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Supplementary Materials for

High-capacity poly(2-oxazoline) formulation of TLR 7/8 agonist extends survival in a chemo-insensitive, metastatic model of lung adenocarcinoma

Natasha Vinod, Duhyeong Hwang, Salma H. Azam, Amanda E. D. Van Swearingen, Elizabeth Wayne, Sloane Christian Fussell, Marina Sokolsky-Papkov, Chad V. Pecot*, Alexander V. Kabanov*

*Corresponding author. Email: pecot@email.unc.edu (C.V.P.); kabanov@email.unc.edu (A.V.K.)

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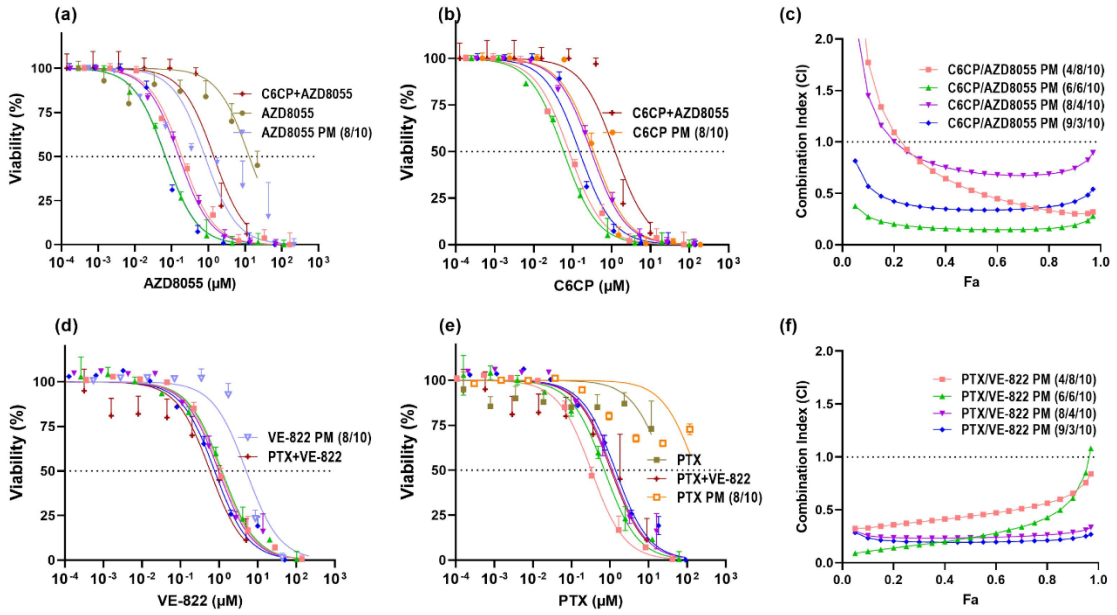
Table S1 | Characterization of POx micelles coloaded with anti-cancer agent and chemosensitizers

mTOR kinase inhibitor (AZD8055) and anticancer agent (C_6CP)							
Feeding ratio (g/L)	LE (%)		LC (%)			Drug concentration in solution (g/L)	
C_6CP/AZD8055/POx	C_6CP	AZD8055	C_6CP	AZD8055	Tot	C_6CP	AZD8055
8/0/10	70.6	-	36.1	-	-	5.7	-
0/8/10	-	43.4	-	25.8	-	-	3.5
4/8/10	22.0	39.13	6.28	22.3	28.6	0.9	3.1
6/6/10	83.2	91.2	24.38	26.7	51.1	5.0	5.5
8/4/10	85.3	91.0	33.33	17.8	51.1	6.8	3.7
9/3/10	80.2	102.0	35.6	15.1	50.7	7.2	3.1
ATR inhibitor (VE-822) and anticancer agent (PTX)							
Feeding ratio (g/L)	LE (%)		LC (%)			Drug concentration in solution (g/L)	
PTX/VE-822/POx	PTX	VE-822	PTX	VE-822	Tot	PTX	VE-822
8/0/10	86.9	-	41.0	-	-	7.0	-
0/8/10	-	83.3	-	40.0	-	-	6.7
4/8/10	83.8	77.8	17.1	31.9	48.9	3.4	6.2
6/6/10	102.0	91.5	28.4	25.4	53.7	6.1	5.5
8/4/10	97.0	89.3	36.4	16.7	53.1	7.8	3.6
9/3/10	95.2	86.7	40.5	12.3	52.8	8.6	2.6



(g)

Drug	C6CP IC50 (μM)	AZD8055 IC50 (μM)	C6CP PM IC50 (μM)	AZD8055 PM IC50 (μM)	IC50 (μM) C6CP/AZD8055 PM 4/8/10	6/6/10	8/4/10	9/3/10	IC50 (μM) C6CP + AZD8055(4+8)
AZD8055	-	12.99	-	0.81	0.19	0.07	0.16	0.07	1.41
C6CP		-	0.27	-	0.08	0.06	0.30	0.15	1.224
	PTX IC50 (μM)	**VE-822 IC50 (μM)**	**PTX PM IC50 (μM)**	**VE-822 PM IC50 (μM)**	**IC50 (μM) PTX/VE-822 PM 4/8/10**	**6/6/10**	**8/4/10**	**9/3/10**	**IC50 (μM) PTX + VE-822 (6+6)**
VE-822	-	5	-	3.05	1.09	1.18	0.97	0.77	0.58
PTX	27.2	-	168.7	-	0.32	0.7	1.15	1.38	1.06

Fig. S1. ***In vitro*** **cytotoxicity of anticancer agent and chemosensitizers in 344SQ lung adenocarcinoma cell line** **(a,b,d,e)** Dose-response curves of free and micelle incorporated drugs and drug combinations in 344SQ cell line after 72h of treatment. Cell viability as a function of individual drug concentrations after treatment with a combination of the drugs AZD8055 and C6CP (**a** and **b**) and VE-822 and PTX (**d** and **e**). The data was fit into sigmodal curve using non-linear regression. Data represent mean ± CV. n=6. **(c, f)** Fa-CI plots of the C_6CP/AZD8055 and PTX/ VE-822 combinations. Data represent mean. n = 6. **(g)** Comparison of the IC_{50} values of POx formulations and free drugs in 344SQ cell line.

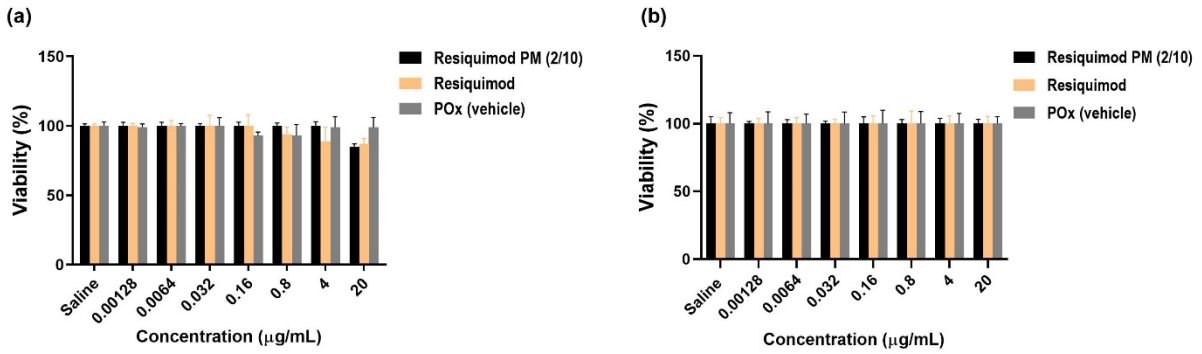


Fig. S2. *In vitro* cytotoxicity of Resiquimod Cell viability of **(a)** 344SQ following 24h treatment with Resiquimod PM, free Resiquimod and POx (vehicle). **(b)** BMDM following 24h treatment with Resiquimod PM, free Resiquimod and POx (vehicle). Data represent mean ± CV. n = 6.

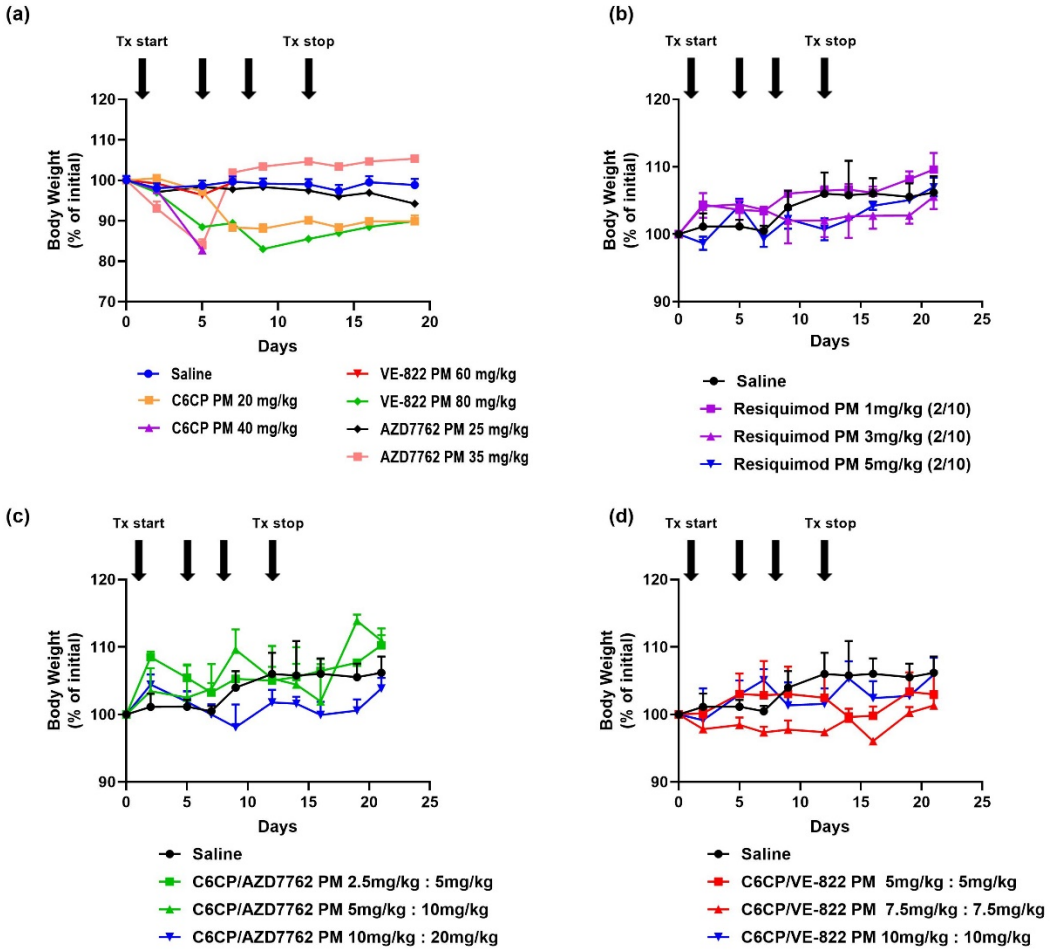


Fig. S3. MTD study in healthy 129/Sv mice Mice body weight (percent of initial) following four injections of **a, b)** Single agent POx micelles **c)** C_6CP/AZD7762 PM **d)** C_6CP/VE-822 PM (q4days x 4doses) and normal saline. A common control (saline) was used for b-d. Data represent mean ± SEM. n = 3.

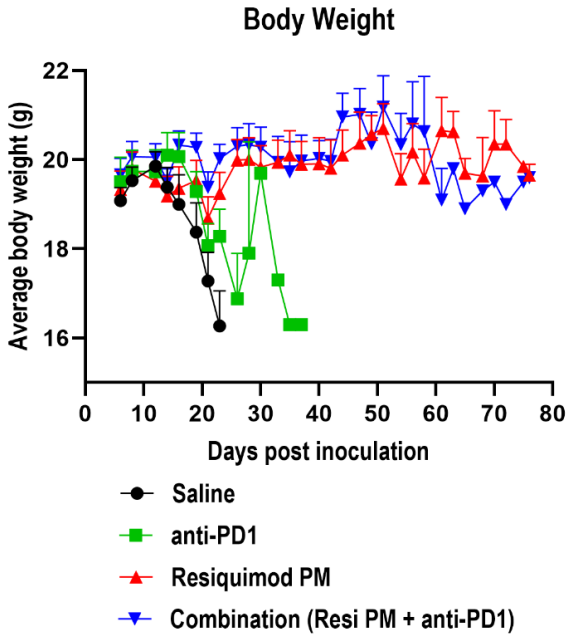


Fig. S4. Changes in the bodyweight of animals subjected to different treatments. Data represent mean ± SEM. n = 3.

Table S2 | Description of Antibody-Fluorochrome pairs

Myeloid Panel		**Lymphoid Panel**	
Antibody	**Fluorophore**	**Antibody**	**Fluorophore**
Ly6C	AF647	CD45	PE
CD11b	BV510	CD8	AF488
CD11c	APC/Cy7	CD3	AF594
Ly6G	AF594	AF700	AF700

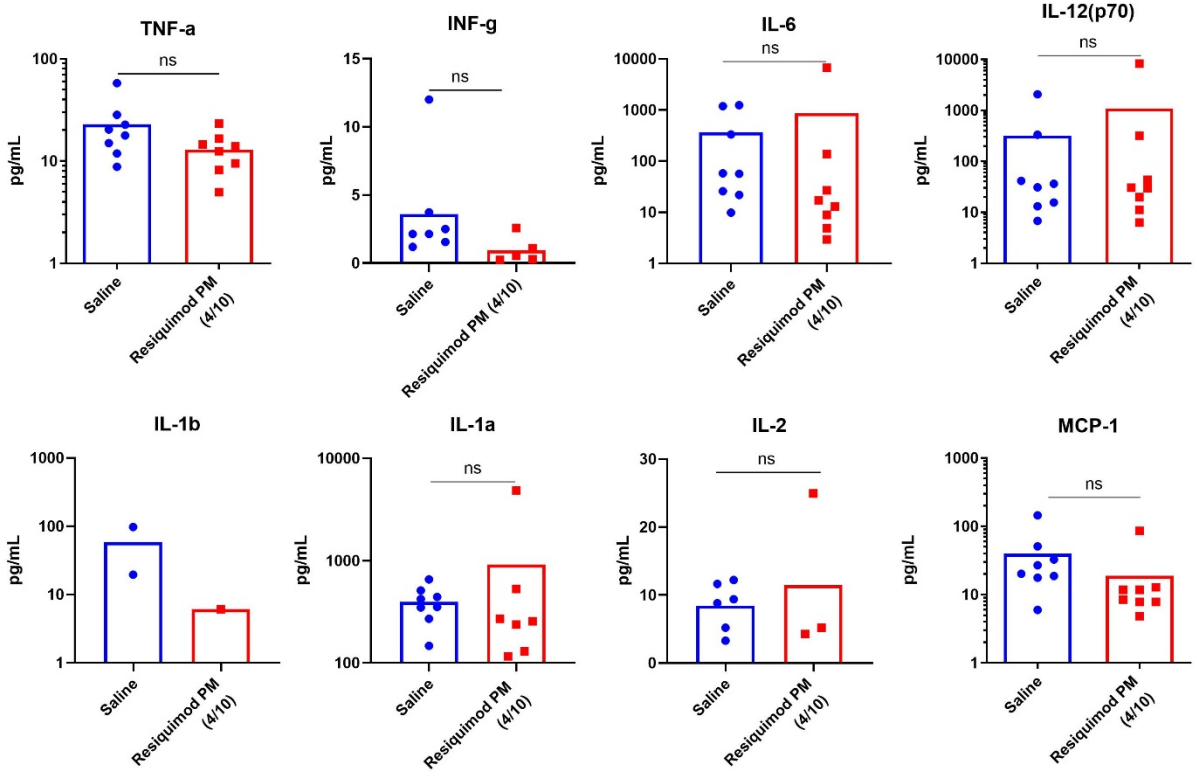


Fig. S5. Alterations in the pro-inflammatory cytokines/chemokines at 48 h following second treatment of Resiquimod PM (q4days x 2doses). n = 8. ns, not significant computed by unpaired student t test with Welch's correction. Significance level (α) was set at 0.05.