# Characterization of novel isoform-specific HSP90 inhibitors

**Daniela Mendoza-Ortiz**[1] **& Ph.D Pia Roos-Mattjus** [2]

[1] Faculty of Technology, University of Turku, [2] Faculty of Science and Engineering - Biochemistry, Åbo Akademi University

edmeor@utu.fi

MOLECULAR SYSTEMS BIOLOGY

## Background

The 90 kDa heat shock protein (HSP90) is a molecular chaperone involved in many cellular processes including malignant cell transformation[1]. In mammals the four HSP90 isoforms are arranged into three cellular compartments; HSP90α and β in the cytoplasm; GRP94 in the endoplasmic reticulum and TRAP-1 in the mitochondria[1]. HSP90 is in charge of folding nascent polypeptides, misfolded proteins, and mutated proteins which are known as client proteins. Most of its client proteins play a central pathogenic role in human diseases such as cancer[1,2]. Hence, targeting HSP90 represents a potential therapeutic target.

At least 15 different inhibitors have been tested in several clinical trials: nevertheless, these have not been approved by the FDA due to their toxicity and side effects in patients[2]. All clinical inhibitors target the interaction between ATP-HSP90 which produces a heat shock response (HSR) (Fig. 1). Hence, alternative approaches to target HSP90 are currently being developed such as selective suppression of HSP90 isoforms.

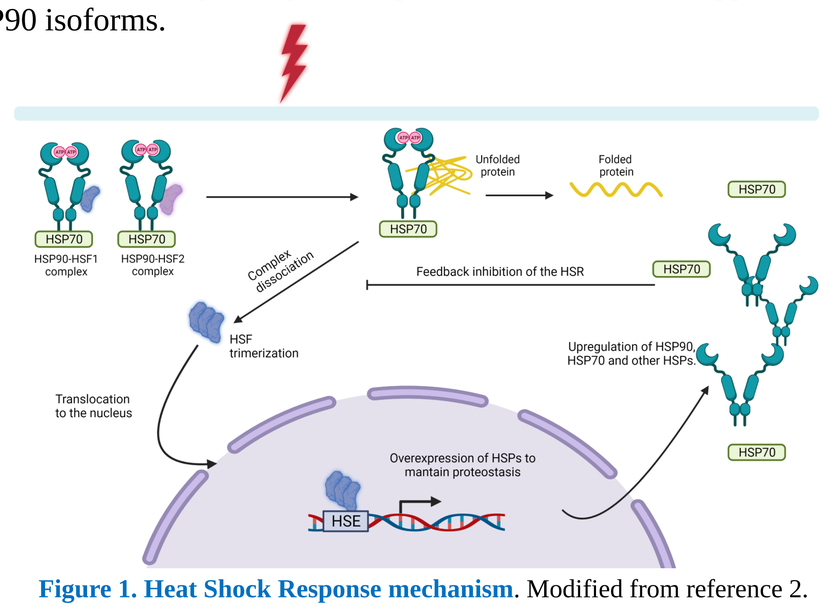


**Figure 1. Heat Shock Response mechanism**. Modified from reference 2.

In collaboration with Prof. Brian Blagg (University of Notre Dame), we aimed to characterize 9 novel isoform-specific HSP90 inhibitors (Table 1) by whether they induced an HSR. Moreover, we aimed to determine whether HSF1KO cells and HSF2KO cells are equally sensitive to these inhibitors as U2OS WT cells.

**Table 1.** Novel isoform-specific HSP90 inhibitors

| COMPOUND ID | TARGET |
|---|---|
| 5 | Alpha N-terminal |
| 6 | Beta N-terminal |
| 7 | |
| 8 | GRP94 |
| 9 | |
| 10 | C-terminal |
| 11 | |
| 12 | |
| 13 | |

## Methods

To answer our questions, this study included two parts (Fig. 2): Treatment of U2OS wild type cells and mutant cells with the inhibitors for 18 h followed by immunoblot analysis (Fig. 2A). Analysis of sensitivity of the mutant cell lines with a Cell Counting Kit-8 (CCK8) assay (Fig. 2B).

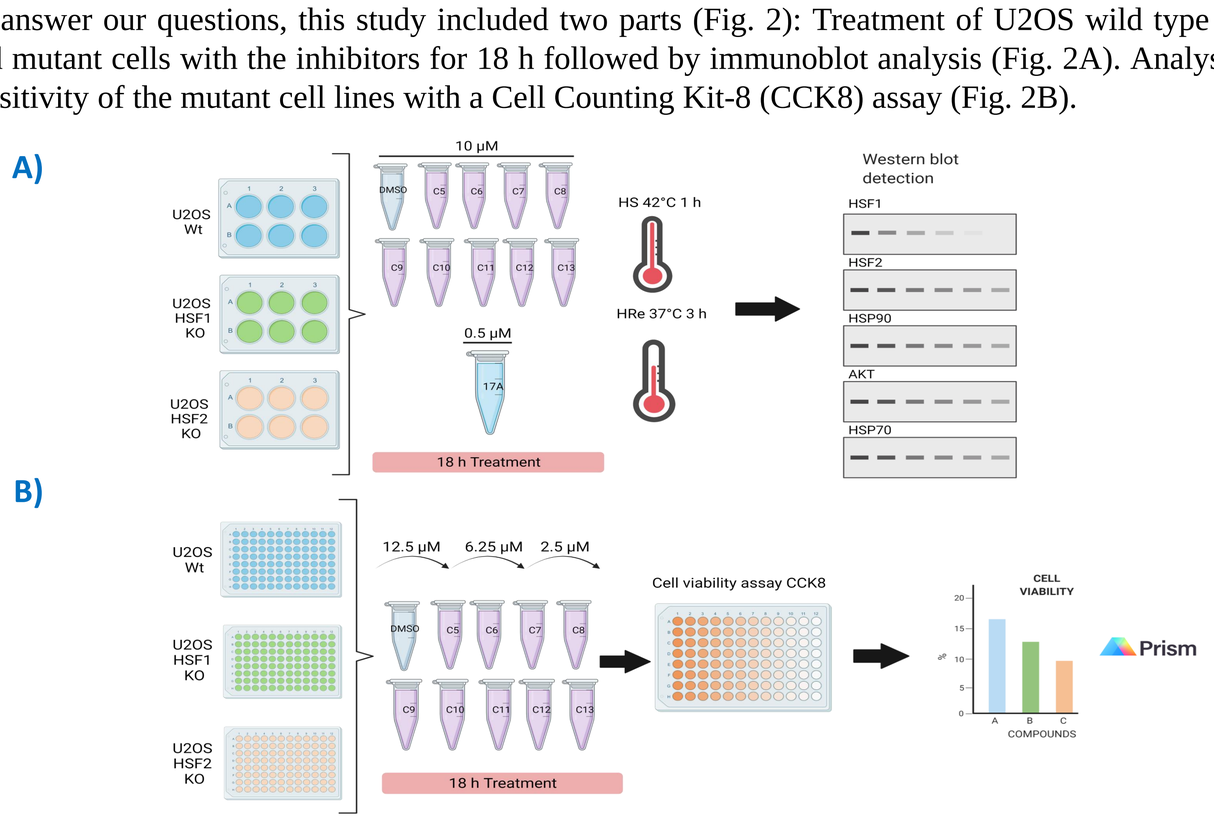


**Figure 2. Methods used in the study**. **A)** U2OS wild type (Wt) cell lines, U2OS HSF1 knock out (KO) and HSF2 knock out (KO) cell lines were treated with 9 novel isoform-specific inhibitors (C5-C13) at 10 µM for 18 h. For heat shock control, cells were treated with heat shock (HS, 42ºC) for 1 h with 3 h recovery (HRe, 37ºC), and 17-AAG (17A) at 0.5 µM. The presence of HSF1, HSF2, HSP90, AKT and HSP70 was determined by immunoblot analysis. **B)** U2OS Wt cell lines, U2OS HSF1 KO and HSF2 knock out KO cell lines were treated for 18 h with 9 novel isoform-specific inhibitors (C5-C13) at 12.5 µM, 6.25 µM and 2.5 µM prepared as serial dilutions. After the treatment, in order to test sensitivity to the compounds, a cell viability assay with CCK8 assay was performed. Statistical analyses were performed with Graph Pad Prism.

## Results

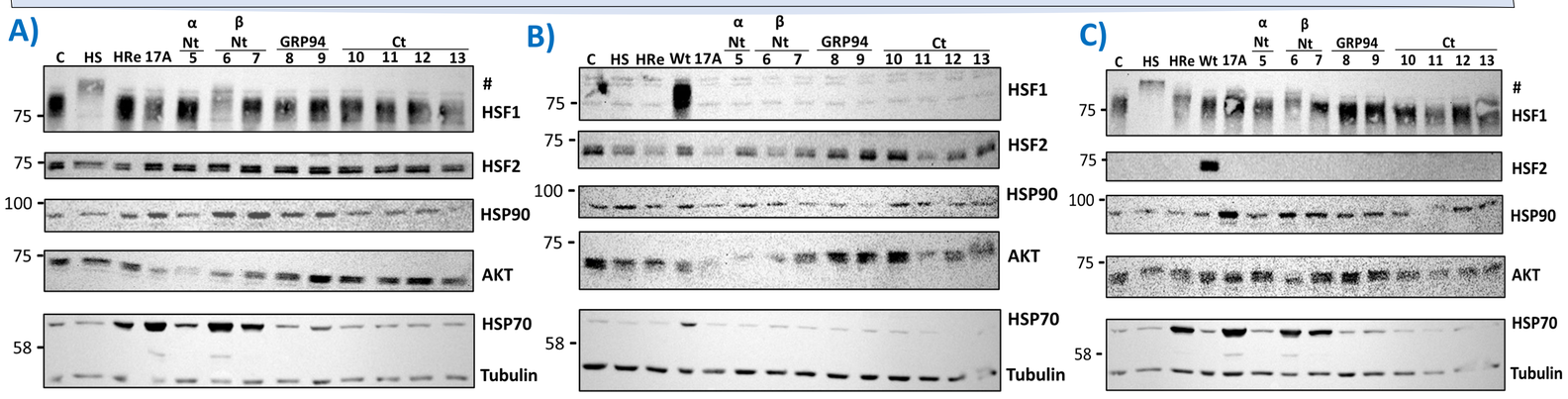


**Figure 3. Beta-N terminal isoform inhibitors induce a heat shock response.** Representative Western blots (n=1) analysis of the 18 h treatment of **A)** U2OS Wt cell lines **B)** HSF1 KO cell lines, **C)** HSF2 KO cell lines with 17A at 0.5 µM and the 9 novel isoform-specific HSP90 inhibitors (5-13) at 10 µM. Cell lines were treated with 1 h heat shock (HS) at 42ºC followed by 3 h recovery (HRe) at 37 ºC. Control was treated with DMSO. DMSO; dimethyl sulfoxide. 17A (17-AAG); 17-(Allylamino-17-demethoxygeldanamycin). # indicates hyperphosphorylation of HSF1.

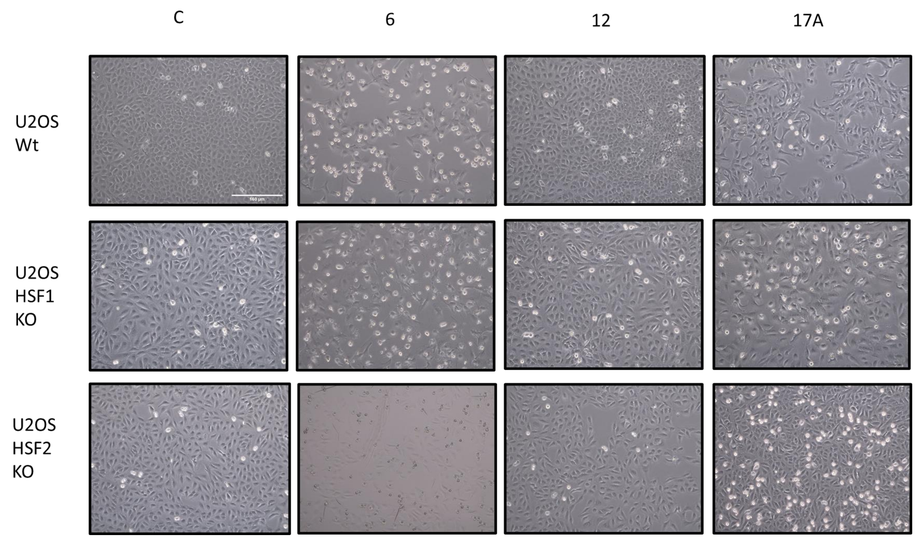


**Figure 4. Beta N-terminal inhibitor induces cytotoxicity in all cell lines.** Cell morphology microscopy pictures showing cytotoxicity of compound 6 in comparison with DMSO (C), compound 12 at 10 µM, and 17A at 0.5 µM in U2OS Wt cell lines, HSF1 KO cell lines and HSF2 KO cell lines. (20x magnification). DMSO; dimethyl sulfoxide. 17A (17-AAG); 17-(Allylamino-17-demethoxygeldanamycin). Scalebar = 160 µm

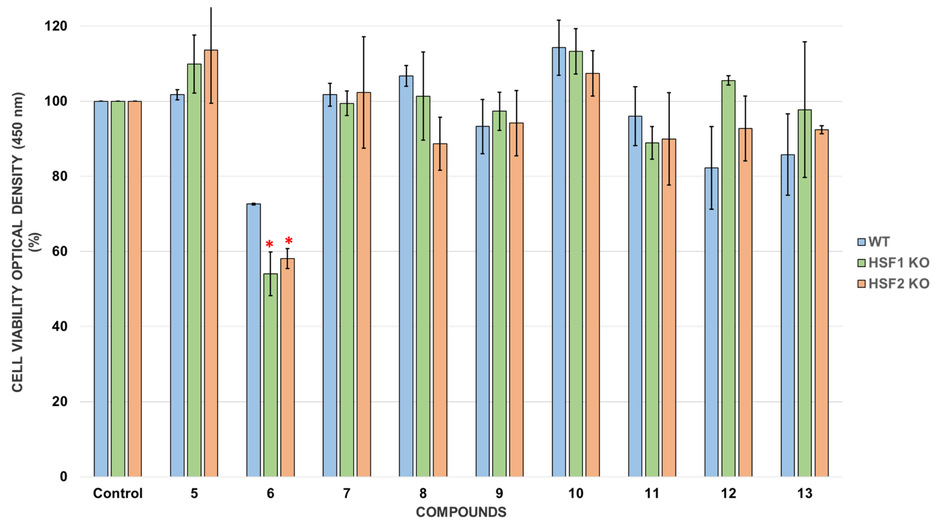


**Figure 5. Comparison of the viability of the cell lines when treated with novel isoform-specific HSP90 inhibitors.** The CCK8 assay results presented as bar graphs for the cytotoxicity effects of the 9 novel isoform-specific HSP90 inhibitors (5-13) at 12.5 µM in U2OS Wt cell lines, HSF1 KO cell lines and HSF2 KO cell lines for 18 h. Cell viability was determined by CCK8 analysis. Data are presented as the means ± standard error (*e.e*) from four independent experiments. One way ANOVA *p<0.05 vs control group. Control was treated with DMSO. DMSO; dimethyl sulfoxide.

## Conclusion

- Beta-N terminal inhibitors induce a heat shock response in both Wt and HSF2 KO cell lines
- GRP94 isoform inhibitors and C-terminal inhibitors seem to induce a weak heat shock response.
- In terms of compound 6, Beta N terminal inhibitors induce a cytotoxic effect on all cell lines.

## Future perspectives

- Is the induction of the heat shock response because of the disruption of HSP90-HSF1 or HSP90-HSF2?
- Why are HSF2 KO cells more sensitive to HSP90 inhibitors?
- Further characterization of GRP94 and C- terminal inhibitors

## References

Figures were created with Biorender.com

1. Hoter, A., El-Sabban, M., & Naim, H. (2018). The HSP90 Family: Structure, Regulation, Function, and Implications in Health and Disease. *Molecular Sciences*, *19*(2560), 1–33
2. Chaudhury, S., Keegan, B. M., & Blagg, B. S. J. (2021). The role and therapeutic potential of Hsp90, Hsp70, and smaller heat shock proteins in peripheral and central neuropathies. *Medicinal Research Reviews*, *41*(1), 202–222. https://doi.org/10.1002/med.21729

UNIVERSITY OF TURKU

Åbo Akademi University