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Supplemental Material to:

Myrna R Rosenfeld, Xiaobu Ye, Jeffrey G Supko, Serena Desideri, Stuart A Grossman, Steven Brem, Tom Mikkelson, Daniel Wang, Claire Chang, Janice Hu, Quentin McAfee, Joy Fisher, Andrea Troxel, Shengfu Piao, Daniel F Heitian, Kay See Tan, Laura Pontiggia, Peter J O'Dwyer, Lisa E Davis, Ravi K Amaravadi

A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme

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Supplementary Tables and Figures

Table S1. Adverse events HCQ 200 mg/dose cohort n=3

Adverse Event	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)
Anemia	2 (66)	1 (33)		
Anorexia	2 (66)			
Anxiety	1 (33)			
Ataxia	1 (33)			
Constipation	1 (33)			
Creatinine Increased	1 (33)			
Dizziness	2 (66)			
Dry skin	1 (33)			
Fatigue	1 (33)			
Headache	2 (66)			
Hyperglycemia	1 (33)			
Nausea	2 (66)			
Neutrophil Count Decreased		2 (66)	1 (33)	
Platelet Count Decreased	2 (66)			
Pruritus	1 (33)			
Purpura	1 (33)			
Vomiting				
White Blood Cell Decreased	1 (33)	1 (33)		1 (33)

Table S2. Adverse events HCQ 400 mg/dose cohort n=7

Adverse Event	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)
Agitation	1 (14)			
Alanine aminotransferase increased	3 (43)			
Alkaline phosphatase increased	2 (29)			
Anemia	2 (29)			1 (14)
Anorexia	2 (29)		1 (14)	
Aspartate aminotransferase increased	2 (29)			
Blood Bilirubin Increased	1 (14)			
Blurred vision	1 (14)			
Constipation	3 (43)			
Diarrhea	2 (29)			
Dizziness	1 (14)			
Dry skin	1 (14)			
Dyspnea	1 (14)			
Erythema multiforme	1 (14)			
Fatigue	4 (57)	3 (43)		
Fever	1 (14)			
Generalized muscle weakness	1 (14)		1 (14)	
Headache	1 (14)			
Hyperglycemia	1 (14)			
Hyperuricemia			1 (14)	

Hypokalemia	1 (14)			
Hypomagnesemia	1 (14)			
Irritability	1 (14)			
Lymphocyte count decreased	1 (14)			
Nausea	4 (57)	1 (14)	1 (14)	
Neutrophil Count Decreased			1 (14)	1 (14)
Photosensitivity		1 (14)		
Platelet Count Decreased	2 (29)			1 (14)
Rash maculo-papular		1 (14)		
Vomiting	2 (29)			
White Blood Cell Decreased	1 (14)	1 (14)		1 (14)

Table S3. Adverse events HCQ 400 mg/dose cohort n=7

Adverse Event	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)
Alanine aminotransferase increased	1 (33)			
Anemia	1 (33)			
Chills	1 (33)			
Constipation	1 (33)			
Fatigue	1 (33)	1 (33)		
Febrile neutropenia			1 (33)	
Nausea	1 (33)	1 (33)		
Neutrophil Count Decreased				1 (33)
Platelet Count Decreased	2 (66)			
Vomiting	1 (33)			
White Blood Cell Decreased	1 (33)			1 (33)

Table S4. Adverse events HCQ 800 mg/dose cohort n=3

Adverse Event	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)
Anemia	1 (33)	2 (66)		
Bicarbonate	1 (33)			
Bruising	1 (33)			
Constipation	1 (33)			
Diarrhea	2 (66)			
Epistaxis	1 (33)	1 (33)		
Fatigue		2 (66)		
Febrile neutropenia			1 (33)	
Hypocalcemia		1 (33)		
Infections			2 (66)	
Nausea		1 (33)		
Neutrophil Count Decreased				2 (66)
Non-cardiac chest pain		1 (33)		
Photosensitivity				
Platelet Count Decreased				2 (66)
Rash maculo-papular		1 (33)		
Vomiting		1 (33)		
White Blood Cell Decreased		1 (33)		2 (66)

Table S5. Individual Estimated Hydroxychloroquine Pharmacokinetic Parameters Steady-State

Patient	HCQ (mg)	Cl_{ss}/F (L/hr)	C_{max} (ng/mL)	C_{min} (ng/mL)	C_{avg} (ng/mL)	AUC (ng*hr/mL)
1.01	200	23.90	578.01	198.65	270.26	6486
1.02	200	14.34	932.09	177.03	450.49	10812
1.03	200	9.02	795.49	654.42	716.16	17188
2.01	400	13.17	1053.96	894.53	981.01	23544
2.02	400	11.16	1949.74	681.70	1116.94	27785
2.03	400	14.61	920.71	834.97	883.82	21212
2.04	400	11.76	1180.29	1006.04	1097.90	26350
2.05	400	11.15	1451.64	1022.94	1158.94	27814
2.06	400	10.17	1342.74	1180.81	1270.60	30494
2.08	400	13.20	1265.41	813.75	978.41	23482
3.02	800	7.48	1896.63	1532.38	1726.74	41442
3.03	800	6.24	2225.77	1900.05	2071.59	49718
4.02	600	9.37	2277.77	1884.46	2067.58	49622
4.03	600	7.47	4842.02	899.54	2592.04	62209
4.04	600	10.90	4368.29	1398.21	1777.16	42652
5.03	600	13.86	1430.43	1348.96	1397.95	33551
5.11	600	19.44	1097.64	901.79	996.52	23916
5.12	600	8.71	2541.51	2007.48	2224.50	53388
5.14	600	13.38	1739.96	1238.02	1447.74	34746
5.17	600	14.86	1764.67	1024.21	1303.93	31294
5.18	600	10.83	1958.98	1630.97	1788.91	42934
5.22	600	13.22	1649.23	1298.51	1465.18	35164
5.23	600	13.61	1491.75	1337.73	1423.73	34170
5.25	600	10.14	2133.16	1724.95	1911.62	45879
5.26	600	11.24	2146.42	1481.16	1724.29	41383
5.27	600	123.95	163.68	149.17	156.31	3751
5.29	600	14.15	1682.68	1206.49	1369.35	32864
5.31	600	11.85	1829.97	1466.91	1635.54	39253
5.32	600	16.06	1287.30	1128.95	1206.39	28953
5.37	600	11.86	2010.75	1400.72	1633.92	39214
5.40	600	11.42	1920.95	1480.93	1696.37	40713
5.43	600	20.29	1384.71	543.17	954.90	22918
5.44	600	17.93	1875.36	622.21	1080.68	25936
5.45	600	10.24	2098.92	1712.14	1892.67	45424
5.50	600	11.23	1884.52	1583.85	1725.60	41414
5.55	600	9.18	2246.26	1960.18	2110.38	50649
7.01	600	14.64	1642.93	1089.80	1323.51	31764
7.02	600	12.91	1658.60	1359.15	1500.50	36012
7.03	600	13.23	1685.26	1287.47	1464.62	35151
7.04	600	18.77	1109.95	934.63	1032.45	24779
7.06	600	13.00	1661.79	1332.76	1490.87	35781
7.07	600	12.80	1603.74	1395.34	1513.13	36315
7.08	600	8.72	5263.97	617.14	2221.35	53312
7.09	600	10.88	1898.79	1645.09	1780.60	42734
7.10	600	6.98	3003.71	2561.61	2777.13	66651
7.11	600	8.83	2371.67	2064.61	2194.42	52666
7.12	600	14.75	1718.14	1077.01	1313.79	31531
7.13	600	9.78	2119.52	1881.42	1980.42	47530
7.14	600	9.28	2245.83	1938.10	2088.12	50115
7.15	600	16.32	1343.60	1073.74	1187.20	28493
7.16	600	19.05	1234.23	874.64	1016.97	24407

Clss/F = Apparent oral clearance at steady-state; C_{max} = Peak blood concentration at steady-state; C_{min} = minimum blood concentration estimate at steady-state; C_{avg} = average blood concentration estimate over 24-hour dosing time period at steady-state; AUC = area under the concentration-versus-time curve over 24-hour dosing time period, estimated at steady-state.

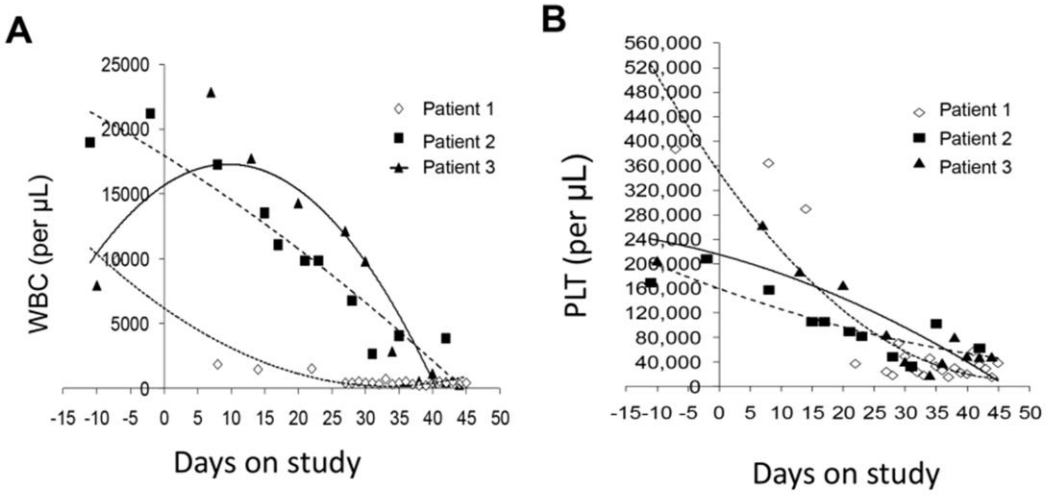

Figure S1. Kinetics of myelosuppression associated with temozolomide, radiation and HCQ 400 mg twice daily. For the three patients enrolled on this cohort: (A) white blood cell count (WBC). (B) Platelet count (PLT).

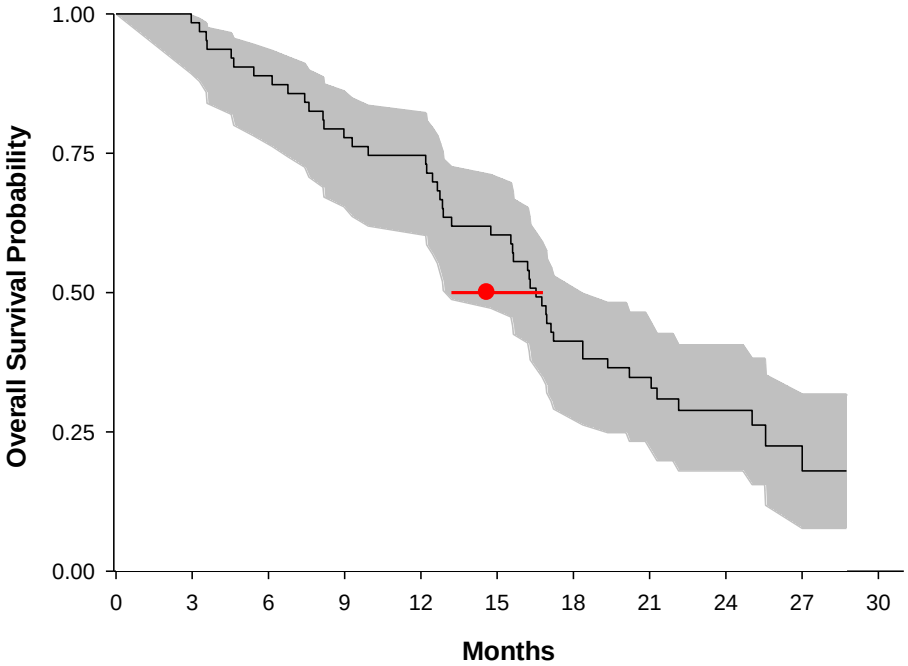

Figure S2. Overall survival. Kaplan Meier survival curve with 95% confidence interval (grey). The red dot with 95% CI is the result of median OS from the EORTC Phase III trial of temozolomide and radiation versus radiation alone.

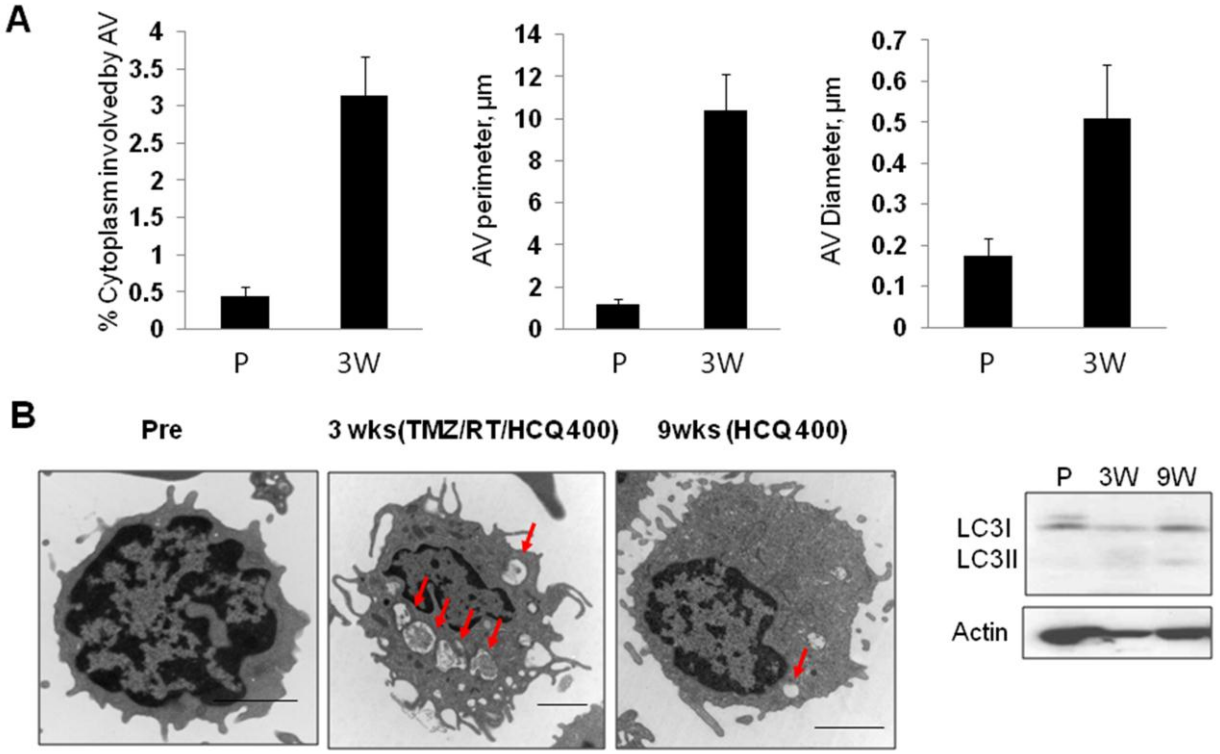

Figure S3. Additional measurements of autophagy inhibition in patients treated with temozolomide, radiation, and hydroxychloroquine . (A) Morphological parameters of AV in electron micrographs of PBMC from a patient treated with HCQ 800 mg. P: pre-treatment. 3W: 3 weeks of treatment. (B) Representative electron micrographs and immunoblotting against LC3 in serial PBMC collected at the indicated timepoints from a patient treated with HCQ 400 mg. Arrows: AV; scale bar 2 µm.

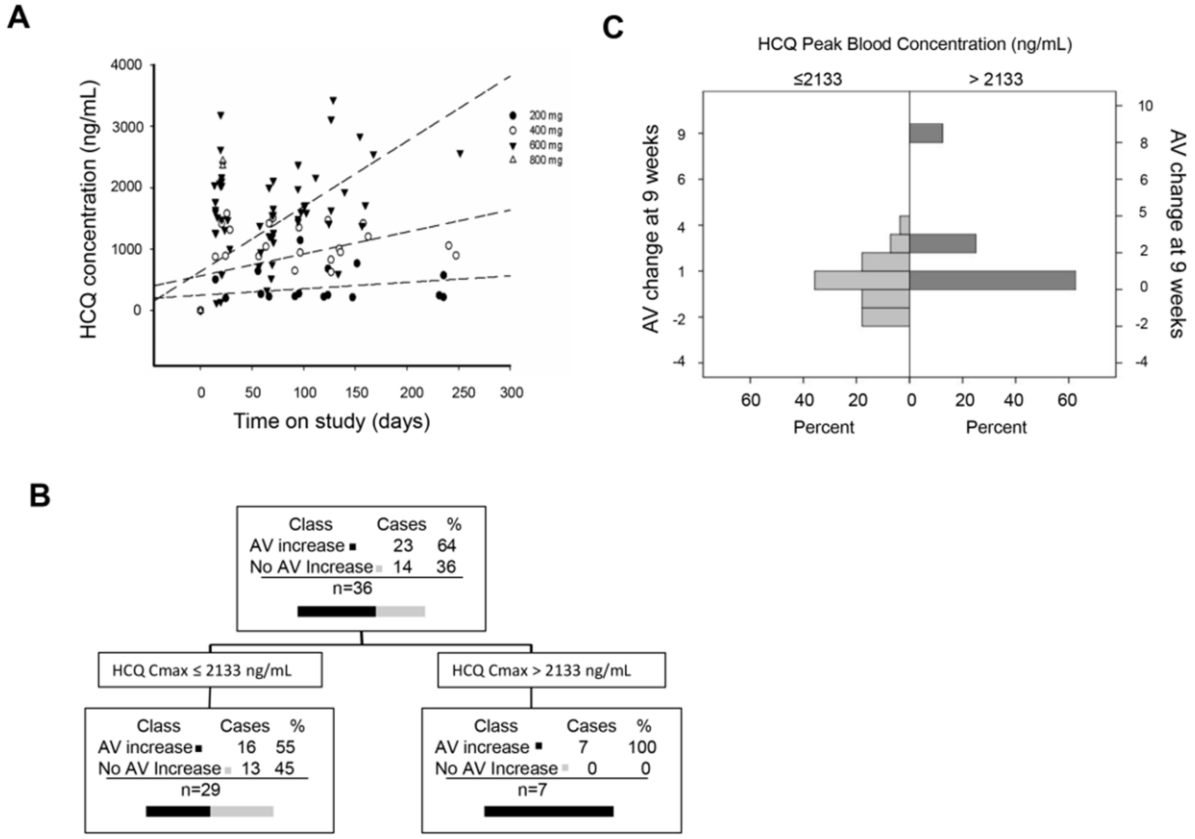

Figure S4. PK-PD analysis. (A) HCQ concentrations in whole blood over time by dose cohort. (B) The CART analysis was conducted using PK and PD data of 36 patients with available data at baseline and 9 weeks of treatment , with the target variable defined as any positive change in number of AV from baseline to 9 weeks and C_{min}, C_{max}, and AUC as predictors.(C) Patients with C_{max} below 2133 ng/mL (N=29) produced a median AV change of 0.28 (IQR: -0.78, 1.27), while patients with C_{max} above 2133 ng/mL (N=7) produced a median AV change of 0.62 (IQR: 0.52, 2.08) (Kruskal-Wallis two-sided p=0.1144, one-sided p=0.0572; Kolmogorov-Smirnov p=0.1443).