ARTICLE - Non-Hodgkin Lymphoma

Targeting glutaminase is therapeutically effective in ibrutinib-resistant mantle cell lymphoma

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Supplementary Data

Supplementary Table S1. Patient information for the samples studied.

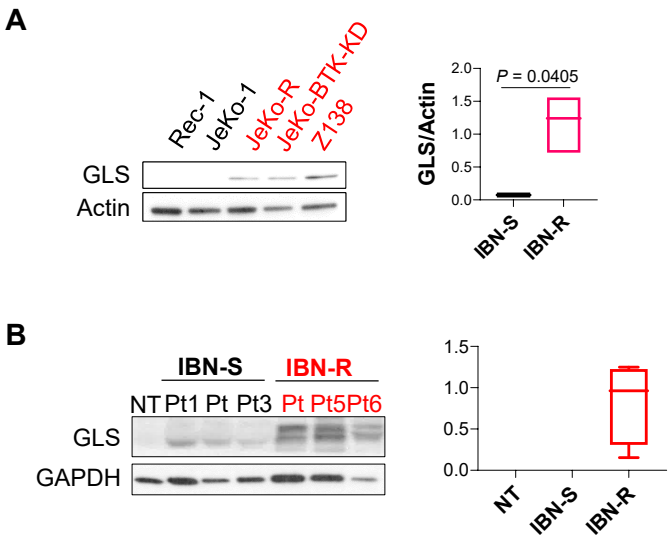
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Sex	Male	Male	Male	Male	Male	Male	Male
Age	51	54	44	76	74	59	74
Bone marrow involvement	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Peripheral blood involvement	Yes	Yes	Yes	N/A	Yes	Yes	Yes
Karyotype	Simple	Simple	Normal	N/A	N/A	Complex	Complex
TP53	Deletion	WT	Deletion	Mutation	N/A	N/A	Deletion and mutation
Ki67	N/A	5%	5%	70%	N/A	60-70%	>50%
Sox11	N/A	N/A	+	-	N/A	+	+
Histology type	Apheresis	Apheresis	Apheresis	Surgical biopsy	Apheresis	Apheresis	Apheresis
Prior therapy lines	2	2	No	10	N/A	5	3
BTKi therapy	Ibrutinib plus Rituxan	Ibrutinib	Ibrutinib	Ibrutinib plus Rituxan	Ibrutinib	Ibrutinib	Not treated
Response to BTKi	Responsive	Responsive	Responsive	Progression	Relapsed	Progression	N/A
Ibrutinib-resistant	No	No	No	Yes	Yes	Yes	N/A

Supplementary Table S2. Combination index (CI) values for drug combinations.

CI *	Dose	JeKo-1	Rec-1	Z-138	JeKo-R	Pt7	PDX-1	PDX-2
Telaglenastat + ibrutinib	1	-	-	-	-	-	-	-
	2	0.250	0.186	0.423	1.472	0.380	0.089	0.166
	3	0.265	0.229	0.399	0.581	0.422	0.174	0.292
	4	0.370	0.215	0.458	0.366	0.773	0.345	0.640
	5	0.311	0.160	0.514	0.567	0.519	0.608	0.929
	6	0.119	0.035	0.556	0.714	0.495	0.879	1.040
	7	0.077	0.006	0.167	0.642	0.408	0.946	0.531
	8	0.110	0.005	0.165	0.116	0.250	1.189	0.578
Telaglenastat + venetoclax	1	-	-	-	-	-	-	-
	2	0.02	0.488	0.195	0.031	0.130	0.117	0.573
	3	0.003	0.588	0.119	0.010	0.182	0.136	0.561
	4	0.001	0.351	0.083	0.008	0.166	0.166	0.552
	5	<0.001	0.224	0.085	0.012	0.197	0.210	0.332
	6	<0.001	0.334	0.053	0.006	0.125	0.255	0.137
	7	<0.001	0.611	0.025	0.006	0.099	0.353	0.123
	8	<0.001	1.256	0.002	0.007	0.028	0.453	0.202

* synergistic: CI < 0.8; non-synergistic: CI ≥ 0.8

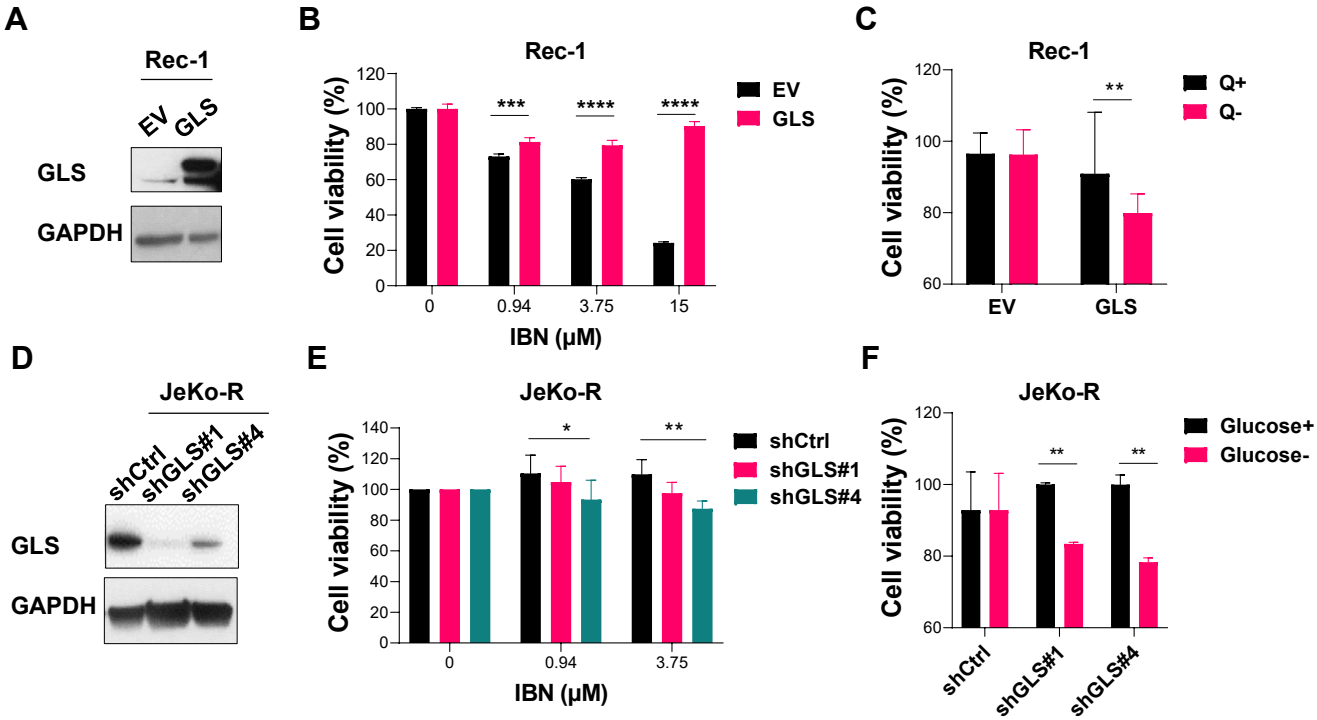
Supplementary Figure S1



Online Supplementary Figure S1. GLS is overexpressed in IBN-R cells

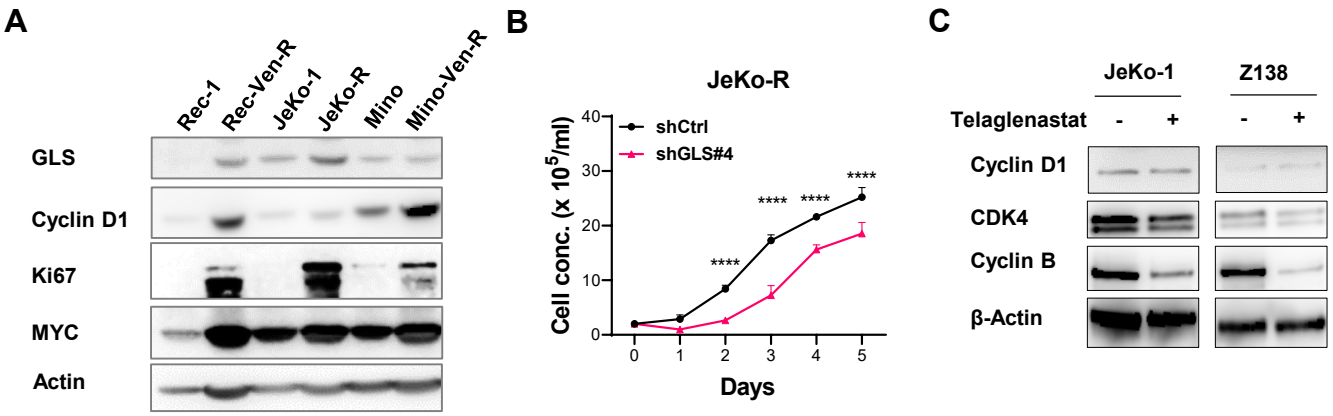
(A) Protein samples from the IBN-S cell lines (Rec-1 and JeKo-1) and IBN-R cell lines (JeKo-R, JeKo-BTK-KD, and Z-138) were analyzed by immunoblotting with the indicated antibodies, and the corresponding grey density normalized to actin was analyzed with ImageJ and shown in the right panel. **(B)** Protein samples from healthy donor (NT), IBN-S (Pt1, Pt2, Pt3), and IBN-R (Pt4, Pt5, Pt6) patients were analyzed by immunoblotting with the indicated antibodies, and the corresponding grey intensity normalized to GAPDH was analyzed with ImageJ and shown in the right panel.

Supplementary Figure S2



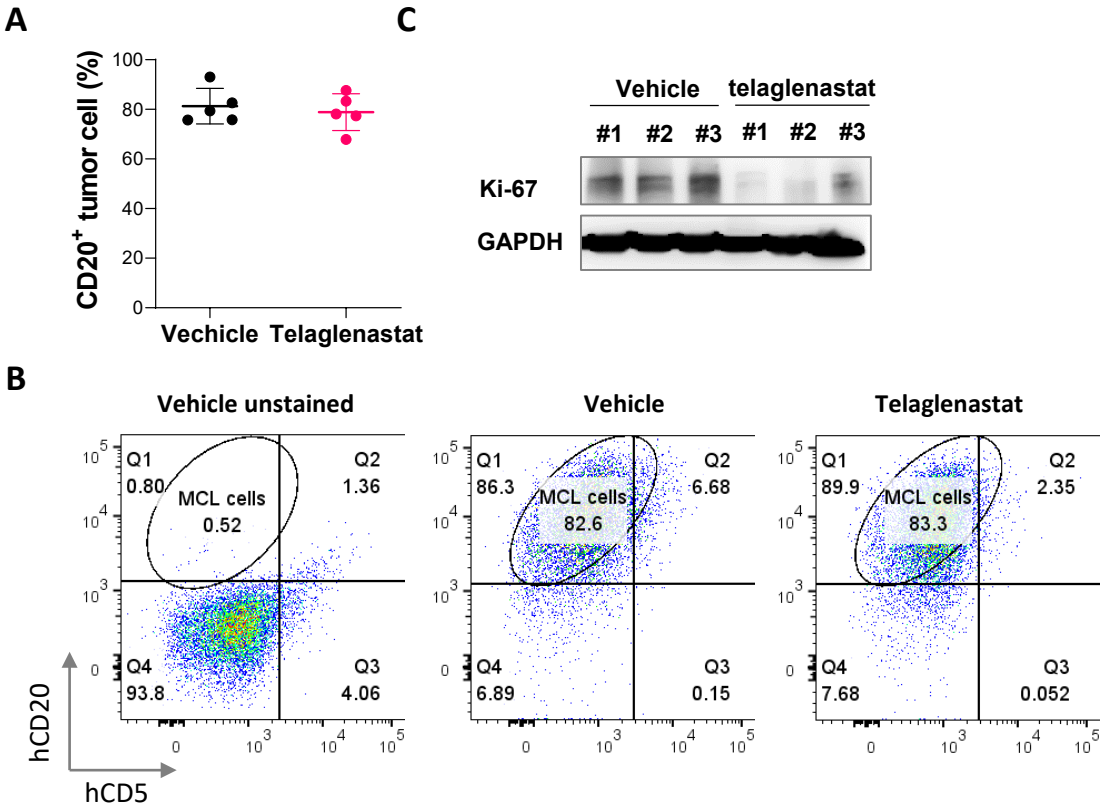
Online Supplementary Figure S2. GLS expression impacts cell proliferation and ibrutinib sensitivity in MCL cell lines. (**A**) Rec-1 cells with or without stable GLS overexpression (Rec-1 EV and Rec-1 GLS) were harvested for immunoblotting with the indicated antibodies. (**B**) Rec-1 EV and Rec-1 GLS cells were treated with ibrutinib for 72 h at the indicated concentrations and the cell viability was determined and plotted. (**C**) Rec-1 EV and Rec-1 GLS cells were seeded for 24 h in culture medium with or without depletion of glutamine (Q) and the cell viability was determined. (**D**) JeKo-R cells with or without stable GLS knockdown by shRNAs (JeKo-R shCtrl, shGLS#1 and shGLS#4) were harvested for immunoblotting with the indicated antibodies. (**E**) JeKo-R shCtrl, shGLS#1 and shGLS#4 cells were treated with ibrutinib for 72 h at the indicated concentrations and the cell viability was determined and plotted. (**F**) JeKo-R shCtrl, shGLS#1 and shGLS#4 cells were seeded for 24 h in culture medium with or without depletion of glucose and the cell viability was determined. Two-way ANOVA was used to determine significance. *, $P < 0.05$; ** $P < 0.01$, ***, $P < 0.001$; ****, $P < 0.0001$.

Supplementary Figure S3



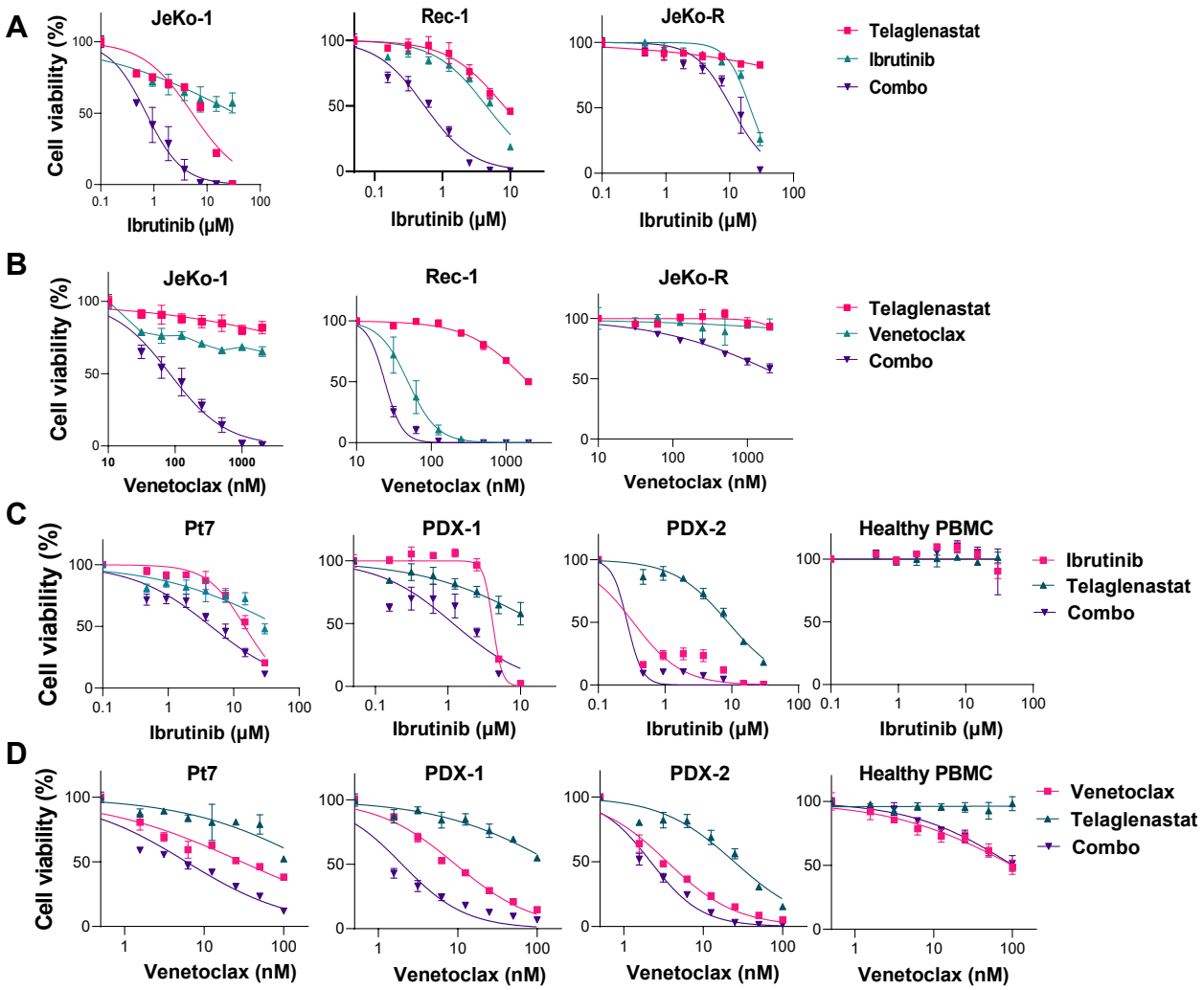
Online Supplementary Figure S3. GLS-mediated glutamine dependency promotes cell growth in ibrutinib-resistant cells. (**A**) Paired MCL cells with acquired ibrutinib resistance (JeKo vs JeKo-R), or with acquired venetoclax resistance (Rec-1 vs Rec-Ven-R, and Mino vs Mino-Ven-R) were subject to immunoblotting using indicated antibodies. (**B**) JeKo-R shCtrl or JeKo-R shGLS#4 cells were cultured for 7 days, and the cell titers were monitored. (**C**) JeKo-1 and Z138 cells were treated with/or without 2 μM telaglenastat for 24 hours and harvested for immunoblotting with indicated antibodies.

Supplementary Figure S4



Online Supplementary Figure S4. Human CD20+ tumor cell population present in Z138 CDX models. Mice carrying Z138 CDX models (n = 5 per group) were treated with vehicle or telaglenastat (200 mg/kg, orally, twice daily). **(A-B)** When tumor size reached 15 mm at the maximum diameter, the mouse was sacrificed, and the tumor cell percentage was determined for each tumor by flow cytometry. Summary data of tumor cell percentage for two groups were plotted **(A)** and representative flow cytometry plots and subset gating of each treatment group with anti-human CD5/CD20 antibodies were shown **(B). (C)** The tumor tissues from Z138 CDX models treated with vehicle (n = 3) or telaglenastat (n = 3) were harvested for immunoblotting with the indicated antibodies.

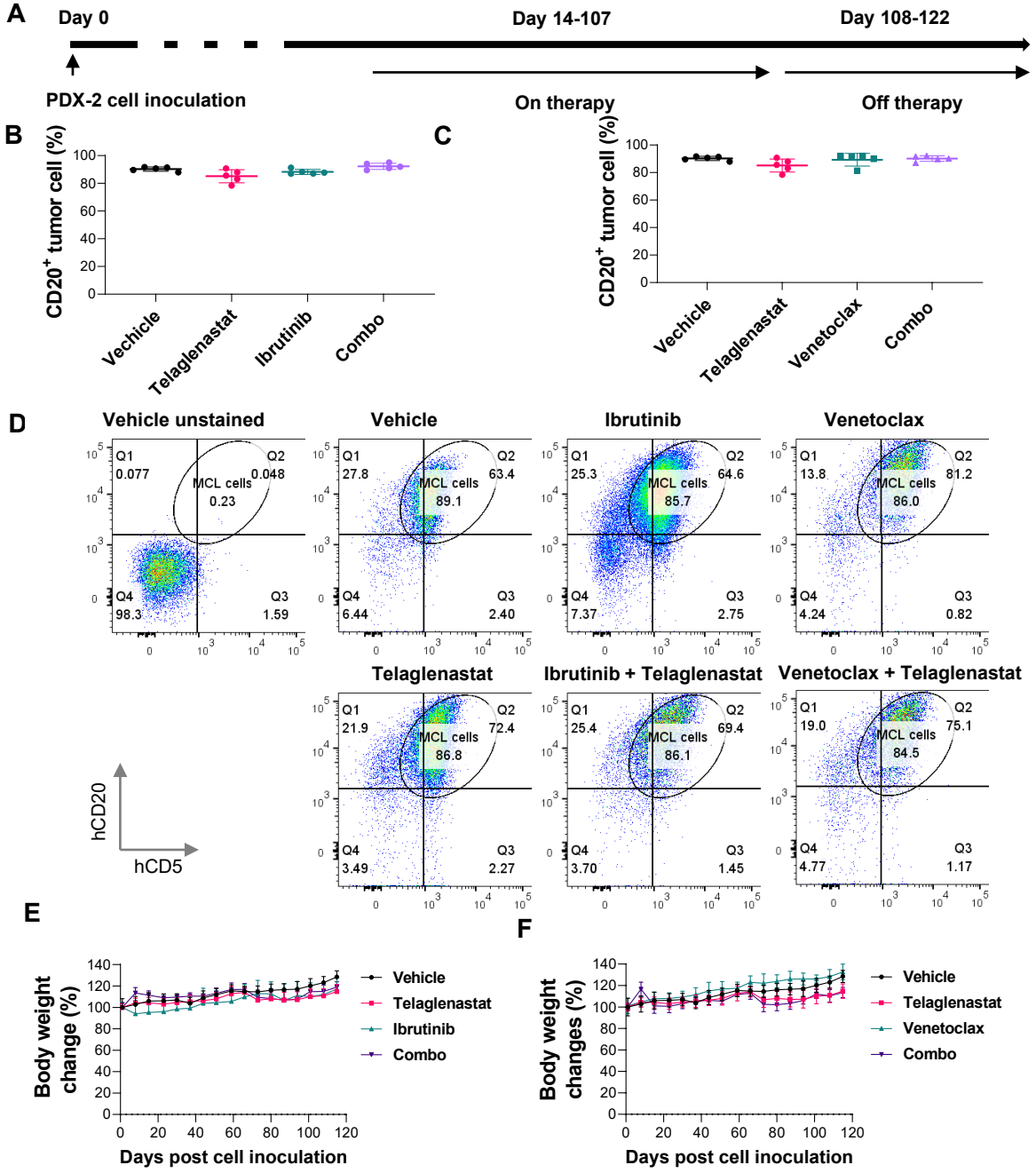
Supplementary Figure S5



Online Supplementary Figure S5. Telaglenastat shows synergistic anti-MCL activity in combination with ibrutinib or venetoclax *in vitro*.

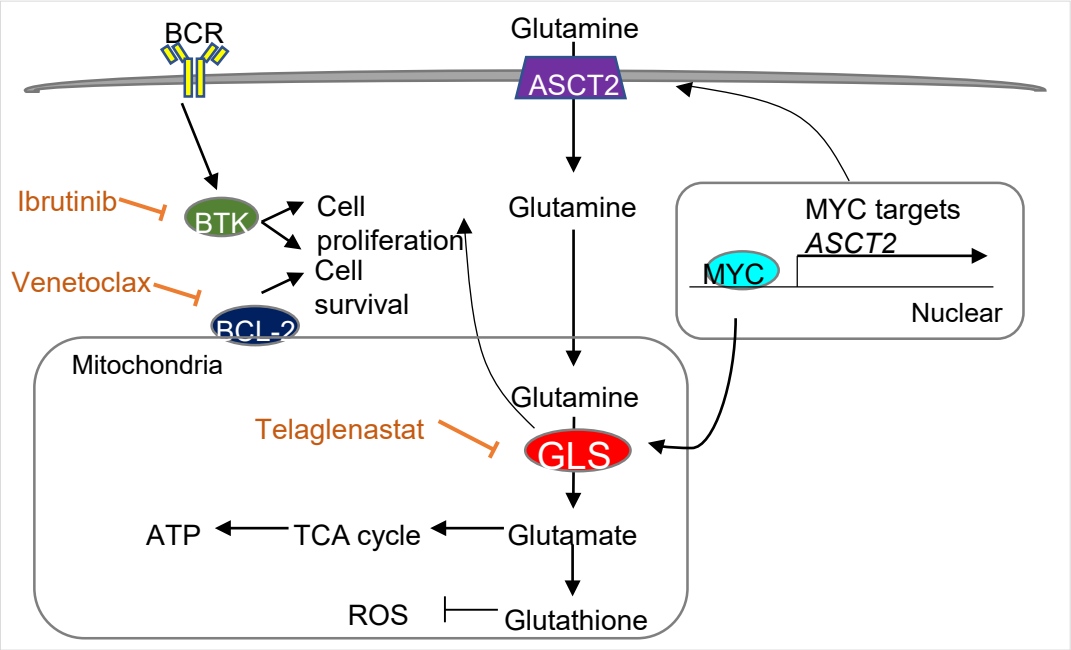
(**A-B**) Cell viability was determined in IBN-S cell lines (JeKo-1 and Rec-1) or IBN-R cell lines (JeKo-R) treated with telaglenastat alone or in combination with ibrutinib **(A)** or venetoclax **(B)** for 72 h. (**C-D**) Cell viability was determined in a primary patient sample (Pt7, left panels), two PDX models (PDX-1 and PDX-2, middle panels), or healthy PBMCs from a donor (right panels) were treated with telaglenastat alone or in combination with ibrutinib **(C)** or venetoclax **(D)** for 24 h. The 2-fold serial dilutions of ibrutinib (**A**) and venetoclax (**B**) are indicated by x-axis. The 2-fold serial dilutions of telaglenastat (0-20 μM) were used alone or in combination with ibrutinib or venetoclax, but not indicated on the x-axis.. Two-way ANOVA was used to determine significance. ns, not significant, *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$.

Supplementary Figure S6



Online Supplementary Figure S6. Human CD5+CD20+ PDX cell population present in PDX models. **(A)** *In vivo* scheme for PDX-2 subcutaneous xenografts treated orally with vehicle (daily), telaglenastat (100 mg/kg, twice daily), ibrutinib (50 mg/kg, daily), venetoclax (10 mg/kg, daily) or in combinations. (**B-C**) When tumor size reached 15 mm at the maximum diameter, the mouse was sacrificed, and the tumor cell percentage was determined for each tumor by flow cytometry. Summary data of tumor cell percentage were plotted separately for each combination telaglenastat plus ibrutinib (**B**) and telaglenastat plus venetoclax (**C**), and representative flow cytometry plots and subset gating of each treatment group with anti-human CD5/CD20 antibodies were shown (**D**). (**E-F**) Mouse body weight was monitored along with the treatments.

Supplementary Figure S7



Supplementary Figure S7. Illustration of the role of MYC-ASCT2-GLS-axis in driving glutaminolysis, glutamine dependency and ibrutinib resistance in mantle cell lymphoma.