



Modification of the Histone Landscape with JAK Inhibition in Myeloproliferative Neoplasms

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Supplementary materials







Figure 1. (A) UKE-1, SET-2 and HEL cells incubated with doses of Ruxolitinib and cell viability assessed by CTG® after 24, 48 and 72 hours. The IC₅₀ was calculated using GraphPad Prism software. **(B)** UKE-1, SET-2 and HEL cells treated for 24 hours with doses of Ruxolitinib and protein extracted. Western blotting performed utilizing antibodies directed at the JAK/STAT pathway.



UKE-1 cell line treated with Ruxolitinib

Figure 2. Protein extracted from vehicle control or 100nM Ruxolitinib treated UKE-1 cells at 2, 4, 8 and 24 hours. Western blots performed to look for effects on Histone H3 with densitometry confirming an increase in mono- and tri-methylation at lysine 9 in treated cells. Acetylation levels were slightly reduced by 24 hours.



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Figure 3. Quantity of H3 modification as a percentage of total H3 present in UKE-1, SET-2 and HEL cell lines. Experiments repeated in duplicate for each cell line and treatment/control.



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24 hour Time (hour) 2 4 8 24 BD dose VC 100 VC 100 VC 100 VC 100 VC 100 Dose Ruxolitinib (nM) **BD** dose Ruxolitinib UKE-1 200-Actin SET-2 Actin HEL 0 Actin UKE-1 SET-2 HĖL + UKE-1 250 + SET-2 + HEL 50 0 ż 4 24 8 Treatment time (hour)

7 of 21



Figure 4. Western blots for H3K36me2, H3K36me3, H3ser28P, H3K4me1, H3K4me2 and H3K4me3 Histone H3 modifications in MPN cell lines (UKE-1, SET-2 and HEL) treated with vehicle control or 100nM Ruxolitinib for 2, 4, 8 or 24 hours (once or twice daily). Densitometry values are normalized for loading control.



Histone H3 validation, correlation of 2 techniques



Figure 5. (A) Pattern of Histone H3 modifications in MPN cell lines with varying duration of Ruxolitinib treatment. **(B)** Densitometry values from western blots correlated with histone array values for each histone H3 modification tested. A positive correlation is seen between the 2 techniques with an R² value of 0.74.

Patient number		1	:	2	1	3		4		5		6
Time point	TE	FU										
H3K36me1	1	-	-	8			-	-	-	-	-	9
Pan-actin	1	-	-	1	-	1	1	1	1	1	1	-
Patient number	,	7	8		9	9	1	10	1	1	1	2
Time point	TE	FU										
H3K36me1			-	-	=	-	*	-		-	-	*
Pan-actin	1		8	1	-	1	1	1		1	8	-
Patient number	1	3	1	4	1	.5	1	16	1	7	1	.8
Time point	TE	FU	ТЕ	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me1		-	8	1	8	-	8		1	-	-	-
Pan-actin	1	1		8		1	8		_	1	-	-

Patient number	1	9	2	20	2	21	2	22	2	3	2	4
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me1	-	-	100			-	-	-	-	-	-	-
Pan-actin			-			0	-	-		-		
Patient number	2	25	2	26		27	2	28	2	9	3	0
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me1	-	-	-	im	-	-	-	-	1	-	-	-
Pan-actin				-	-				8	9	-	-
Patient number	3	51	3	52	3	33	3	34	3	5	3	6
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me1	-	-		0					-		-	-
Pan-actin	-	8			1	8	-	-	-	-	-	-

Patient number	3	37	3	38	3	39		40	4	1	4	2
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me1		-	-			-		-				
Pan-actin	-	-		-		-					-	-
Patient number	4	13	4	44 TE FU		45		46	4	7	4	18
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me1	-	-	3	1	3	-	-	-	-	-	-	-
Pan-actin				8	8	8		-	-	8		
Patient number	4	19	5	50	:	51						
Time point	TE	FU	TE	FU	TE	FU	1					
H3K36me1	-			-	-	-]					
Pan-actin	-			-	-							

Patient number	3	7	3	8	3	39	4	10	4	1	4	2
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me1		-	-		-	*			-			1
Pan-actin	-	-		9		8		0			-	1
Patient number	4	3	4	44 TE EU		15	4	16	4	7	4	8
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me1	-	-	3	-	3	-	-	-	-	-	-	-
Pan-actin	8				8	8		-	-	8	8	
Patient number	4	9	5	50	5	51						
Time point	TE	FU	TE	FU	TE	FU						
H3K36me1	(regen			-	-	-						
Pan-actin	-			-	-	-						

Patient number	1	9	2	20	2	21		22	2	3	2	4
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me2		+=	•		-		12	-	the second	-	-	-
Pan-actin	1		_	0		0		-	-		-	
Patient number	2	25	2	26		27		28	2	9	3	0
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me2	-	-		-	-	-		-		-	•	-
Pan-actin	-	-			-			8	8	8	-	1
Patient number	3	51	3	32	3	33		34	3	5	3	6
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me2	-	-	-			-	-	-	-	-	-	-
Pan-actin	-		-		1			-			-	-

Patient number	3	57	3	38	3	39	4	40	4	1	4	2
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me2	-		-	-		-	-	-			-	-
Pan-actin		-	-		-	8	-			-		1
Patient number	4	13	44 TE EU		4	15	4	46	4	7	4	8
Time point	TE	FU	TE	TE FU		FU	TE	FU	TE	FU	TE	FU
H3K36me2	-	-	-	-	ŝ	*	-	Fritte	-	-	-	-
Pan-actin				8		9		-	1		8	(
Patient number	4	19	5	50	5	51						
Time point	TE	FU	TE	FU	TE	FU						
H3K36me2	-		1	-	6	-						
Pan-actin	-		-	1	0							

Patient number		1	:	2	3	3		4	:	5		6
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me3	-	-	×			-	-	-	-	-	-	I
Pan-actin	No. of Street,	-		8			1		1		1	1
Patient number		7 8 7 8		9	9	1	0	1	1	1	2	
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me3	ł.	-	-	-	-				-	-	-	1
Pan-actin	1	1	8	1	-	1	8	8	8	1	8	1
Patient number	1	3	1	4	1	5	1	6	1	7	1	.8
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me3	-	-		-		-			-	-		
Pan-actin	1	i		8	0	1		8		-		

Patient number	1	9	2	20	2	21	2	22	2	3	2	24
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me3	1	2	-			1	-	-	-	-	-	-
Pan-actin	-	-	-	6			-	-	1	-		-
Patient number	2	25	2	26		27	2	28	2	:9	3	60
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me3	-	-		1	- 24			Alas I			-	Jack .
Pan-actin			1		-			8			1	1
Patient number	3	51	3	32	3	33	3	34	3	5	3	6
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me3	-	-		9	-	4	and the second	-	-	-		-
Pan-actin	-	-	-	-			-	-	-	-	-	

Patient number	3	57	3	8	3	i9	4	40	4	1	4	2
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me3	-	-	-	-		-	-			-	-	-
Pan-actin	1	-			-	-	-	9			-	-
Patient number	4	13	4	44 TE FU		15	4	46	4	7	4	8
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K36me3	-		-	-	1	-	-	-	-	-	-	-
Pan-actin	-	8	-			-	-		-	-	8	0
Patient number	4	19	5	50	5	51						
Time point	TE	FU	TE	FU	TE	FU						
H3K36me3	-	-	-	1	-	-						
Pan-actin	-		-	-								

Patient number		1	1	2	1	3		4	:	5		6
Time point	TE	FU										
H3K4me1		-		-	-	-	-			-	-	
Pan-actin		-	-	-	1	1	1	1	-	1	-	1
Patient number		7	1	8		9	1	0	1	1	1	2
Time point	TE	FU										
H3K4me1	-		-	-	-	-		-	-	-	-	
Pan-actin	I	I	-	١		1	1	-		-	8	١
Patient number	1	3	1	4	1	.5	1	6	1	7	1	8
Time point	TE	FU										
H3K4me1	-		-	-		-					-	-
Pan-actin	1	I	8	8		1	9		_	-	1	1

Patient number	1	9	20 TE FU 26 TE FU		2	21	:	22	2	23	2	4
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me1	-	100	-		:30	100	-		-	-	-	3
Pan-actin				26		0	-		-	-	-	8
Patient number	2	25	2	26		27	:	28	2	:9	3	0
Time point	TE	FU	TE	TE FU		FU	TE	FU	TE	FU	TE	FU
H3K4me1	-	-	-	-	12		-	-				-
Pan-actin	-	-	-	-		-	-	-	-	-	1	1
Patient number	3	51	3	32	3	33		34	3	5	3	6
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me1	-		-		-			1	-	-	-	-
Pan-actin	-	-	-	-	-	-	-		1			

Patient number	3	57	3	8	3	39	4	40	4	1	4	2
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me1		-				-	-	-	-			
Pan-actin	0		-	-	-			-	-	1	-	-
Patient number	4	13	44 TE FU		4	15	4	16	4	7	4	8
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me1	-			-		-	-	-	-	-	-	-
Pan-actin	-	0	-	8		8	-					
Patient number	4	19	5	50	5	51						
Time point	TE	FU	TE	FU	TE	FU						
H3K4me1	-			-	-	-						
Pan-actin	-		138									

Patient number	1 2		:	3		4	:	5	6			
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me2	-	-	-	-	-	-	-	und .		-	-	
Pan-actin	-	-	-		-		-	-	-	-	-	-
Patient number	,	7	8	8	9	9	1	.0	1	1	1	2
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me2	-	-	-		-	-	-	page 1	1	100	-	-
Pan-actin	-	1		-	-	403	-			1		-
Patient number	1	3	1	4	1	5	1	6	1	7	1	.8
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me2		-	-			The second		-	-	-	1	-
Pan-actin		-	-	-		N.	<		_	-	-	-

Patient number	19		20		2	21	1	22	2	23	2	4
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me2	-	-	-		-	-	-			-	-	-
Pan-actin	1				0		-	-	-	-	-	-
Patient number	2	5	2	26	2	27		28	2	:9	3	0
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me2	-	-	-	-	(COR)		-	-		-	-	Annes.
Pan-actin	1		-	1	-			Į	8	0	1	-
Patient number	3	1	3	32	3	33	:	34	3	35	3	6
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me2		-	-	-	12	-	1000	-	1212		-	
Pan-actin	8	8	-	-	-			1	-	-	-	-

Patient number	3	37 3		38 39		4	40	4	1	42		
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me2				-	-	=	-	-	-	-		
Pan-actin		-	1			-	8	8				-
Patient number	4	3	4	4	4	45	4	46	4	7	4	8
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me2							-	-		-	-	-
Pan-actin	-							-	-	-	-	
Patient number	4	9	5	50	5	51						
Time point	TE	FU	TE	FU	TE	FU	1					
H3K4me2	-	-	-	-	-	-]					
Pan-actin			-		-							

Patient number		1	:	2	3	3		4	:	5		6
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me3	-	-				-	-	-	-	-	-	-
Pan-actin	-	-	1	-	-	-	-	-	-	1	-	-
Patient number	7		:	8	9	9	1	0	1	1	12	
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me3	-	-	-		-	form.	201.		100	1		
Pan-actin		1	-	-		danie i	-		-	1	6	and the second
Patient number	1	3	1	4	15		16		17		18	
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me3	-	-	-			-		-		1		-
Pan-actin		-		I		1			0			-

Patient number	1	9	2	20		21	:	22	2	23	24	
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me3	-	-	-		-	-	-	0	-	-	-	-
Pan-actin	-	-	-	-	-	-	-	-	-		-	
Patient number	2	25	2	:6	2	27	:	28	2	9	3	0
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me3	-	-	-	100	*** 3			-		-	-	
Pan-actin					-	-	-	-		-	-	-
Patient number	3	51	3	52	3	33		34	3	85	3	6
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me3	-	-	-	-	-	-		-	-	-		- Andrew
Pan-actin	-	-	-	-	-			-	-		-	-

Patient number	3	37 38		3	9	4	10	4	1	4	2	
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me3	-	-	-		-	11			-	0		
Pan-actin			-		-		9		8		-	
Patient number	4	3	4	4	4	5	4	16	4	7	4	8
Time point	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU	TE	FU
H3K4me3		-	-	-	-	-	-	-	-	-	-	-
Pan-actin	-	8		8	-			-	-	-		0.
Patient number	4	9	5	50	5	51						
Time point	TE	FU	TE	FU	TE	FU	1					
H3K4me3	-	-			*	-]					
Pan-actin	-			-	0							

Figure 6. Western blots for mono, di and tri-methylation at lysine 36 and lysine 4 on histone H3 and corresponding pan-actin for all 51 MAJIC patients examined.









Figure S7: For each patient the normalized densitometry values before and after therapy for each histone mark examine



Figure 8. CHIP-seq analysis pipeline.





Table 1. Histone arrays, rank order of *p* values.

Histone modification	UKI	E-1	SET	ſ -2	HE	L	All cell	lines	UKE-1 +	- SET-2	UKE-1	+ HEL	SET-2 +	- HEL	Sum of Ranks	Overall rank
	p value	Rank	<i>p</i> value	Rank	p value	Rank	p value	Rank	<i>p</i> value	Rank	<i>p</i> value	Rank	p value	Rank		
H3K4me1	0.148	5	0.026	1	0.263	9	0.745	21	0.328	17	0.468	20	0.021	1	74	12
H3K4me2	0.166	7	0.498	11	0.443	14	0.057	3	0.106	5	0.072	3	0.231	7	50	3
H3K4me3	0.326	17	0.032	2	0.418	12	0.106	4	0.183	14	0.154	8	0.117	3	60	5
H3K9me1	0.230	13	0.671	16	0.954	20	0.139	9	0.154	11	0.162	10	0.644	15	94	17
H3K9me2	0.531	20	0.975	21	0.966	21	0.393	19	0.421	19	0.421	19	0.944	21	140	21
H3K9me3	0.136	4	0.629	13	0.284	10	0.198	13	0.095	4	0.238	13	0.314	11	68	6
H3K27me1	0.447	19	0.613	12	0.461	15	0.342	17	0.441	20	0.275	16	0.939	20	119	20
H3K27me2	0.317	16	0.466	8	0.119	2	0.355	18	0.331	18	0.271	15	0.168	5	82	15
H3K27me3	0.040	1	0.687	18	0.560	16	0.046	2	0.053	1	0.056	2	0.417	12	52	4
H3K36me1	0.092	3	0.637	14	0.160	4	0.123	7	0.079	2	0.145	7	0.243	8	45	2
H3K36me2	0.084	2	0.671	17	0.016	1	0.029	1	0.095	3	0.020	1	0.124	4	29	1
H3K36me3	0.167	8	0.825	20	0.320	11	0.145	10	0.127	7	0.157	9	0.212	6	71	10
H3K79me1	0.150	6	0.472	9	0.423	13	0.130	8	0.194	15	0.078	4	0.883	19	74	13
H3K79me2	0.354	18	0.667	15	0.716	17	0.231	15	0.224	16	0.254	14	0.600	14	109	19
H3K79me3	0.204	10	0.491	10	0.806	18	0.123	6	0.139	9	0.142	6	0.477	13	72	11
H3K9ac	0.218	11	0.323	6	0.193	6	0.147	11	0.134	8	0.185	11	0.855	17	70	7
H3K14ac		21	0.352	7	0.135	3	0.577	20	0.920	21	0.676	21	0.044	2	95	18
H3K18ac	0.171	9	0.298	5	0.218	7	0.240	16	0.106	6	0.295	17	0.276	10	70	8
H3K56ac	0.266	14	0.202	4	0.845	19	0.166	12	0.168	13	0.204	12	0.876	18	92	16
H3ser10P	0.283	15	0.200	3	0.170	5	0.208	14	0.146	10	0.296	18	0.774	16	81	14
H3ser28P	0.220	12	0.783	19	0.252	8	0.122	5	0.159	12	0.132	5	0.246	9	70	9

Table 2. STRING pathway analysis of downregulated genes following ruxolitinib administration to HEL cells.(2).

Term Description	observed gene count	background gene count	false discovery rate
PI3K-Akt signaling pathway	13	348	4.00E-05
Transcriptional misregulation in cancer	9	169	9.92E-05
Jak-STAT signaling pathway	8	160	0.00041
Cytokine-cytokine receptor interaction	9	263	0.0013
Focal adhesion	8	197	0.0013
Inflammatory bowel disease (IBD)	5	62	0.0014
Pathways in cancer	12	515	0.0017
HTLV-I infection	8	250	0.0033
Hippo signaling pathway	6	152	0.0068

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2 of 21

Th17 cell differentiation	5	102	0.0068
AGE-RAGE signaling pathway in diabetic complications	5	98	0.0068
TNF signaling pathway	5	108	0.008
Human papillomavirus infection	8	317	0.0094
Proteoglycans in cancer	6	195	0.0154
Measles	5	133	0.0158
Wnt signaling pathway	5	143	0.0174
ECM-receptor interaction	4	81	0.0174
Cell adhesion molecules (CAMs)	5	139	0.0174
Primary immunodeficiency	3	37	0.0174
MAPK signaling pathway	7	293	0.0178
MicroRNAs in cancer	5	149	0.0178
Breast cancer	5	147	0.0178
Hematopoietic cell lineage	4	94	0.0204
Malaria	3	47	0.0229
Insulin resistance	4	107	0.0293
Viral myocarditis	3	56	0.0338
Platelet activation	4	123	0.0435