Ruxolitinib in combination with prednisone and nilotinib exhibit synergistic effects in human cells lines and primary cells from myeloproliferative neoplasms

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ID	Dx	JAK2	Age Dx	Sex	Treatment	Previous treatments
P02	PV-MF	V617F	83	Male	Ruxolitinib	Hydrea
P03	TE-MF	wt	73	Male	Ruxolitinib	Hydrea, melfalan
P05	TE-MF	wt	70	Male	Anagrelide	
P08	PV-MF	V617F	80	Female	Ruxolitinib	
P09	PV-MF	V617F	49	Female	Hydrea+INF	Ruxolitinib
P24	MFP	V617F	58	Female	Ruxo+BKM120	Ruxolitinib, hydrea
P25	MFP	V617F	83	Male	Hydrea+EPO	
P26	TE-MF	wt	72	Male	Hydrea	
P31	MFP	V617F	61	Female	Hydrea	

Supplementary table 1: Description of the patients used in the drug screening assay. ID: identification code; Dx: diagnosis.

ID	Dx	JAK2	Age Dx	Sex
P03	ET-MF	wt	73	Male
P05	ET-MF	wt	71	Male
P06	PMF	wt	70	Male
P07	PMF	V617F	54	Male
P08	PV-MF	V617F	80	Female
P09	PV-MF	V617F	49	Female
P11	PV-MF	V617F	71	Male
P14	PMF	V617F	62	Male
P19	PV-MF	V617F	66	Female
P20	PMF	wt	46	Male
P24	PMF	V617F	59	Female
P26	ET-MF	wt	72	Male
P27	PMF	V617F	75	Male
P29	PMF	wt	63	Male
P33	PMF	V617F	78	Female
P34	PMF	V617F	83	Male

Supplementary table 2: Description of the patients used in the dose response curves (all) and synergy analysis (grease) of ruxolitinib, nilotinib and prednisolone. ID: identification code; Dx: diagnosis.

Supplementary Table 3: Rationale of drugs tested in screening: We tested several drugs related to signaling pathways and the fibrosis process, and other drugs used in the treatment of myeloproliferative neoplasm.

Drug	
Nilotinib	It is used to treat chronic myeloid leukemia (CML), has been shown to efficiently eliminate CD34+ cells in combination with ruxolitinib (1) and can decrease the expression of collagen I (2), two important characteristics for decreasing pathological cells and achieving histological response in myelofibrosis.
Midostaurin	It has been shown that FLT3 has a role in the dysregulation of megakaryopoiesis in an inflammatory context (3), a process related to myelofibrosis physiopathology. For this reason, it is interesting to study the combination of a FLT3 inhibitor, such as midostaurin, with ruxolitinib, a JAK2 inhibitor.
Bosutinib	It is a member of the so-called "second-generation" BCR-ABL inhibitors for CML; it is also an SRC inhibitor. Interestingly, a gain-of-function mutation of SRC causes myelofibrosis (4).
Sorafenib	In a previous work, it showed a strong synergy with ruxolitinib in chronic myeloproliferative neoplasm (5).
Buparlisib dactolisib everolimus	PI3K signaling is important in both normal and PV erythroid differentiation (6), so it seems interesting to introduce PI3K inhibitors such as buparlisib and dactolisib. Furthermore, several kinases from PI3K signaling are constitutively phosphorylated in NMPc (6).
LCL161	It is an SMAC mimetic, increases apoptosis induced by chemotherapy in a TNF-α-dependent manner (8), so it is a good candidate for a combination therapy. Moreover, interestingly, myelofibrosis is characterized by increasing levels of inflammatory cytokines, such as TNF-α among others (9).
Bortezomib	It is a proteasome inhibitor, inhibits the degradation of proteins related to cell cycle regulation inducing cell death. Moreover, it blocks the degradation of IkB among others, preventing the action of NFkβ, which induced the expression of TGF-β1 (10), implicated in bone marrow fibrosis in myelofibrosis.
Danazol	It is well known that **danazol** achieves an anemic response in myelofibrosis (11) patients and could counteract the hematologic toxicity of ruxolitinib.
Prednisone	**Prednisone** is used to improve anemia and thrombocytopenia in myelofibrosis (12) and, interestingly, it has been shown that prednisone can decrease levels of TGF-β and prevent collagen accumulation (13)
Anagrelide	**It** is used to treat thrombocytopenia in myeloproliferative neoplasms, but, the most interesting thing is that it can prevent maturation of megakaryocytes (14), one of those responsible for the increase of TGF-β levels, which produces fibrosis in bone marrow in myelofibrosis (15).

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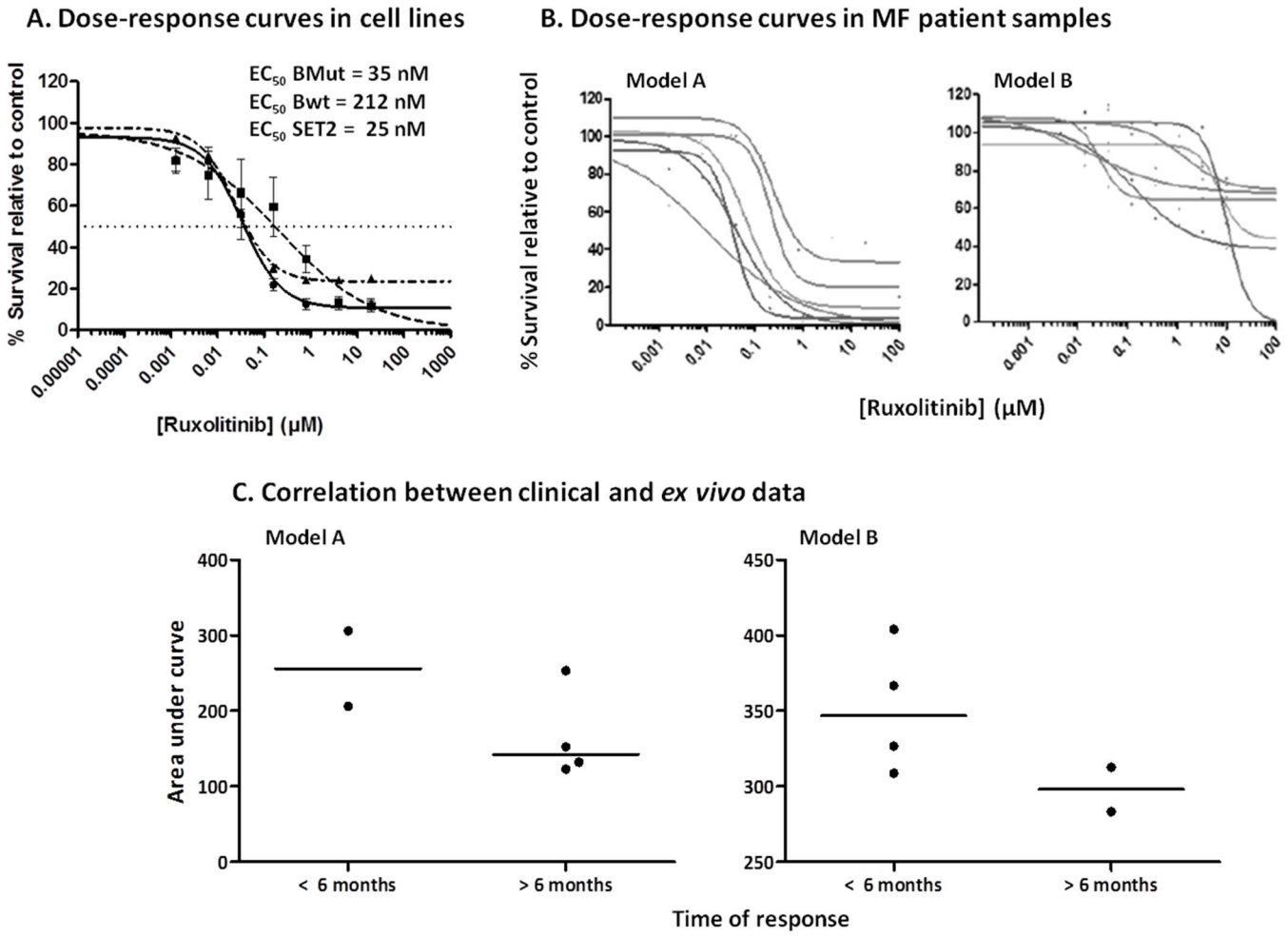
	E_{max} (% SUPERVIV.)	EC_{50} (µM)	AUC
MODEL A	9.3 (1.9-20.7)	0.043 (0.023-0.140)	256.6 (237.6-323.0)
MODEL B	54.3 (39.8-67.2)	0.747 (0.054-5.852)	446.2 (430.2-484.6)

Supplementary Table 4: Ruxolitinib activity in ex vivo model A and B: Median (interquartilic range) of E_{max}, EC_{50} and area under curve (AUC).

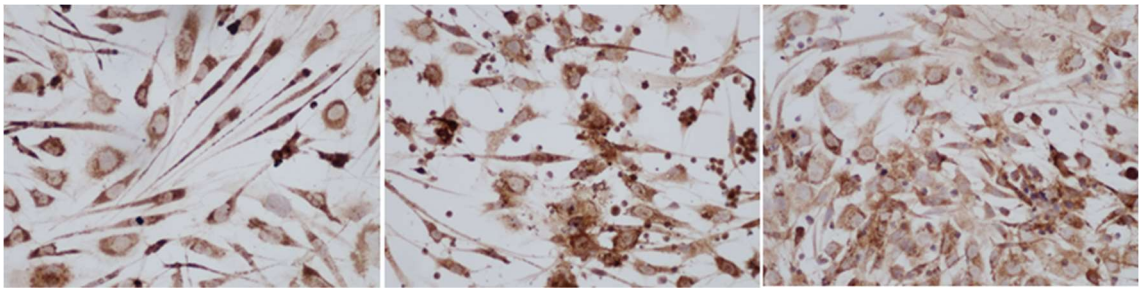
BA/F3 JAK2 V617F	EC_{50} (µM)		Emax (% Survival)	
Drugs	**Mean**	**SD**	**Mean**	**SD**
Panobinostat	0.041	0.001	0.0	0.0
Bortezomib	0.041	0.001	0.0	0.0
HSP990	0.045	0.008	4.0	5.2
BEZ235	0.153	0.154	23.7	8.1
Midostaurin	0.374	0.026	0.0	0.0
LCL161	0.934	0.952	0.0	0.0
BKM120	3.331	1.409	9.5	13.4
Ponatinib	5.530	0.860	0.0	0.0
Bosutinib	9.192	4.190	0.0	0.0
Sorafenib	12.473	1.201	0.0	0.0
Perifosine	14.718	4.170	0.0	0.0
Everolimus	23.131	9.058	0.0	0.0
Nilotinib	40.779	8.733	44.0	41.9
SB431542	94.353	17.511	0.0	0.0
Prednisolone	0.000		100.0	
Danazol	0.000		100.0	
Pomalidomide	0.000		100.0	

Supplementary table 5: Results of the dose-response curves of drugs in monotherapy in cell lines after 24h of incubation with drugs. SD: Standard deviation.



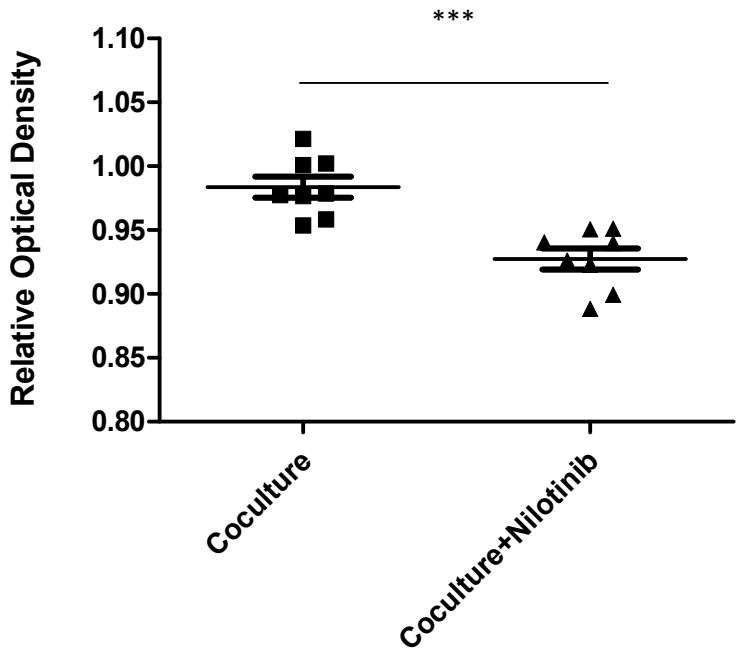
Supplementary Figure 1: Ruxolitinib activity. A) Dose-response curves of ruxolitinib in cell lines. B) Dose-response curves of ruxolitinib using patient samples and two ex vivo models. C) Representation of area under curve of dose response curves of ruxolitinib in ex vivo model A or B depending on time of response of patients treated with ruxilitinib. Bmut: BA/F3 JAK2 V617F; Bwt: BA/F3 wild type.

A.



Control Coculture Coculture+Nilotinib

B.



Supplementary Figure 2. Collagen production. (A) Representative images (20 X magnification) of HS27a cells (Control), HS27a-SET2 cocultures (Coculture) and nilotinib treated HS27a-SET2 cocultures (Coculture + Nilotinib) stained with anti-Collagen-I. (B) Relative optical density quantified by Image J and normalized to control. Values given are the means ± sem of 8-9 randomly chosen fields. Data are representative of two independent experiments. ***P<0.001.