

## Supplementary Materials

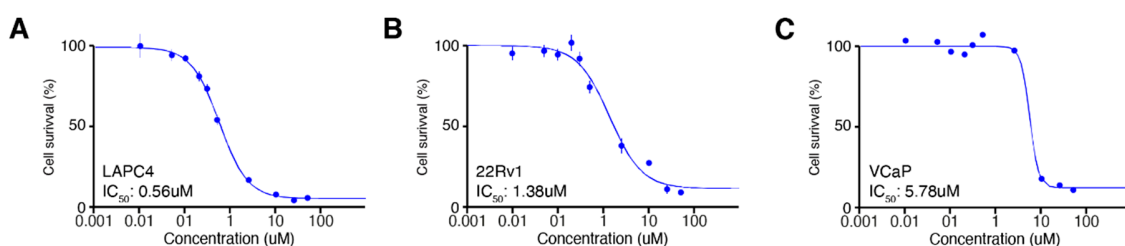
# An Aminosteroid Derivative Shows Higher In Vitro and In Vivo Potencies than Gold Standard Drugs in Androgen-Dependent Prostate Cancer Models

Donald Poirier,<sup>1,2,\*</sup> Jenny Roy,<sup>1</sup> René Maltais,<sup>1</sup> Cindy Weidmann,<sup>1</sup> Étienne Audet-Walsh<sup>1,2</sup>

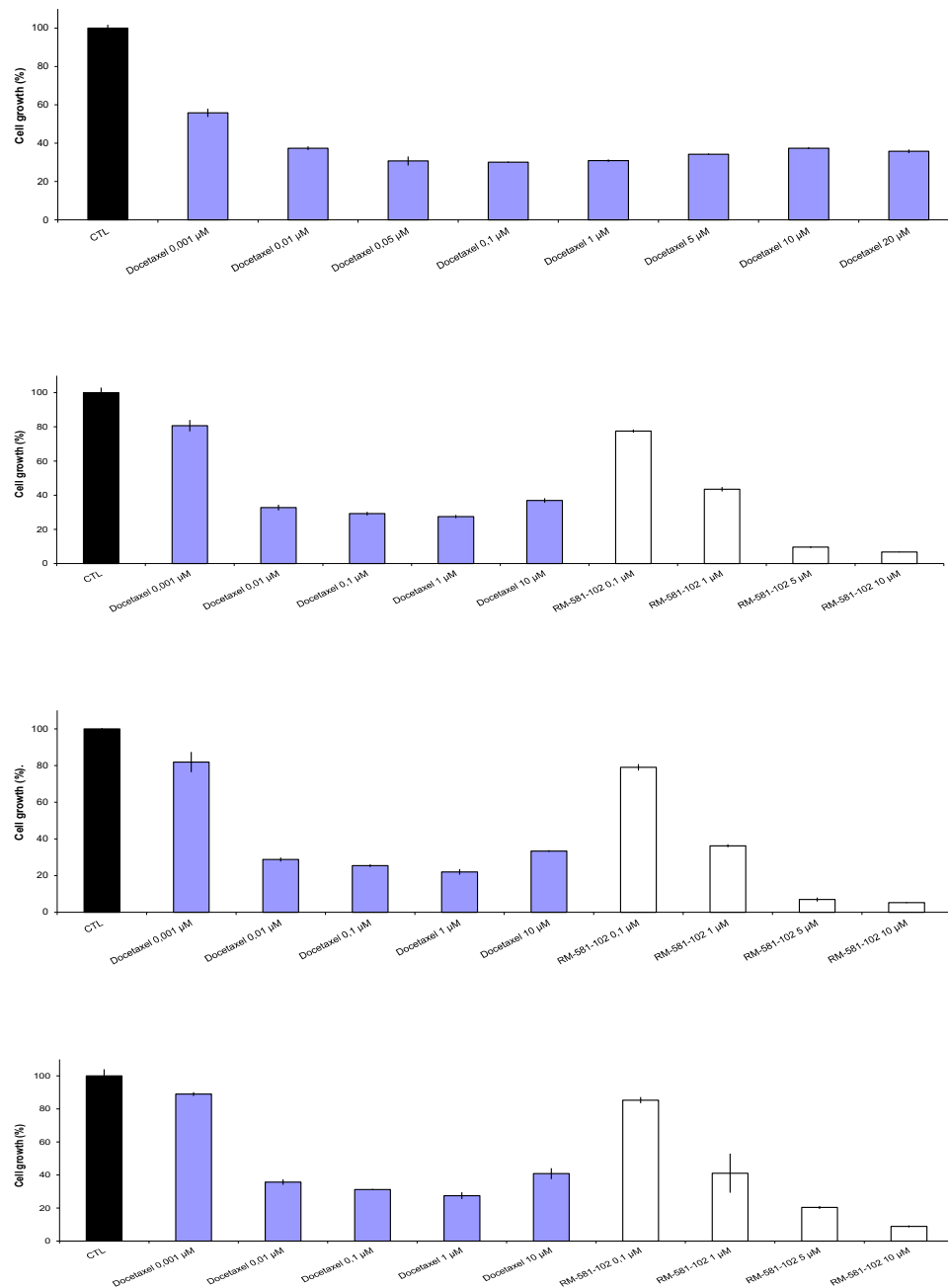
<sup>1</sup>Endocrinology and Nephrology Unit, CHU de Québec Research Center-Université Laval, Pavillon CHUL, Québec, QC, G1V 4G2, Canada

<sup>2</sup>Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec, QC, G1V 0A6, Canada

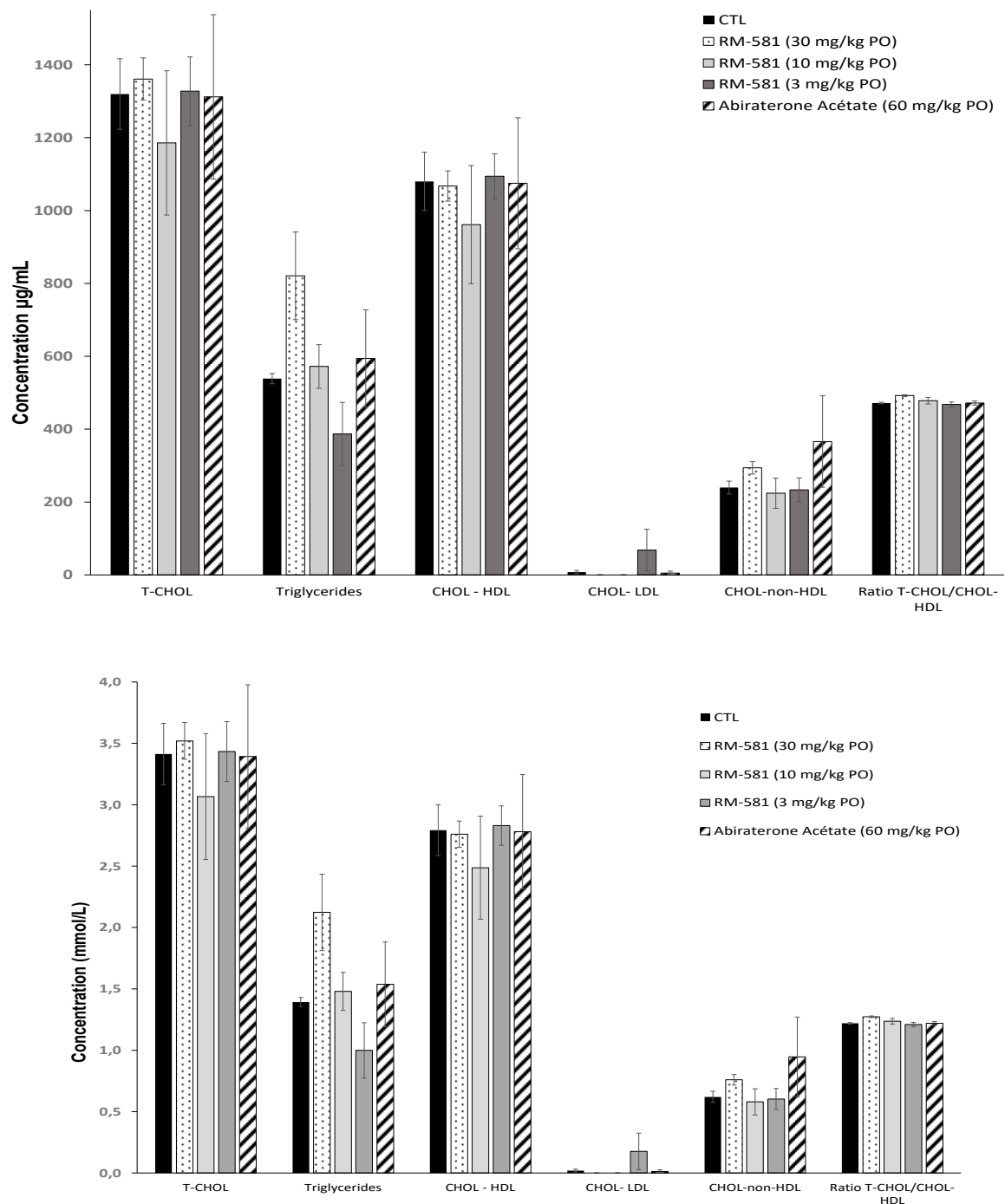
**Content:** Figure S1, Figure S2, Figure S3, Table S1, Table S2 and Table S3



**Figure S1.** Efficacy of RM-581 in reducing the LAPC-4 (A), 22Rv1 (B) and VCaP (C) cell proliferation after 3 days of treatment (LAPC-4 and 22Rv1) or 7 days of treatment (VCaP). Results from one representative experiment out of 2-4 independent experiments performed with 8 replicates per conditions are shown. Concentration is shown in log<sub>10</sub> scale.



**Figure S2.** Efficacy of docetaxel and RM-581 in reducing LAPC-4 cell proliferation after 6 days of treatment. **A)** Standard cell culture protocol with change of medium, **B)** with an initial washing of the cells, **C)** with an initial washing of the cells and without change of medium, and **D)** without an initial washing of the cells and with change of medium. For docetaxel, results are the mean of 4 experiments performed in triplicate. For docetaxel,  $IC_{50}$  value was not calculated. For RM-581, results are the mean of 3 experiments performed in triplicate with  $IC_{50} = 0.51, 0.47$  and  $0.73 \mu M$ . Mean  $IC_{50} = 0.57 \pm 0.14 \mu M$ .



**Figure S3.** Concentrations in µg/mL (up) or mmol/L (down) of total cholesterol, triglycerides, cholesterol-HDL, cholesterol-LDL, cholesterol non-HDL and ratio of total cholesterol/cholesterol-HDL obtained from LAPC-4 xenograft experiments with RM-581 (3, 10 and 30 mg/kg/PO) and abiraterone acetate (60 mg/kg/PO). Method B (Medical Laboratory of CHU de Québec/L'Enfant Jésus Hospital).

**Table S1.** Content of 56 fatty acids detected in tumors treated with RM-581 (10 mg/kg) or not (CTL) from LAPC-4 xenograft experiment in mice.

<b>Fatty Acid (FA) Content<sup>1</sup></b>	<b>Series</b>	<b>FA (double bond)</b>	<b>FA RM-581 % of total</b>	<b>FA CTL (mg/g)</b>	<b>FA RM-581 (mg/g)</b>	<b>%<sup>2</sup></b>
<b><i>More abundant FA (&gt; 0.5%)</i></b>						
Palmitic acid	-	16:0	20.8	4.395	5.095	+ <b>16</b>
Oleic acid (9c)	w9	18:1	20.1	3.665	4.920	+ <b>34</b>
Linoleic acid (9c12c)	w6	18:2	18.1	2.840	4.435	+ <b>56</b>
Stearic acid	-	18:0	9.61	2.275	2.355	+ 3.5
Arachidonic (5c8c11c14c)	w6	20:4	8.77	2.195	2.150	- 2.1
Palmitoleic acid (9c)	w7	16:1	4.23	0.680	1.035	+ <b>52</b>
Vaccenic acid (11c)	w7	18:1	2.82	0.655	0.690	+ 5.3
Dihomo- $\gamma$ -linolenic acid (8c11c14c)	w6	20:3	2.35	0.520	0.580	+ 12
Cervonic acid (4c7c10c13c16c19c)	w3	22:6	2.18	0.530	0.530	0
Adrenic acid-1 (7c10c13c16c)	w6	22:4	1.54	0.350	0.380	+ 8.6
Myristic acid	-	14:0	1.12	0.220	0.275	+ <b>25</b>
Adrenic acid-2 (4c7c10c13c16c)	w6	22:5	1.07	0.295	0.260	- 12
Alpha-linolenic acid (9c12c15c)	w3	18:3	0.94	0.140	0.230	+ <b>64</b>
Dimethoxyhexadecanoic acid	-	16:0	0.68	0.170	0.170	0
Lignoceric acid	-	24:0	0.65	0.130	0.160	+ 23
Docosapentaenoic acid(7c10c13c16c19c)	w3	22:5	0.57	0.120	0.140	+ 17
Nervonic acid (15c)	w9	24:1	0.52	0.110	0.130	+ 18
<b><i>Less abundant FA (&lt; 0.5%)</i></b>						
Caprylic acid	--	8:0	0	0	0	--
Caproic acid	--	10:0	0.02	0	0	--
Lauric acid	--	12:0	0.10	0.02	0.02	--
-- (9t)	w5	14:1	0.03	0.01	0.01	--
Myristoleic acid (9c)	w5	14:1	0.06	0.01	0.02	--
Pentadecanoic acid	--	15:0	0.17	0.03	0.04	--
-- (5t)	w10	15:1	0	0	0	--
-- (5c)	w10	15:1	0	0	0	--
-- (1c)	w14	15:1	0	0	0	--
Palmitelaidic acid (9t)	w1	16:1	0	0	0	--
Isobranched	--	17:0	0.08	0.02	0.02	--
Dimethoxyoctadecanoic acid	--	18:0	0.38	0.075	0.09	--
-- (7t)	w10	17:1	0.09	0.02	0.02	--
Dimethoxyoctadecenoic acid (9c)	w9	18:1	0.29	0.055	0.07	--
-- (7c)	w10	17:1	0.20	0	0.05	--
Petroselaidic acid (6t)	w12	18:1	0	0	0	--
Elaidic acid (9t)	w9	18:1	0	0	0	--

Trans-vaccenic acid (11t)	w7	18:1	0.13	0.03	0.03	--
Petroselinic acid (7c-n11/6c-n12)	w11/12	18:1	0	0	0	--
-- (12c)	w6	18:1	0	0	0	--
-- (13c)	w5	18:1	0.07	0.02	0.02	--
Linolelaidic acid (9t12t)	w6	18:2	0.14	0.03	0.03	--
-- (9c12t)	w6	18:2	0.09	0.02	0.02	--
-- (9t12c)	w6	18:2	0	0	0	--
-- (9t12t15t)	w3	18:3	0.03	0.01	0.01	--
Arachidic acid	--	20:0	0.20	0.04	0.05	--
Gamma-linolenic acid (6c9c12c)	w6	18:3	0.19	0.04	0.05	--
-- (8c)	w12	20:1	0	0	0	--
Gondoic acid (11c)	w9	20:1	0.32	0.05	0.08	--
Stearidonic acid (6c9c12c15c)	w3	18:4	0	0	0	--
-- (11c14c)	w6	20:2	0.34	0.06	0.08	--
Behenic acid	--	22:0	0.35	0.07	0.09	--
-- (9t)	w13	22:1	0	0	0	--
-- (11c14c17c)	w3	20:3	0	0	0	--
Erucic acid	w9	22:1	0.10	0.02	0.025	--
-- (8c11c14c17c)	w3	20:4	0.16	0.015	0.04	--
-- (13c16c)	w6	22:2	0.04	0.01	0.01	--
Timnodonic acid (5c8c11c14c17c)	w3	20:5	0.10	0.02	0.025	--
-- (13c16c19c)	w3	22:3	0	0	0	--

<sup>1</sup> RM-581 (10 mg/kg) was administrated orally (gavage) in mice (28 days of treatment). Control (CTL) mice received only the vehicle (PG:DMSO/92:8). <sup>2</sup> Calculated only for the 17 most abundant fatty acids (> 0.5%) whose concentration was measured by GC-FDI.

**Table S2.** Total cholesterol concentration in LAPC-4 cells treated 18, 36 and 72 h with the aminosteroid RM-581 (2  $\mu$ M) or atorvastatin (10  $\mu$ M).

Time (h)	Control	RM-581 (2 $\mu$ M)	Atorvastatin (10 $\mu$ M)
18	65 $\pm$ 0.7	68 $\pm$ 3	59 $\pm$ 3.5
36	62 $\pm$ 1	69 $\pm$ 6	61 $\pm$ 0.4
72	66 $\pm$ 2	69 $\pm$ 4	70 $\pm$ 1
72	108 $\pm$ 6.5	--	50 $\pm$ 2

**Table S3.** Primer sequences for different genes

<b>Gene</b>	<b>Name</b>	<b>Primer sequences</b>
<i>ACLY</i>	ACLY_F	CGGACTTCGGCAGAGGTTAGA
	ACLY_R	GTTGACCCCAACGAGACCAA
<i>HMGCS1</i>	HMGCS1_F	GTCACGCTTGTGCCCCGAA
	HMGCS1_R	GCCGCCCAATGCAATCATAG
<i>HMGCR</i>	HMGCR_F	TTCGGTGGCCTCTAGTGAGA
	HMGCR_R	AAAGCTTCATTCAAGCCTGTCA
<i>MVK</i>	MVK_F	GAGCCATGTTGTCAGAAGTCC
	MVK_R	GTGACATCACCTTGCTCCAGAA
<i>MVD</i>	MVD_F	TCATCAAGTACTGGGGCAAGC
	MVD_R	GGTTTTTAACTGGTCCTGGTGC
<i>ACAT1</i>	ACAT1_F	GGAGGCTGGTGCAGGAAATA
	ACAT1_R	TCCTGATGTCCACACATAAGACT
<i>FASN</i>	FASN_F	ATGGAGGAGGTGGTGATTGC
	FASN_R	CTGGGCCCTCTGAAGTCGAA
<i>SCD1</i>	SCD1_F	AACAGCAGGAGCTCATCGTC
	SCD1_R	AAGTTGATGTGCCAGCGGTA
<i>LDLR</i>	LDLR_F	GAGGGCTCTGTCCATTGTCC
	LDLR_R	ACCATCTGTCTCGAGGGGTA