Supplementary Tables for Cells 4641985

Chaperoning the Hallmarks of Cancer: Onco-Client Proteins and Cytosolic Hsp90 Isoforms

Table S1. SANRA criterion-by-criterion self-appraisal

Criterion-by-criterion appraisal using the six domains of Baethge et al. [14]. This qualitative mapping makes the evidence for each criterion inspectable; it does not claim an independently rated score, a validated pass threshold or comprehensive elimination of selection bias.

SANRA domain	Appraisal	Location	Basis and remaining limitation
1 Importance	Addressed	Introduction; Sections 6 and 7	Explains proteostasis dependence, unmet therapeutic needs and translational failures.
2 Specific aims	Addressed	Introduction final paragraph; Abstract	Defines cytosolic isoforms, cancer hallmarks, extracellular activity and therapeutic translation.
3 Literature search	Partially addressed	Section 2	Sources, revision date, query concepts and selection priorities are described. Search is purposive and non-exhaustive; no independent duplicate screening.
4 Referencing	Addressed within scope	Citations adjacent to mechanistic and clinical claims	Primary evidence supports new isoform, PTM, immune and clinical statements. Citation coverage is selective rather than exhaustive.
5 Scientific reasoning	Strengthened	Sections 3.1, 4.3, 6.5 and 7	Distinguishes physical binding from dependence, compensation from nonredundancy, and preclinical inference from clinical evidence.
6 Relevant endpoint data	Addressed where available	Table 1; Sections 4.4 and 6.3–6.5	Reports clinical response/survival endpoints and negative results; missing verified estimates remain explicitly identified.

The original SANRA instrument rates each domain from 0 to 2. No numerical total is assigned here because this is a transparent qualitative author appraisal rather than an independently calibrated rating. All six domains are addressed, with the search limitation explicitly retained. Reference numbers match the accompanying manuscript.

Table S2. Isoform-resolved PTM evidence

PTM	Site / isoform	Evidence	Observed consequence	Limit
Phosphorylation	Hsp90β S365	Biochemical and phosphomimetic analysis	Changes conformation, Cdc37 interaction and client activation [38].	β-resolved example; not a universal β-only client mechanism.
Tyrosine phosphorylation	α Y197 / β Y192	Paired isoform mutants and inhibitor-binding experiments	Phosphomimetics reduced geldanamycin binding; PU-H71 was less affected [39].	Drug-binding result; does not prove patient response prediction.
Tyrosine phosphorylation	α Y309 / β Y301	Comparison of corresponding-site mutants	β Y301 phosphomimetic affected geldanamycin binding differently from α Y309 [39].	Corresponding residues can have unequal effects; phosphomimetics are not endogenous site-occupancy measurements.
Acetylation	Hsp90 K294 in the studied construct	Site mapping and mutational analysis	Changes client/cochaperone association [40].	Do not copy numbering or consequences to β without alignment and experimental validation.
Other PTMs	Ubiquitination, SUMOylation and related modifications	Broader chaperone literature	Additional regulation of processing and turnover [37].	Paired α/β functional evidence is uneven; no universal isoform rule assigned.

Residue numbers are retained as reported in the cited experiments. PTMs are candidate mechanisms and exploratory biomarkers; none of these entries is presented as a clinically validated predictive assay.

Table S3. Proposed biomarker validation experiments and advancement criteria

Stage	What to test	Readout	Advancement criterion
1 Intended use	Specify tumor, agent, compartment and decision	Prediction of benefit versus prognostic association	Lock population, treatment, endpoint and sampling times before assay testing.
2 Analytical validity	Validate α/β antibodies with knockout controls; unique-peptide MS; fixed tissue/plasma processing	Precision, specificity, detection limits, matrix effects and stability	Prespecify acceptance limits and reproduce across runs/sites; reject cross-reactive assays.
3 Causal dependence	Inducible single/dual depletion; rescue; tumor and matched normal models	Client stability/activity, cell phenotype, α/β compensation and HSR	Reproducible rescue and orthogonal confirmation; abundance alone does not qualify.
4 Pharmacodynamics	Measure free exposure and cellular/compartment target engagement before death	Client loss, pathway suppression; PTM occupancy where justified	Mechanism-consistent temporal relationship at tolerated exposure; Hsp70 induction alone is insufficient.
5 Clinical qualification	Independent blinded longitudinal cohort; locked assay and threshold	Incremental discrimination/calibration; tumor burden and inflammation adjustment	External reproducibility and added value over clinical covariates; avoid outcome-driven threshold selection.
6 Predictive utility and safety	Comparative trial with biomarker strata; immune and organ monitoring	Biomarker-by-treatment interaction; clinical benefit and adverse events	Evidence that the marker changes treatment benefit with acceptable safety; single-arm prognosis cannot establish prediction.

Exploratory candidates include the α:β protein ratio, defined PTM sites, client–chaperone complexes, epichaperome imaging and circulating Hsp90α. For plasma, challenge processing delay, hemolysis, platelet/cell contamination, renal function and EV separation. Thresholds must be fitted to the assay and intended use, then locked before validation; no numerical cutoffs are asserted from heterogeneous studies. These steps are proposals, not experiments performed in this review.

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