Deciphering the landscape of Allosteric Glutaminase 1 inhibitors as anticancer agents

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Table S1. Structural data of GLS1 structures, from Protein Data Bank, https://www.rcsb.org/

PDB	Res.	Chains	Residues	Mutations	Ligand	Literature and other info's
3CZD	2.40 Å	A	221-533 (out of a total of 669 aa)	F219S, V220M. The protein has no additional mutations in comparison to the wild-type sequence.	L-glutamate	https://doi.org/10.1073/pnas.1116573109 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. In the paper are reported also crystal structures of the protein with L-glutamine and with BPTES (see following items 3VOY, 3VOZ, 3VP0, 3VP1, 3VP2, 3VP3, 3VP4.
3UNW	2.56 Å	A/B/C/D	71-597	From residue 550 to 597 several mutations have been inserted. Only residues 557, 569, 580,	L-glutamate	https://doi.org/10.1021/bi201613d Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION.

				596, 602 and 607 have not been mutated Residues 556, 568 and from 583 to 595 are missing.		The protein is arranged as a tetramer in the asymmetric unit with both long (~1600 $Å^2$ buried) and short (~640$Å^2$ buried) interfaces. Both the N- and C-termini exhibit two disordered regions, residues 71−135 and 547−598. Comparison between wild type sequence and 3UNW, highlights only differences in the last part of 3UNW sequence (547-598, connecting loop), while the first residues appear to be conserved. Two different mutant forms of GAC are reported: an F318Y/F322S double mutant and a Y394L single mutant. Both mutants exhibit similar kinetics for glutamine binding in respect to the wild-type .
3UO9	2.30 Å	A/B/C/D	71-597	See comments on 3UNW	BPTES 04A	See comments on 3UNW
3VOY	2.20 Å	A	221-533	F219S, V220M. The protein has no additional mutations in comparison to the wild-type sequence.	Sulfate ion	https://doi.org/10.1073/pnas.1116573109 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The paper reports the crystal structure of the catalytic domain of human apo KGA and its complexes with substrate (L-glutamine), product (L-glutamate), BPTES, and its derived inhibitors. Authors solved the crystal structure of glutaminase (cKGA)L-glutamate complex PDB code 3CZD by molecular replacement with MolRep using the coordinates of a homolog from Micrococcus luteus K-3. Similarly, here, the apo cKGA and other complexes are determined by molecular replacement with MolRep using the cKGA coordinates from cKGA–glutamate complex. Manual fitting of the inhibitor and refinements were carried out.
3VOZ	2.40 Å	A	221-533	See comments on 3VOY	BPTES 04A Sulfate ion	https://doi.org/10.1073/pnas.1116573109 Human. Expression System: *Escherichia coli.*

						Method: X-RAY DIFFRACTION. The same comments reported for 3VOY.
3VP0	2.40 Å	A	221-533	See comments on 3VOY	L-glutamine with Sulfate ion	https://doi.org/10.1073/pnas.1116573109 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The same comments reported for 3VOY.
3VP1	2.30 Å	A	221-533	See comments on 3VOY.	L-glutamate and BPTES 04A	https://doi.org/10.1073/pnas.1116573109 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION.The same comments reported for 3VOY.
3VP2	2.70 Å	A	221-533	See comments on 3VOY.	L-glutamate and Inhibitor **2** BP0 Sulfate ion	https://doi.org/10.1073/pnas.1116573109 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The same comments reported for 3VOY.
3VP3	2.70 Å	A	221-533	See comments on 3VOY.	L-glutamate and Inhibitor **3** BP8 Sulfate ion	https://doi.org/10.1073/pnas.1116573109 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The same comments reported for 3VOY.
3VP4	2.45 Å	A	221-533	See comments on 3VOY.	L-glutamate and Inhibitor **4** BP9	https://doi.org/10.1073/pnas.1116573109 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The same comments reported for 3VOY.
4O7D	2.30 Å	A	221-531	F219S, V220M. The protein has no additional mutations in comparison to the wild-type sequence.	5-oxo-L-nor leucine	https://doi.org/10.1038/srep03827 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. cKGA-DON complex was solved with the molecular replacement method, using coordinates derived from apo cKGA (PDB code 3VOY).
5D3O	2.79 Å	A/B	72-597	A268V, from residues 72 to 550 the protein has no additional mutations in	Apo-form	Human. Expression System: *Escherichia coli BL21(DE3).* Method: X-RAY DIFFRACTION.

				comparison to the wild-type sequence. From residue 550 to 613 the sequence is almost completely different from the wild type one, with some truncations and missing or mutated residues (n.d. this should not affect the binding site)		Crystal structure of full length human glutaminase C expressed in *E. coli*. Huang Q. et al. *To be published.* The pdb structure has been released and deposited in 2015. Authors publishedhttps://doi.org/10.1016/j.bmc.2016.03.009, containing three solved BPTES-GLS1 crystal structures.
5FI2	2.50 Å	A/B/C/D	72-597	See 5D3O. Even if 5D3O has not been published, 5FI2, 5FI6, 5FI7, 5I94 and 5HL1 (not published) share the same protein sequence and publication.	Compound **7d** 5XX	https://doi.org/10.1016/j.bmc.2016.03.009 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. In the paper Authors analyze the crystal structures of 3UO9, 3VOZ and 3VP1. They use the pdb structure of 3UO9 (GAC + inhibitors) to solve 5FI2 X-ray structure
5FI6	2.52 Å	A/B/C/D	72-597	See comments on 5FI2.	Compound **7e** 5XY	https://doi.org/10.1016/j.bmc.2016.03.009 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. See the same comments reported for 5FI2.
5FI7	2.50 Å	A/B/C/D	72-597	See comments on 5FI2.	Compound **14b**	https://doi.org/10.1016/j.bmc.2016.03.009 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. See the same comments reported for 5FI2.
5HL1	2.40 Å	A/B/C/D	72-597	See comments on 5FI2.	CB-839 63J	Human. Expression System: *Escherichia coli BL21(DE3).* Method: X-RAY DIFFRACTION. Huang Q. et al. *To be published.* The pdb structure has been released and deposited in 2015. Authors published the X-ray data of GLS1-CB-839 and GLS1-UPGL00004 complexes in https://doi.org/10.1074/jbc.M117.810101
5I94	2.98 Å	A/B/C/D	72-597	See comments on 5FI2.	Compound **14d**	https://doi.org/10.1016/j.bmc.2016.03.009

					69V	Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The same comments reported for 5FI2.
5JYO	2.10 Å	A/B/C/D/ E/F/G/H	221-533	Residues from 201 to 221 completely differ from the wild-type sequence. From 222 to 533 the protein has no additional mutations in comparison to the wild-type sequence.	CB-839 63J	https://doi.org/10.1016/j.bmc.2016.03.009 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. Structures of the cKGA:inhibitor complexes were obtained by molecular replacement with the program Phaser-MR using the coordinates of *apo* cKGA as template (PDB code 3VOY)
5JYP	2.74 Å	A	221-533	See comments on 5JYO	1*S*, 3*S*-CBTBP	https://doi.org/10.1016/j.bmc.2016.03.009 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The 1*S*, 3*S* stereoisomer displayed higher preference to complex with cKGA over the 1*R*, 3*R* form. The 1*S*, 3*S*-CBTBP molecule was positioned using a *Fo-Fc* Simulated Annealing omit map. BPTES and 1S, 3S-CBTBP share similar symmetric halves, and have identical hydrogen bonding interactions with cKGA.
5U0I	1.42 Å	A/B C-terminal ankyrin repeats from KGA - monoclinic crystal form	551-669	Residues 530-550 differ from the wild-type protein. From residue 551 to 669 the protein has no mutations in comparison to the wild-type sequence.	Apo form	https://doi.org/10.1074/jbc.M117.787291 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The authors studied the C termini of both kidney-type glutaminase (KGA) and GLS2 isoforms (glutaminase B and liver-type glutaminase) to obtain information on ANK folding.
5UOJ	1.72 Å	A/B C-terminal ankyrin	551-669	See comments on 5U0I.	Apo form	https://doi.org/10.1074/jbc.M117.787291 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. See comments reported for 5U0I.

		repeats from KGA - monoclinic crystal form				
5UQE	3.60 Å	A/B/C/D/F	137-656	F220L	BPTES 04A	https://doi.org/10.1074/jbc.M117.787291 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. Authors solved also the novel crystal structure of KGA containing the C-terminus, bound to a BPTES. Phasing was achieved by molecular replacement using the coordinates of the previously solved N-terminal and glutaminase domains from GAC bound to BPTES (PDB code 4JKT) and the KGA.ANK.
5WJ6	2.44 Å	A/C/D	72-550	V268A. The N terminal of the protein 60-71 shows some mutated residues in comparison with the wild-type sequence. The portion 551-613, is truncated compared to the wild type.	UPGL-00004 B4A	https://doi.org/10.1074/jbc.M117.810101 Human. Expression System: *Escherichia coli.* Method: X-RAY DIFFRACTION. The structures were solved by molecular replacement using wild type GAC (PDB code 5D3O) as search model. Four molecules of GAC are present in an asymmetric unit. In the manuscript Authors describe also the complex GLS1-CB-839
6LOX	3.20 Å	A/C/D	71-600	From 71 to 550 the protein sequence shows no mutations. From 551 to 619 the sequence almost completely differs from the wild-type one (different residues and truncation)	Compound **13b** LL202 EN3	**https://doi.org/10.1021/acs.jmedchem.0c02044** Human. Expression System: *Escherichia coli BL21(DE3).* Method: X-RAY DIFFRACTION. Two molecules of the inhibitor bind the tetramer in the tunnel region formed by Leu321, Phe322, Leu323, and Tyr394 of GLS1 (same pocket involved in the binding of CB-839). When the 13b-GLS1

						complex is superimposed with apo GLS1 X-rays structure, a major conformational change is observed at the Glu312-Pro329 loop for the 13b−GLS1complex
6UJG	3.00 Å	A/B/C/D/ E/F/G/H	72-550	From residues 72-550 the protein corresponds to the wild-type sequence except for a single mutation V268A; as in the case of structures 5WJ6, 5D3O, 5FI2, 5FI6, 5FI7, 5I94 and 5HL1	Compound **7g** Q9A UPGL00012	Human. Expression System: Escherichia coli 'BL21-Gold(DE3)pLysS AG. Method: X-RAY DIFFRACTION. The crystal structure have been obtained with a novel technique, called serial room temperature crystallography. Crystal structure coordinates have been deposited in the PDB in 2020 for the crystals of GAC in complex with compounds **7d** (PDB: 5FI2), **7e** (PDB: 5FI6), **14b** (PDB: 5FI7) and **14d** (PDB: 5I94). Crystallographic data on GLS1 in complex with **7g** (PDB:6UJG), **7h** (PDB:6UJM), **13d** (PDB:6UK6), **14i** (PDB:6UKB) can be found in ref https://doi.org/10.1016/j.bmc.2016.03.009. Authors published some X-ray data of GLS-ligand complexes also in https://doi.org/10.1016/j.jbc.2021.101535
6UJM	2.50 Å	A/B/C/D	72-550	See comments on 6UJG.	Compound **7h** Q94 UPGL00013	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. See comments on 6UJG.
6UK6	2.90 Å	A/B/C/D	72-550	See comments on 6UJG.	Compound **13d** Q9S UPGL00018	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. See comments on 6UJG.
6UKB	3.00 Å	A/B/C/D	72-550	See comments on 6UJG.	Compound **14i** Q9V UPGL00020	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. See comments on 6UJG.

6UL9	2.50 Å	A/B/C/D	72-550	See comments on 6UJG	Compound **14a** Q9M UPGL00023	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. See comments on 6UJG
6ULA	2.95 Å	A/B/C/D/ E/F/G/H	72-550	See comments on 6UJG	Compound **14j** QA4 UPGL00030	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. Crystal structure coordinates have been deposited in the PDB in 2019. Authors published the X-rays data of GLS1-**14j** complex in https://doi.org/10.1016/j.jbc.2021.101535
6ULJ	2.69 Å	A/B/C/D	72-550	See comments on 6UJG	Compound **26b** QAA UPGL00045	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. Crystal structure coordinates have been deposited in the PDB in 2019. Authors published the X-rays data of GLS1-**26b** complex in https://doi.org/10.1016/j.jbc.2021.101535
6UMC	2.75 Å	A/B/C/D	72-550	See comments on 6UJG	Compound **14c** U27	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. Crystal structure coordinates have been deposited in the PDB in 2019. Authors published the X-rays data of GLS1-**14c** complex in https://doi.org/10.1016/j.jbc.2021.101535
6UMD	2.70 Å	A/B/C/D	72-550	The deposited sequence 72-550 completely corresponds to the wild-type sequence. From 551 to 613 some residues differ from the wild-type sequence.	UPGL0046 or UPGL00012 QAJ	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. Crystal structure coordinates have been deposited in the PDB in 2019. Authors published the X-rays data of GLS1-ligand complex in https://doi.org/10.1016/j.jbc.2021.101535 Discrepancy has been observed between the ligand deposited in PDB and the one reported in the paper
6UME	2.90 Å	A/B/C/D	72-550	The indicated sequence 72-550 corresponds to the wild-type sequence except for a single mutation V268A	UPGL0046 or UPGL00012 QAJ	Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. Crystal structure coordinates have been deposited in the PDB in 2019. Authors published the X-rays data of GLS1-ligand complex in

						https://doi.org/10.1016/j.jbc.2021.101535 Discrepancy has been observed between the ligand deposited in PDB and the one reported in the paper. 6UMD and 6UME present folding differences in some regions
6UMF	2.68 Å	A/B/C/D	72-550	See comments on 6UME.	Compound **16d** QAM	Human Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. Crystal structure coordinates have been deposited in the PDB in 2019. Authors published the X-rays data of GLS1-ligand complex in https://doi.org/10.1016/j.jbc.2021.101535 Discrepancy has been observed between the ligand deposited in PDB (named UPGL00012, **7g**) and the one reported in the paper and co-crystallized with GLS1, **16d**.
7REN	2.80 Å	A/B/C/D	72-550	From residues 60 to 71 and from 551 to 613 some residues differ from the wild type GLS1 sequence. From residues 72 to 550 the protein has no additional mutations	UPGL0004 B4A Same ligand of 5WJ6.	https://doi.org/10.1016/j.jbc.2021.101535 Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION.
7RGG	3.00 Å	A/B/C/D	72-550	See comments on 7REN.	BPTES 04A	https://doi.org/10.1016/j.jbc.2021.101535 Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION.
7SBM	2.80 Å	A/B/C/D	72-550	Y466W. From residues 60 to 71 and from 551 to 613 some residues differ from the wild-type sequence.	Glutamine, open conformation w/o phosphate ion	https://doi.org/10.1016/j.jbc.2022.101564 Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. The Y466W mutant is unable to catalyze the hydrolysis of glutamine to glutamate; however, the fluorescence of W466 is quenched specifically upon the binding of substrate (glutamine) but not upon the product (glutamate), thereby providing a direct

						readout for the interactions of glutamine with the enzyme. Structural difference in the protein conformation and orientations could be observed with or without phosphate in the active site; it could also affect the tetramer formation. Authors also prepared a double mutant (K320A and Y466W) but X-rays data were not deposited in PDB.
7SBN	2.14 Å	A/B/C/D	72-550	Y466W. See comments on 7SBM.	Glutamine, closed conformation with phosphate ion	https://doi.org/10.1016/j.jbc.2022.101564 Human. Expression System: Escherichia coli. Method: X-RAY DIFFRACTION. The structure shows four GAC monomers in the asymmetric unit, with electron density for glutamine being present in all four active sites. Inorganic phosphate appears to induce a symmetrical arrangement of the GAC tetramer, such that all four subunits bind glutamine with high affinity and closed lids, as evident by Y249 engaging in hydrogen bonding with E381.
8BSK	2.10 Å	A	221-533	F219S, V220M. From 221 to 533 the protein does not present additional mutations in comparison to the wild-type sequence.	BPTES 04A Sulfate ion	https://doi.org/10.1021/acs.jmedchem.9b00260 Human. Expression System: Escherichia coli BL21. Method: X-RAY DIFFRACTION. Two molecules of the inhibitor are associated with four units of protein. Each BPTES sits between the interfaces of two GLS1 subunits occupying equivalent space on each protein and appears to hold the protein in a nonfunctional tetrameric form.
8BSL	2.38 Å	A/B/C/D	123-550	K121G, E122S. From residues 123 to 550 the protein does not present additional mutations in comparison to the wild-type sequence. Residues from 550 to 613 are	Compound **12** R90	https://doi.org/10.1021/acs.jmedchem.9b00260 Human. Expression System: Escherichia coli BL21. Method: X-RAY DIFFRACTION.

				different from the wild-type sequence.		
8BSM	2.78 Å	A	221-533	See comments on 8BSL	Compound **18** 5XX Sulfate ion	https://doi.org/10.1021/acs.jmedchem.9b00260 Human. Expression System: Escherichia coli BL21. Method: X-RAY DIFFRACTION.
8BSN	2.49 Å	A	221-533	F219S, V220M. The protein has no additional mutations in comparison to the wild type sequence.From residues 221 to 533 the protein does not present additional mutations in comparison to the wild type sequence.	Compound **27** R0C	https://doi.org/10.1021/acs.jmedchem.9b00260 Human. Expression System: Escherichia coli BL21. Method: X-RAY DIFFRACTION.
8GWR	2.80 Å	A/B	1-669	No mutations.	DDP HZO	https://doi.org/10.1111/febs.16658 Human. Expression System: Escherichia coli 'BL21-Gold(DE3)pLysS AG. Method: X-RAY DIFFRACTION. Authors state that they used a mutant D386K DFL-KGA to facilitate the crystallization, but the mutation is not present when analysing the protein sequence. Authors identify a second allosteric site, allosteric site II for BPTES.
8IMA	2.90 Å	A/B/C/D	123-550	From 123 to 550 no mutations are present. Residues from 550 to 613 are different from the wild-type sequence.	Phosphate ion	https://doi.org/10.1038/s41422-023-00886-0 Human. Expression System: Escherichia coli 'BL21-Gold(DE3)pLysS AG. Method: ELECTRON MICROSCOPY.
8IMB	2.90 Å	A/B/C/D	123-550	See comments on 8IMA.	Phosphate ion	https://doi.org/10.1038/s41422-023-00886-0 Human. Expression System: Escherichia coli 'BL21-Gold(DE3)pLysS AG. Method: ELECTRON MICROSCOPY.

8JUB	2.01 Å	A/B/C/D	71-550	From 63 to 70 the protein presents mutations in comparison with the wild-type sequence. From 71 to 550 the protein does not present additional mutations in comparison to the wild-type sequence. Residues 551-610 are mutated.	Compound **27** V4I	https://doi.org/10.1021/acsmedchemlett.3c00375 Human. Expression System: Escherichia coli BL21(DE3). Method: X-RAY CRYSTALLOGRAPHY.
8JUE	2.39 Å	A/B/C/D	71-550	From 63-70 different residues are mutated in comparison to the wild-type sequence. From 71 to 550 the protein does not present additional mutations. Residues 551-610 are mutated.	Compound **11** V59	https://doi.org/10.1021/acsmedchemlett.3c00375 Human. Expression System: Escherichia coli BL21(DE3). Method: X-RAY CRYSTALLOGRAPHY.
8SZJ	3.35 Å	A/B/C/D/ E/F/G/H/ I/J/K/L	1-550	Y466W. From residue 1-550 the protein does not present additional mutations in comparison to the wild-type sequence. From 550 to 613 the residues are different from the wild-type sequence.	L-glutamine Phosphate ion	https://doi.org/10.1038/s41467-024-46351-3 Human. Expression System: Escherichia coli 'BL21-Gold(DE3)pLysS AG. Method: ELECTRON MICROSCOPY. In the manuscript, thanks to cryo-EM technique the authors demonstrate that the filament formation guides an “activation loop” to assume a specific conformation that works together with the lid to close over the active site and correctly position glutamine which undergoes a nucleophilic attack by a serine residue. The cryo-EM structure of the human GAC