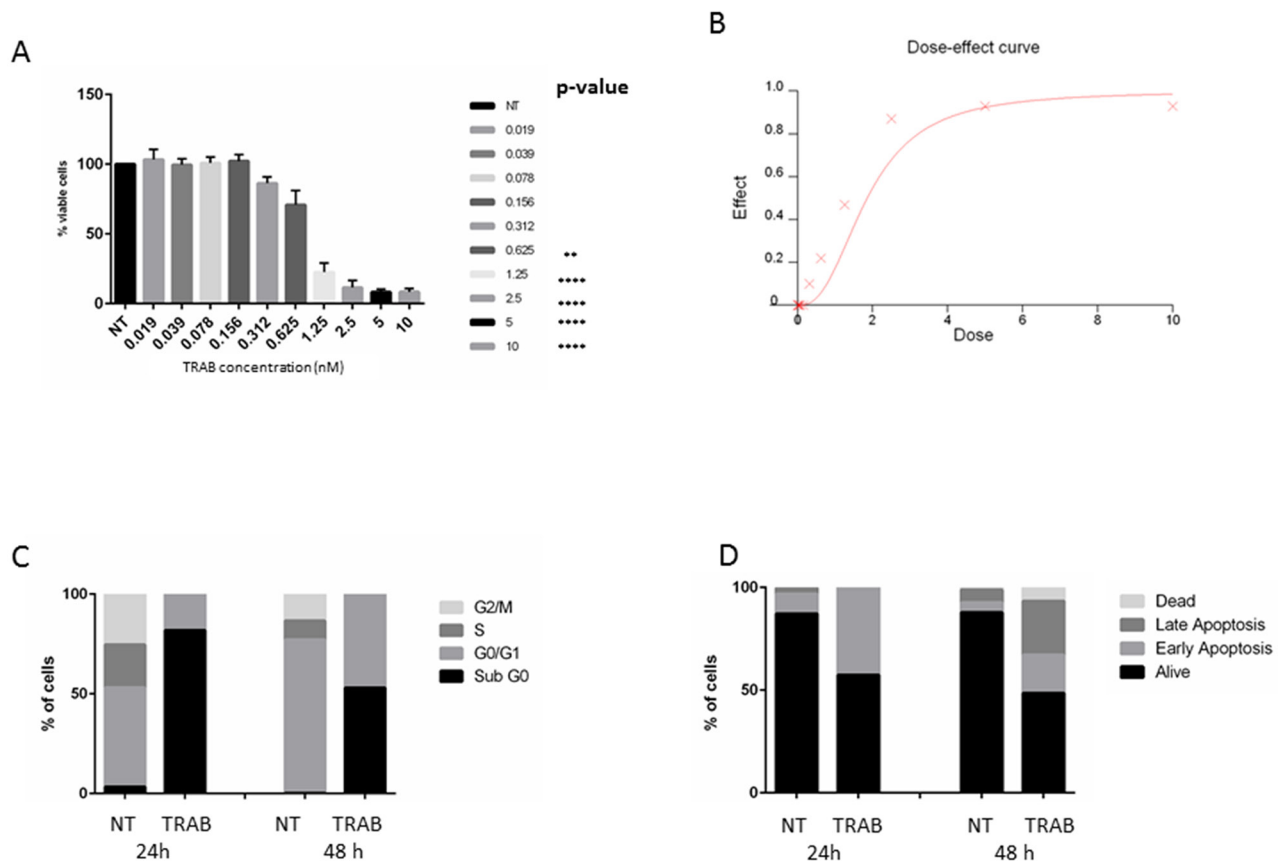
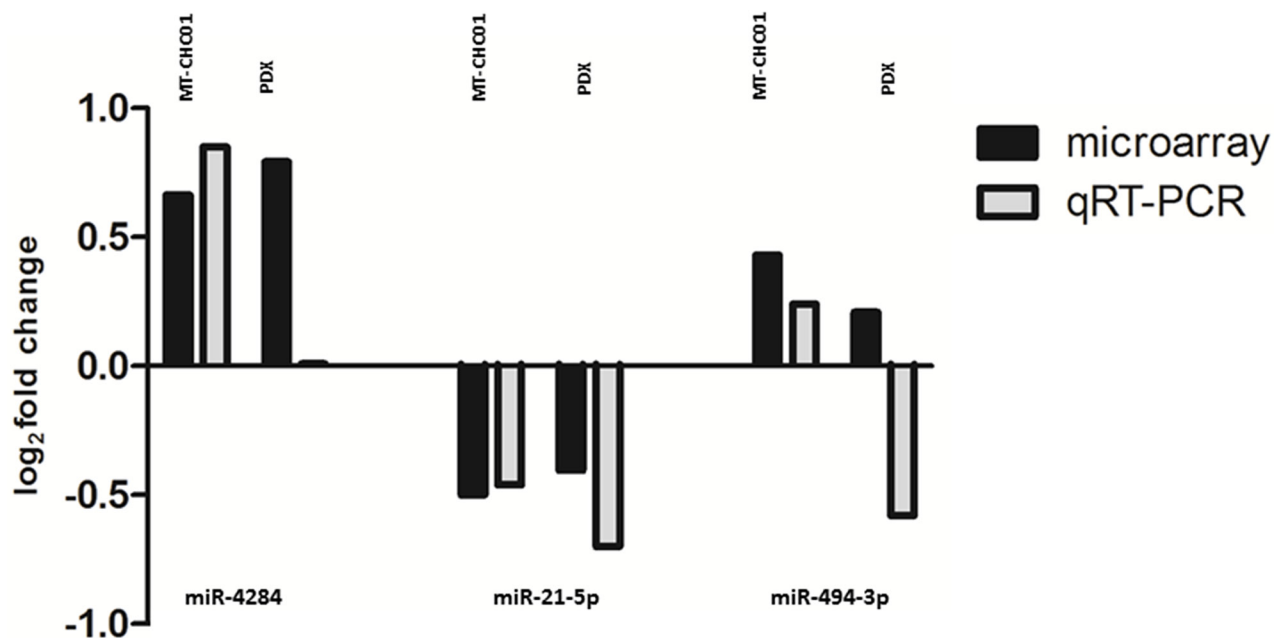


# Gene and microRNA modulation upon trabectedin treatment in a human intrahepatic cholangiocarcinoma paired patient derived xenograft and cell line

## SUPPLEMENTARY FIGURES AND TABLES



**Supplementary Figure S1: Effect of trabectedin on proliferation, cell cycle and apoptosis in MT-CHC01 cells. Panel A:** Statistical analysis of cell viability after 72 hours of treatment with escalating doses of trabectedin. A statistical significant reduction of proliferation was revealed until 0.625 nM. **Panel B:** Dose effect curve of trabectedin; the IC50 value is 1.84 nM. **Panels C-D:** Effect of trabectedin on cell cycle (C) and on apoptosis (D) *in vitro*. MT-CHC01 cells were treated with 5 nM of trabectedin for 24 and 48 h and subjected to cell cycle analysis and apoptosis detection by AnnexinV/PI staining by flow cytometry as described in methods. The bars represent the average with SEM of percentage of cells in each phase (C) and apoptotic cells (D) of three independent experiments. NT: not treated cells



**Supplementary Figure S2: Comparison between the expression values of selected microRNAs obtained by array and qRT-PCR after trabectedin treatment.** X axis: gene expression of MT-CHC01 and PDX respectively. Y axis: log<sub>2</sub> fold change expression obtained by microarray and 2<sup>-ΔΔCt</sup> for qRT-PCR.

**Supplementary Table S1: Biological processes significantly enriched within down- and up- regulated probes after trabectedin treatment of MT-CHC01 cells.**

See Supplementary File 1

**Supplementary Table S2: Pathways enriched within down- and up-regulated genes (Kegg database) after trabectedin treatment of MT-CHC01 cells**

Name	p-value	Expression upon trabectedin
Focal adhesion	0.000001	down
Fc gamma R-mediated phagocytosis	0.001	down
Endocytosis	0.001	down
Regulation of actin cytoskeleton	0.003	down
Tight junction	0.009	down
Glioma	0.01	down
O-Glycan biosynthesis	0.01	down
Axon guidance	0.01	down
ErbB signaling pathway	0.03	down
Fc epsilon RI signaling pathway	0.04	down
Adherens junction	0.04	down
Insulin signaling pathway	0.04	down
Cytokine-cytokine receptor interaction	0.01	up
Pathways in cancer	0.02	up
p53 signaling pathway	0.03	up

**Supplementary Table S3: Biological processes significantly enriched within down and up regulated probes after trabectedin treatment of PDX**

See Supplementary File 2

**Supplementary Table S4: Pathways enriched within down- and up-regulated genes (Kegg database) after trabectedin treatment of PDX**

Name	p-value	Expression upon trabectedin
Focal adhesion	0.001	down
ECM-receptor interaction	0.002	down
Complement and coagulation cascades	0.001	down
Systemic lupus erythematosus	0.004	down
Basal cell carcinoma	0.008	down
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0.03	down
Hedgehog signaling pathway	0.04	down
MAPK signaling pathway	0.02	up
Inositol phosphate metabolism	0.02	up
Complement and coagulation cascades	0.02	up

**Supplementary Table S5: Common deregulated genes between *in vitro* and *in vivo* models upon trabectedin treatment**

See Supplementary File 3

Supplementary Table S6: Differentially expressed microRNA upon trabectedin of MT-CHC01 cells

miRNA	logFC	Function	PMID
hsa-miR-27b-3p	-0.56	5-FU resistance	25356050
hsa-miR-28-3p	-0.56	Migration/invasion	22240480
hsa-miR-628-5p	-0.55	Sunitinib resistance	24475095
hsa-miR-21-3p	-0.53	BTC progression	24699315/ 23417858/ 22213236
hsa-miR-21-5p	-0.50	BTC progression	22213236
hsa-miR-210-3p	-0.47	Hypoxia	24517586
hsa-miR-331-3p	-0.44	proliferation	24825302
hsa-miR-31-3p	-0.44	BTC progression	22213236
hsa-miR-27a-3p	-0.42	Hypoxia	24517586
hsa-miR-200a-5p	-0.40	Tumor progression	25275448
hsa-miR-151a-3p	-0.40	No cancer related	
hsa-miR-29a-3p	-0.39	Marker for AML NCS Prognostic marker in NSCLC	21678057 24898736 24928469
hsa-miR-574-5p	0.39	Marker in ESCC Tumor suppressor in CRC metastasis	23124769 24936152
hsa-miR-3162-5p	0.39	SCC progression marker	23799609
hsa-miR-1915-3p	0.40	Apoptosis	24814047/ 22121083
hsa-miR-494-3p	0.43	Proliferation/invasion	24644030
hsa-miR-3656	0.46		
hsa-miR-3665	0.46		
hsa-miR-1268a	0.57		
hsa-miR-1207-5p	0.61	Tumor progression	24481448
hsa-miR-1225-5p	0.66	No cancer related	
hsa-miR-4284	0.67	Apoptosis	24732116
hsa-miR-1246	0.71	Cervical cancer progression	24806621
hsa-miR-4281	0.79	Melanoma marker	23111773

Supplementary Table S7: Primers sequences for qRT-PCR validation

Gene Name	Forward	Reverse
CDH2	CCAGTATCCGGTCCGATCTG	ATAGTCCTGCTCACCACCAC
WNT7B	GAAGGAGAAGTACAACGCGG	ACACCAGGTCTGTCTCCATG
PMEPA1	ACCATCTTCGACAGTGACCT	GATGACCTCGCTGTAGGTGG
NAV2	AGTTACGGCCTGAGGATGTC	GTTGCTGTTGGAGTCGCTG
ATF3	TCGGAGAAGCTGGAAAGTGT	TTCTGAGCCCGACAATACA
NOV	AGCTGTGGTATGGGGTTCTC	AGGTGGATGGCTTTGAGTGA
CD68	GCATCCCTTCGAGATCTCCA	GCAGGAGAACTTTGCCCAA
RASD1	GCCAAGAAGAACAGCAGCC	AGTACTGCACCGAGACCTTG
SYK	AGCAAAAGCGATGTCTGGAG	ATCTCTTTGGACACCCTGC
LGALS1	CTGTCTTCCCTTCCAGCCT	GCGGTTGGGGAACCTGAATT
PGK	AGCTGCTGGGTCTGTCATCCT	TGGCTCGGCTTTAACCTTGT