Reflections and Outlook on Targeting HSP90, HSP70 and HSF1 in Cancer: A Personal Perspective

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Abstract

This personal perspective focuses on small-molecule inhibitors of proteostasis networks in cancer—specifically the discovery and development of chemical probes and drugs acting on the molecular chaperones HSP90 and HSP70, and on the HSF1 stress pathway. Emphasis is on progress made and lessons learned and a future outlook is provided. Highly potent, selective HSP90 inhibitors have proved invaluable in exploring the role of this molecular chaperone family in biology and disease pathology. Clinical activity was observed, especially in non small cell lung cancer and HER2 positive breast cancer. Optimal use of HSP90 inhibitors in oncology will likely require development of creative combination strategies. HSP70 family members have proved technically harder to drug. However, recent progress has been made towards useful chemical tool compounds and these may signpost future clinical drug candidates. The HSF1 stress pathway is strongly vali-

dated as a target for cancer therapy. HSF1 itself is a ligandless transcription factor that is extremely challenging to drug directly. HSF1 pathway inhibitors have been identified mostly by phenotypic screening, including a series of bisamides from which a clinical candidate has been identified for treatment of ovarian cancer, multiple myeloma and potentially other cancers.

Keywords

HSP90 · HSP70 · HSF1 · Chemical probes · Drug discovery · Biomarkers · Translational research

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11.1 Introduction

It seems very fitting that a collection of articles on proteostasis networks in cancer – and one that is dedicated to the extraordinary life and work of Sue Lindquist – should conclude with a piece on experience with preclinical and clinical translation in this area. I feel privileged to be invited to provide a short article on my reflections on progress made, lessons learned and an outlook for the future in this field. This brief commentary is written very much as a personal perspective – based on more than two decades of involvement working on chemical biology and drug discovery

around HSP90, HSP70 and HSF1 as molecular targets. I will mainly illustrate the progress and challenges based on the work carried out in my own laboratory – in association with many talented colleagues and collaborators. I will also refer to the work of others, but I apologise to the many excellent scientists in the field that it is in the nature of a short personal perspective that exhaustive referencing is not possible.

Sue Lindquist was an outstanding scientist and true pioneer in the field of proteostasis. Her curiosity and great aptitude for the basic science coupled with a strong desire to make a difference to human disease led her to work and collaborate across many research boundaries. Such an approach is often critically important to make real breakthroughs in fundamental research and is certainly essential to translate basic research into clinical benefit. In this and in her contributions to the development of the field and the careers of scientists who work in it, Sue was an inspiration to us all.

11.2 Therapeutic Targeting of HSP90

My lab started working on the Heat Shock Protein (HSP) and molecular chaperone HSP90 in the mid-to-late 1990's as a serendipitous result of the convergence of the two main strands of our molecular pharmacology and drug discovery research at the time. The first strand was the design of drugs that would selectively kill cancer cells by virtue of their preferential bioreductive conversion to cytotoxic metabolites – either as a result of the hypoxic nature of solid tumours or/and the increased expression of enzymes that catalyse their bioreductive activation (Workman and Stratford 1993). The second strand was the design of drugs that exploit and counteract the oncogenic pathways involved in the maintenance and progression of cancer, now referred to as 'oncogene addiction' (Brunton and Workman 1993).

The coalescing of these two strands is illustrated by a study carried out in my lab to test two hypotheses to explain the molecular mode of action by which the natural product geldanamycin – a member of the benzoquinone ansamycin class of natural product antibiotic – exerts its

nonclinical anticancer activity in human colorectal cancer cells. See Fig. 11.1 for the chemical structure of geldanamycin and other selected compounds and drugs referred to in this article. The two possibilities we tested were that the anticancer effect was mediated either through: (1) bioreductive activation of the quinone moiety catalysed by the flavoenzyme oxidoreductase NAD(P)H: quinone oxidoreductase (EC 1.6.99.2; known as NQO1 and previously as DT-diaphorase); or (2) depletion of the cellular c-SRC oncoprotein as a result of inhibition of the molecular chaperone HSP90. These two hypotheses did not appear from thin air. Exemplified by research on another natural product called mitomycin C, the bioreductive metabolic activation of quinone-group containing agents by NQO1 and other oxidoreductases to produce DNA-binding metabolites was well precedented as an approach to killing cancer cells through the work of the groups of Alan Sartorelli (Sartorelli 1988) and later David Ross (Ross et al. 2000) and others. Indeed, our own lab showed that cancer cell sensitivity to both mitomycin C and the synthetic indoloquinone EO9 is determined by the level of expression of NQO1 – which is often very high in some cancer cell lines that tend to be sensitive, or alternatively low or absent in others that tend to be resistant (Walton et al. 1992; Fitzsimmons et al. 1996). The alternative hypothesis that we tested was that the anticancer effect of geldanamycin and related agents such as herbimycin is a result of the inhibition of the activity of cellular SRC – a kinase that has been implicated in colorectal cancer initiation and progression (Cartwright et al. 1990; Garcia et al. 1991).

In our initial study we largely ruled out that geldanamycin kills human colorectal cancer cells through either bioreductive metabolism by NQO1 or inhibition of cellular c-SRC kinase activity (Brunton et al. 1998). Firstly, we showed that although NQO1 does reduce geldanamycin this could not explain its anticancer activity. And secondly, we observed no effect on cellular c-SRC at pharmacologically active concentrations. We did observe a decrease in c-SRC kinase activity in the colorectal cancer cells at very high concentrations and prolonged exposure to geldanamycin, but this is due to a reduction in

Fig. 11.1 Chemical structures of selected examples of compounds and drugs referred to in this article

overall c-SRC protein levels. Indeed, there had been previous reports of depletion of SRC and other tyrosine kinase oncoproteins in response to geldanamycin and herbimycin A. Furthermore, in what is now regarded as a seminal discovery in the field published by Luke Whitesell working in Len Neckers lab, it was shown that the major target to which geldanamycin is bound in the cell is a 90 kDa protein that was identified as the heat shock protein HSP90 – and moreover it was demonstrated that this led to the disruption of the known interaction between HSP90 and the oncogenic viral form of the tyrosine kinase v-SRC (Whitesell et al. 1994). These findings explained

the previously demonstrated ability of geldanamycin to revert the oncogenic transformation of mammalian cells by viral SRC, rather than inhibiting SRC's intrinsic tyrosine kinase activity, and pointed to HSP90 as a potential target in cancer by bringing about the depletion of oncogenic client proteins by proteosomal degradation. Despite this interesting and attractive mechanism of action, geldanamycin did not progress to clinical studies due to concerns about liver toxicity.

However, semi-synthetic derivatives of geldanamycin subsequently showed more promise as drugs. Thus we later returned, with more translational success this time, to study both the impact of

bioreductive metabolism of the quinone moiety and also the depletion of oncogenic kinases and other 'client' proteins of the HSP90 molecular chaperone following exposure of cancer cells to the analogue of geldanamycin that was commonly (and still is) referred to as 17-AAG (17-allylamino-17-demethoxygeldanamycin) – subsequently named tanespimycin. This drug was progressed to the clinic because it maintained the anticancer effect of geldanamycin but was better tolerated in animals and has an acceptable therapeutic index. In collaboration with colleagues at the US National Cancer Institute (NCI) we showed that in contrast to geldanamycin – but similar to the bioreductive quinones mitomycin C and EO9 (see above) – there is a clear correlation between sensitivity to tanespimycin and NQO1 expression in the NCI collection of 60 human cancer cell lines (the so-called 'NCI 60' panel), with greater sensitivity seen in those lines with high NQO1 and lower sensitivity in those with low expression, including lines with a polymorphism resulting in reduced enzyme activity and stability (Kelland et al. 1999). We confirmed a causal relationship in an isogenic pair model either lacking NQO1 or exhibiting high expression. The strong dependence of cancer cell sensitivity on NQO1 of tanespimycin in contrast to geldanamycin was explained by our demonstration in the same paper that 17-AAG is a much better substrate for NQO1 than geldanamycin. Subsequent work by the Ross lab and others showed that the reduced hydroquinone form of tanespimycin is a more potent binder of HSP90 than the parent quinone 17-AAG itself (Guo et al. 2005).

I would like to make three observations about our discovery of NQO1 as a mechanism-based biomarker of tanespimycin sensitivity. The first point is that although we emphasized in several publications that NQO1 expression should be considered when comparing the sensitivity of different cancer cell lines to tanespimycin *in vitro*, this predictive biomarker relationship is generally ignored. Note, however, that although important in cell culture, NQO1 is likely to be less important in animal (and human) studies due to the metabolism of tanespimycin to 17-amino-geldanamycin – which we showed behaves like

geldanamycin in being independent of NQO1 (Kelland et al. 1999). The second point is that the NQO1 effect is, of course, absent from non-quinone HSP90 inhibitors and not relevant for these (ref. (Kelland et al. 1999) and see later). The third point is that it was pleasing when our demonstration of NQO1 as a predictor of cancer cell sensitivity to tanespimycin – that we made using the NCI-60 cell panel – was subsequently confirmed as one of the strongest predictive genetic-pharmacology relationships in the first two major publications on the much bigger panels of hundreds of cancer cell lines that are now commonly used for such correlative analysis and predictive biomarker discovery (Barretina et al. 2012; Garnett et al. 2012). We discussed these findings in a commentary (Workman et al. 2012).

In our first above-mentioned publication on tanespimycin (Kelland et al. 1999) we demonstrated depletion of CRAF and mutant p53 as representative client proteins in cancer cells and the increased expression of HSP72 as a representative heat shock gene product that is upregulated in an HSF1-dependent manner following HSP90 inhibition. Following earlier work on geldanamycin and another natural product radicicol by others, we showed in human colon cancer models that tanespimycin inhibits oncogenic signal transduction in the MAP kinase and PI3 kinase pathways through depletion of HSP90 clients such as CRAF and AKT, resulting in cytostasis and apoptosis (Hostein et al. 2001). Similar findings were made by other labs, including the Neal Rosen group, showing for example that tanespimycin induces G1 cell cycle arrest, apoptosis and morphological and functional differentiation in breast cancer cells and cell cycle arrest and anti-tumour activity in prostate cancer models, both associated with depletion of the highly sensitive oncogenic HSP90 client protein HER2/ERBB2, steroid hormone receptors and other client proteins (Münster et al. 2001; Basso et al. 2002; Solit et al. 2002). Both the Rosen team and also the Richard Marais lab in collaboration with our group discovered independently that many mutant forms of BRAF, including the common V600E mutant that is the key oncogenic driver of melanoma and other cancers, are more highly

dependent on HSP90 and are more rapidly depleted by 17-AAG treatment (Grbovic et al. 2006; da Rocha Dias et al. 2005) – an effect subsequently seen with other mutant/wild type protein pairs.

Our lab was an earlier adopter of gene expression microarrays which we used to profile large-scale transcriptional changes in response to HSP90 inhibition (Clarke et al. 2000) and we also used mass spectrometry-based proteomics to profile broad changes in cellular proteins at scale (Maloney et al. 2007). Studies from many labs have detailed the depletion by proteosomal degradation of multiple oncogenic HSP90 client proteins following inhibition of the molecular chaperone. An online listing of the very large number of client proteins and other HSP90 interacting proteins is maintained by Didier Picard (HSP90 Interactors [n.d.](#)). A large-scale study from the Lindquist lab provided a systematic and quantitative survey – using the LUMIER assay system – of human kinases, transcription factors, and E3 ligases with respect to interaction with HSP90 and its cochaperone CDC37 (Taipale et al. 2012). This comprehensive analysis confirmed and enhanced our appreciation that many kinases interact with HSP90 but with different binding affinities – concluding (1) that kinase client binding specificity is dictated by CDC37 and (2) that the extent of depletion after HSP90 inhibition is greatest for strong binders and least for weak binders. A further conclusion was that the interaction of kinases with the HSP90/CDC37 system is dictated by the thermodynamic stability of their kinase folds. HSP90 client kinases are intrinsically less stable than non-client kinases. This finding helped us to understand why the ability to act as an HSP90 client protein cannot be predicted from the DNA or protein sequence. The study also revealed that relatively few transcription factors interact with HSP90, in contrast to the many E3 ligases and the majority of kinases (Taipale et al. 2012).

A number of protagonists supported the concept of progression of HSP90 inhibitors, including our own lab and those of Len Neckers, Neal Rosen, Ed Sausville, Gabriella Chiosis and Francis Burrows. For example, in ref. (Workman

et al. 2007) a group of us reviewed the potential advantages of HSP90 inhibitors, especially the simultaneous and combinatorial degradation of multiple oncogenic client proteins, resulting in the blockade of multiple oncogenic pathways and antagonism of all of the pathological hallmark traits of malignancy – with cancer selectivity being achieved by exploiting cancer dependencies and vulnerabilities, including both oncogene addiction and the stressed state of malignant cells. We also argued that multiple downstream effects of HSP90 inhibitors should make the development of resistance more difficult compared to drugs exhibiting more limited effects. Of course the other side of the coin is the potential for significant toxicity, even though many of the affected proteins and pathways are more important to cancer compared to normal cells.

Working with our collaborators and supported by the US National Cancer Institute and what is now Cancer Research UK, it was exciting for us when we took tanespimycin into a first-in-human clinical trial in the Drug Development Unit of the Institute of Cancer Research and our hospital partner the Royal Marsden. To enable pharmacokinetic-pharmacodynamic (PK-PD) studies, we validated a protein biomarker signature of HSP90 inhibition, comprising depletion of HSP90 client proteins CRAF and CDK4 together with increased expression of HSP72 (Banerji et al. 2005a). CRAF and CDK were selected as biomarkers that are depleted, because of their applicability for measurement in peripheral blood lymphocytes (PBLs) and multiple cancers. In addition, HSP72 represents an ‘up’ biomarker that is robustly induced upon HSP90 inhibition across a wide range of biological contexts. It should be noted that HSP72 is a more sensitive biomarker of HSP90 inhibition, occurring at lower tanespimycin concentrations and doses; however, depletion of client proteins, which requires higher exposures, is more likely to be indicative of therapeutic effect. Evidence of HSP72 induction in the absence of client protein depletion is not sufficient to conclude that the degree of target modulation will have a reasonable chance of seeing a therapeutic effect. The combined biomarker signature of HSP72 induc-

tion and client protein depletion was used in our hypothesis-testing, PK-PK biomarker-led Phase I study of tanespimycin (Banerji et al. 2005b) that applied the Pharmacological Audit Trail (PhAT) framework that my colleagues and I developed for use in nonclinical and clinical studies to link target modulation to biological effects (Banerji and Workman 2016; Workman 2003). PK properties were consistent with target coverage and the PD biomarkers demonstrated HSP90 inhibition in PBLs and tissue biopsies. Our Phase I clinical study of tanespimycin provided proof-of-concept that HSP90 could be inhibited at doses that were tolerated by cancer patients. Dose-limiting side-effects were defined as diarrhoea and liver toxicity. Two patients with metastatic melanoma had stable disease for 15 and 49 months respectively; of note is that these patients' tumours had a V600E BRAF and a G13D NAS mutation, respectively, and thus both cancers would have contained active forms of the HSP90 client protein BRAF (Banerji et al. 2008).

Tanespimycin progressed to a large number of Phase II studies and showed its most promising activity in combination with the HER2 antibody trastuzumab in patients with HER2-positive metastatic breast cancer who were progressing on trastuzumab (Modi et al. 2011). The trial definitively showed objective responses according to Response Evaluation Criteria in Solid Tumors (RECIST) in these patients and the overall response rate was 22% with a clinical benefit rate of 59%. The effectiveness of tanespimycin in HER2-driven cancers makes mechanistic sense given that HER2 is one of the most highly sensitive HSP90 client proteins to HSP90 inhibition. However, despite its promise the clinical development of tanespimycin as a cancer therapy was terminated by the company for nonclinical, potentially commercial reasons such as costly production/formulation and patent expiry concerns (Arteaga 2011; Neckers and Workman 2012; Garcia-Carbonero et al. 2013). Subsequently, early clinical trials were carried out with additional geldanamycin analogues – specifically alvespimycin which is less dependent on NQO1 (see earlier) and the soluble stabilized hydroquinone form of tanespimycin,

retaspimycin, but these have not progressed further. Factors that have been considered as limitations for tanespimycin and its analogues include insufficient depletion of key client proteins, the requirement for activation by NQO1, and side-effects – including the liver toxicity that may have been related to the quinone moiety undergoing bioreductive activation, as discussed above (see also ref. (Neckers and Workman 2012)).

Nevertheless, the proof of concept and promising activity in breast cancer shown by tanespimycin stimulated research to discover second generation, wholly synthetic, non-quinone inhibitors that might overcome the limitations of the geldanamycin class. Two particular new classes initially led the way, namely (1) the purine inhibitors based on PU-3 from Chiosis and colleagues at Memorial Sloan Kettering leading to the clinical candidate PU-H71 and others from Conformia/Biogen (Chiosis et al. 2001; Speranza et al. 2018) and (2) the resorcinol-type compounds exemplified by ganespib from Synta Pharmaceuticals (Ying et al. 2012) and luminespib from ICR/Vernalis (Eccles et al. 2008; Sessa et al. 2013). Numerous other structurally diverse inhibitors were subsequently discovered and many progressed to the clinic (Travers et al. 2012).

Our own drug discovery research on HSP90 began with our identification by high-throughput screening at the ICR of the diaryl pyrazole resorcinol CCT018159 (Cheung et al. 2005; Sharp et al. 2007a). Improvements on this chemical starting point were enabled by the crystal structure of HSP90 solved by our collaborator Laurence Pearl and colleagues (Prodromou et al. 1997) which revealed precisely how CCT018159 is bound at the nucleotide site in the N-terminal domain of HSP90, mimicking the binding mode of the resorcinolic natural product radicicol (Roe et al. 1999). Our structure-based optimization based on the screening hit CCT018159 yielded more potent, advanced leads and chemical tools, specifically the potent resorcinolic pyrazole/isoxazole analogues VER-49009 and VER-50589 (Sharp et al. 2007b) and in turn resulted in our intravenously administered clinical candidate, the 4,5-diaryl isoxazole resorcinol luminespib

(Eccles et al. 2008; Brough et al. 2008). In addition, our collaboration between ICR and Vernalis yielded the oral back-up clinical candidate NVP-BEP800 from a 2-aminothieno[2,3-*d*]pyrimidine series, derived from a fragment-based and *in silico* hit-finding approach and optimized by structure-based design (Brough et al. 2009). Both series were licenced to Novartis.

Luminespib was the first of the second generation non-geldanamycin HSP90 inhibitors for which full results of a phase I study were reported (Sessa et al. 2013). In this Phase I clinical trial of intravenous luminespib led by Udai Banerji, the main side-effects were diarrhoea, asthenia/fatigue, anorexia, atrial flutter and visual symptoms and the recommended phase II dose (RP2D) was 70 mg/m². Application of the PhAT showed that the plasma concentrations of luminespib that were achieved were consistent with those that produced therapeutic effects in a range of human tumour xenograft models. In addition, evidence of target modulation was obtained in peripheral blood mononuclear cells (dose-dependent HSP72 induction) and tumour tissue (HSP70 induction and depletion of client protein AKT in two ER-positive patients at 70 mg/m²). There were no radiological responses, which was attributed potentially to the fact that patients were not molecularly prioritized based on client protein status. However, dose-dependent metabolic responses were observed in a number of patients as measured by a reduction of ¹⁸F-fluorodeoxyglucose uptake by PET scan. Based on these Phase I data, Phase II studies were initiated with the dose of 70 mg/m² in breast, gastric and non-small cell lung cancers with appropriate HSP90 client protein dependencies.

Evidence of activity of luminespib was observed in HER2-positive and ER-positive breast cancer (Schroder et al. 2011; Kong et al. 2016). A Phase II trial with luminespib in NSCLC included patient cohorts with EGFR-mutant, ALK-rearranged and KRAS-mutant disease (Felip et al. 2018). The overall response rate of 13% was modest and no objective responses were observed in patients with oncogenic KRAS mutation. In contrast, the most favourable outcome

was seen in patients with oncogenic ALK rearrangement, for whom the objective response rate was 32%, although the median progression-free survival was less than 3 months and no activity was detected against brain metastases, which is a common problem in patients with ALK-rearranged NSCLC. Overall, it was concluded that luminespib had a manageable safety profile and is active in patients with both ALK rearrangement and EGFR mutations, including the gatekeeper mutant T790M, which exhibited resistance to EGFR tyrosine kinase inhibitors. Of interest was the activity of luminespib in a Phase II trial in NSCLC patients harbouring oncogenic EGFR exon 20 insertion mutants who at the time were refractory to available EGFR tyrosine kinase inhibitors, as also seen in nonclinical models (Piotrowska et al. 2018).

Ganetespib has progressed all the way to a Phase III study (known as (GALAXY-2) comparing the HSP90 inhibitor plus the taxane docetaxel versus docetaxel alone in advanced NSCLC (Pillai et al. 2017). The trial showed that there was no significant difference in median overall survival for the two arms (10.9 months for the combination versus 10.5 months with docetaxel alone. Of interest, ganetespib appeared to have lower ocular toxicity than luminespib at the respective doses used, potentially related to the higher exposure of the retinal epithelium to the hydrophilic luminespib compared to the more hydrophobic ganetespib (see ref. (Piotrowska et al. 2018). A weakness of the trial design was that the patients were not molecularly stratified, for example to include only ALK-rearranged and EGFR-mutant. It is also possible that once or twice a week dosing may not give sufficient depletion of key oncogenic client proteins. A commentary on the study (Pillai and Ramalingam 2018) suggested that newer orally administered HSP90 inhibitors could be trialled in molecularly stratified patients with NSCLC, particularly if it was possible to dose the drug so as to maintain continued suppression of the relevant HSP90 client oncoproteins. However, the authors pointed out that there are now a range of kinase inhibitors and immunotherapies in NSCLC, together with chemotherapy combinations, which may poten-

tially decrease the interest in pursuing HSP90 inhibitors in this setting at this time (Pillai and Ramalingam 2018).

In a retrospective review of 158 patients treated with various HSP90 inhibitors (tanespimycin, alvespimycin, retaspimycin, ganetespib and CNF2024) at Memorial Sloan Kettering Cancer Center, sufficient tissue to allow biomarker assessment was available for 51 patients and it is noteworthy that 13 of 16 responses strongly correlated with HER2-positive status (Jhaveri et al. 2016). The authors concluded that their findings were consistent with the preclinical data demonstrating that HER2 is the most sensitive client protein of HSP90 inhibition and suggested that this biomarker requires prospective validation in larger studies.

Overall, the experience to date with HSP90 inhibitors in the clinic is that doses can be given that provide evidence of pharmacological inhibition of HSP90. However, greater reliance on HSP72 as a PD biomarker and relatively little information on depletion of oncogenic client proteins, which requires higher exposures, means that it remains unclear whether in the trials described above the key client oncoproteins have been depleted to a sufficient extent and for adequate time to reveal the full therapeutic potential of HSP90 inhibitors. Concerning side-effects, the first-generation geldanamycin analogues exhibited liver toxicity that is potentially related to the quinone moiety – whereas ocular effects, typified by reversible night blindness, blurred vision, and flashing lights, were more of a limitation with non-geldanamycin inhibitors, to a degree that varies between the different second generation inhibitors and may relate to physical properties and retinal exposure.

In a review of HSP90 inhibitors in 2015, we focused on potential approaches to maximize the therapeutic potential of these agents (Butler et al. 2015). Possibilities included optimizing dosing and schedule through use of PD biomarkers; dissecting and exploiting the complex molecular and cellular response to HSP90 inhibition, including effects of co-chaperones; and use of combinatorial drug strategies, including the application of a one-two punch approach –

employing a combination of drugs that directly inhibit the function of a key client protein (eg. kinase activity using a kinase inhibitor) together with an HSP90 inhibitor to deplete the overall protein level. Of interest in this regard is our observation that ATP-competitive protein kinase inhibitors can, as single agents, deplete the levels of the target kinase by blocking protein kinase recruitment to the HSP90-CDC37 system (Polier et al. 2013). In support of the kinase-HSP90 inhibitor one-two punch idea, it has been shown that inhibition of HSP90 by the resorcinol drug onalespib delays the emergence of resistance to BRAF kinase inhibition and overcomes resistance to dual BRAF and MEK inhibition in melanoma models (Smyth et al. 2014). In addition, relatively low level HSP90 inhibition blocks the emergence of resistance to anti-oestrogens in breast cancer models (Whitesell et al. 2014). Note also that we have shown that onalespib blocks mRNA splicing of androgen receptor variant 7 in prostate cancer cells (Ferraldeschi et al. 2016). This suggests an approach to overcome resistance mediated via the splice variant, in addition to the more conventional HSP90 inhibitor mechanism of proteasomal depletion of the wild type receptor.

Efforts continue to design novel classes of HSP90 inhibitors with distinct properties. One example is the identification of inhibitors with different HSP90 paralog selectivity profiles (Huck et al. 2019). Another is a series of inhibitors based on the novobiocin structure that bind at or close to the HSP90 C-terminal dimerization domain, potentially involving a putative second nucleotide-binding pocket, from which some analogues are reported to have reduced propensity to induce the heat shock response (Neckers et al. 2018 and see Chap. 9). In addition, we need to remain alert to the identification of cancers not considered previously that might be responsive to HSP90 inhibitors. A possible example is our recent demonstration of the promising activity of HSP90 inhibitors in nonclinical models of bile duct cancers known as cholangiocarcinomas – for which microRNA 21 (miRNA21) appears to mediate resistance by decreasing levels of the DnaJ Heat Shock Protein family (Hsp40) member B5 (DNAJB5) (Lampis et al. 2018). It was

suggested that miRNA21 could be a potential marker of sensitivity for HSP90 inhibitor sensitivity in these hard-to-treat cancers (Lampis et al. 2018).

11.3 Therapeutic Targeting of HSP90 Co-Chaperones, HSP70 and HSF1

It was mentioned above that it may be possible to exploit the complex molecular and cellular response to HSP90 inhibition to enhance therapeutic effectiveness or provide alternative therapeutic targeting. Our lab (often in collaboration with the Pearl group) and others have explored the consequences of depleting co-chaperones such as CDC37 (Smith et al. 2009, 2015), AHA1 (Holmes et al. 2008) and the E3 ubiquitin ligase Cullin-5 (Samant et al. 2014). We showed that depletion of CDC37 sensitizes cancer cells to HSP90 inhibitors and leads to client kinase depletion – but greatly reduced inhibition of binding of CDC37 to HSP90 inhibition does not. Indeed, rather surprisingly, we demonstrated that CDC37 is able to stabilise kinase clients by a mechanism that is not dependent on a substantial direct interaction between CDC37 and HSP90, but nevertheless requires HSP90 activity. These results indicate that pharmacological inhibition of CDC37-HSP90 binding is unlikely to be effective for cancer therapy (Smith et al. 2015). We also showed that knockdown of the HSP90 ATPase-promoting co-chaperone AHA1 decreases client protein activation and increases cancer cell sensitivity to the HSP90 inhibition (Smith et al. 2009). In our work with Cullin-5 (CUL5), we again obtained somewhat surprising results in that knockdown of this E3 ligase modulates multiple molecular and cellular responses to HSP90 inhibition in human cancer cells (Smith et al. 2015). CUL5 was found to be required for degradation of a number of HSP90 clients after treatment with an HSP90 inhibitor. Unexpectedly, silencing CUL5 also slows the earlier loss of HSP90 client protein activity while also delaying co-chaperone dissociation from inhibited HSP90-client complexes. In addition, depleting CUL5

decreased the sensitivity of cancer cells to three distinct HSP90 inhibitors, across four tumour types that are driven by different protein kinases. These findings show that the role of E3 ligases in the pharmacology of HSP90 inhibitors is more complex than previously considered, and not simply concerned with client protein degradation. Pharmacological modulation of E3 ligases is of course of interest. In addition CUL5 may be a potential biomarker for sensitivity to HSP90 inhibitors.

Inhibition of the heat shock response or components of it (other than HSP90 discussed above) is an alternative approach to cancer therapy in the proteostasis area. The heat shock response was of course famously discovered by Ferruccio Ritossa when he observed the effect of the inadvertent increase in a lab incubator temperature on the ‘puffing’ of polytene chromosomes in the salivary glands of *Drosophila*, which he then linked to new RNA synthesis (Ritossa 1962, 1996). The heat shock response was subsequently shown to be mediated by heat shock transcription factor HSF1 – the major transcriptional regulator of the eukaryotic heat shock response and the primary mediator of transcriptional responses to proteotoxic stress – which binds to Heat Shock Elements in heat shock-regulated genes (Rabindran et al. 1991; Ankar and Sistonen 2011). Studies by Voellmy and colleagues showed that the heat shock response can be activated by the release of HSF1 from the inhibitory stress-sensitive complex it forms with HSP90 (Zou et al. 1998). This can be triggered by unfolded proteins binding to HSP90. The mechanisms are, however, quite complex and certainly involve trimerization, nuclear accumulation and post-translational modification of HSF1, leading to activation of induction of a fraction of heat shock genes by increasing RNA polymerase II release from promoter-proximal pause (Mahat et al. 2016).

The Whitesell lab published an important study on the effect of HSP90 inhibitors in mouse embryo fibroblasts derived from Ivor Benjamin’s HSF1 knockout mice as compared with wild type controls (Bagatell et al. 2000). They showed that HSF1 knockout cells exhibited significantly

greater sensitivity to the effect of HSP90 inhibitors, which was linked to failure to activate the cytoprotective heat shock response in knockout cells in contrast to wild type cells; there was no change in response to the cytotoxic agents doxorubicin or cisplatin. Also to note is that, in the same paper, the authors demonstrated that treatment with tanespimycin induced the expression of the representative heat shock protein HSP72 both in normal mouse tissues and human tumour xenografts – an effect subsequently used as part of the pharmacodynamic biomarker signature for HSP90 inhibition in drug discovery and clinical trials (see above).

Given the challenges of inhibiting the HSF1 transcription factor directly, our lab set out to investigate whether inhibiting key components of the heat shock response (other than HSP90) could exert selective anticancer activity and also whether this approach could sensitize cancer cells to HSP90 inhibitors (Powers et al. 2008). HSP70 isoforms are known to contribute to tumorigenesis, for example through their anti-apoptotic activity and their involvement as co-chaperones for HSP90. We showed that silencing the expression of either HSP72 – a well-studied heat shock inducible gene – or heat shock cognate 70 (HSC70) in human cancer cell lines has no effect on the activity of HSP90 in chaperoning client proteins or on cell proliferation. In contrast, simultaneous combinatorial depletion of both of these isoforms induces the proteasome-dependent degradation of HSP90 client proteins, causes G1 cell-cycle arrest and triggers extensive apoptosis – the latter to a much greater extent than pharmacological HSP90 inhibition – all in the absence of HSP90 inhibitors. In contrast, combinatorial silencing of the two HSP70 isoforms in non-tumorigenic cell lines does not cause comparable growth arrest or induction of apoptosis, indicating a potential therapeutic window for this combinatorial approach. The need for HSC70 inhibition in addition to HSP72 was explained by the ability of HSC70 to replace HSP72 in the heterochaperone complex with HSP90. Sensitization to HSP90 inhibitors was also seen in cancer cells. Similar effects of dual

knockdown of HSP72 and HSC70 have also been confirmed by others.

These findings provided validation for the discovery of HSP70 inhibitors. HSP70 proteins are members of the actin family of ATPases for which the ATP site is much more challenging to drug than HSP90 and which has delivered very little success in the discovery of high affinity ligands. One challenging feature is the flexibility of the ATP site, which undergoes numerous conformational changes. The Vernalis team identified novel adenosine-derived ligands through structure-based design and found selectivity towards the GRP78 isoform (Williamson et al. 2009). In our efforts to design HSP70 inhibitors we have also adopted structure-based design strategies (Cheeseman et al. 2016; Jones et al. 2016). In particular, our ICR team took the approach of exploiting protein conformational change to optimize adenosine-based inhibitors of HSP70 (Cheeseman et al. 2016). We provided evidence that such adenosine-derived HSP70 ligands have the potential to bind to the protein with a novel mechanism of action, which involves the stabilization by desolvation of an intramolecular salt-bridge that in turn induces a conformational change in HSP70, leading to high affinity ligands. We also demonstrated that through the application of this mechanism, adenosine-derived HSP70 inhibitors can be optimized in a rational manner. We concluded that improved understanding of the flexibility of HSP70 and the impact of this on the affinity of ligands should contribute to better assay design and enhanced efficiency of inhibitor optimization. We also carried out a comprehensive fragment-based exploration of an HSP70 family enzyme, resulting in the discovery of an amino-quinazoline fragment which we then elaborated to produce novel ATP site binders that exhibited physicochemical properties distinct from previously known adenosine-based HSP70 inhibitors (Jones et al. 2016). In addition, my ICR colleagues discovered an irreversible inhibitor of HSP72 that unexpectedly targets lysine-56 and enhanced this series of targeted covalent inhibitors using rational design and kinetic optimization to yield Compound 18

(Pettinger et al. 2017, 2019). Other groups have also reported HSP70 family inhibitors, in particular Gabriella Chiosis and Jason Gestwicki (Rodina et al. 2013; Gestwicki and Shao 2019). However, to date HSP70 inhibitors have not progressed to the clinic.

Recent attention has turned to the HSF1 transcription factor as a potential target for cancer therapy (Whitesell and Lindquist 2009; De Billy et al. 2009). Although the available structure of the DNA binding domain indicates that it would be extremely tough to drug, HSF1 has been strongly validated, especially by key work in the Lindquist lab, as a critical protein involved in supporting oncogenesis and the tumour state (Dai et al. 2007). Other important work by the Lindquist group showed that HSF1 drives a transcriptional programme that is distinct from heat shock and which supports the malignancy of human cancers; moreover, HSF1 expression is prognostic in several clinical cancers (Mendillo et al. 2012). In addition to cell autonomous effects, HSF1 supports the reprogramming of tumour stroma, contributing to malignancy (Scherz-Shouval et al. 2014).

Recognising the druggability challenge posed by HSF1, we carried out a mechanism-based phenotypic screen to identify small-molecule inhibitors of the HSF1-mediated heat shock response; through this we discovered the bisamide amide class of inhibitors that exhibit a potent and selective effect on the HSF1 pathway in intact cancer cells (Cheeseman et al. 2017). We optimized this series, in particular to enhance intrinsic solubility, resulting in the advanced lead compound CCT251236 which showed therapeutic activity in models of human ovarian cancer and multiple myeloma (Cheeseman et al. 2017; Fok et al. 2018). Using chemical proteomics and validated by biophysical methods of surface plasmon resonance and x-ray crystallography, we identified the putative co-transcriptional regulator pirin as a molecular target that may be responsible for the anti-migration properties of CCT251236 (Cheeseman et al. 2017). Moreover, we designed and synthesized a pirin degrader (CCT367766) which showed potent and specific depletion of pirin in cancer cells; this can be used to

demonstrate in-cell target engagement with pirin (Chessum et al. 2018). Meanwhile we continue to search for additional molecular targets of CCT251236 while progressing a clinical candidate towards first-in-human studies.

11.4 The Importance of Chemical Probes

CCT251236 has been rated positively as a ‘chemical probe’ for pirin at the Chemical Probes Portal (The Chemical Probes Portal [n.d.](#)). High-quality chemical probes are important reagents, used alongside genetic and biochemical tools, for probing biology and disease mechanisms and validating drug targets (Workman and Collins 2010; Frye 2010). A number of small-molecules discussed herein and also other compounds are important chemical probes or ‘tools’ in the chaperone/proteostasis area (Gestwicki and Shao 2019; Shrestha et al. 2016). To be useful, and not misleading, high-quality chemical probes must exhibit essential features or ‘fitness factors’ – particularly high potency and selectivity towards the desired target, together with cellular permeability and evidence of modulation of the target in cells (Workman and Collins 2010; Frye 2010). Unfortunately, the literature is full of examples of the use of chemical agents that lack the characteristics of high quality chemical probes and may even act broadly as chemically reactive compounds or behave as detergents or aggregators. Compounds such as apoptozole and 2-phenylethynylsulfonamide (also known as PES and pifithrin- μ) have been used as chemical probes for HSP70 proteins but have subsequently been shown to be very non-specific (Evans et al. 2015; Schlecht et al. 2013).

My colleagues and I at ICR have provided chemical probes for use by the community, and have also contributed to guidelines for best practice in the selection and use of chemical probes for biological and biomedical research (Workman and Collins 2010; Arrowsmith et al. 2015; Blagg and Workman 2017). We are now hosting the Chemical Probes Portal – a public resource pro-

viding expert peer-viewed recommendations for chemical probes (Arrowsmith et al. 2015). We have also developed a public resource called Probe Miner (Antolin et al. 2018) that is complementary to the Portal in providing objective, quantitative, data-driven assessment of chemical probes, building on our canSAR knowledgebase (Coker et al. 2019). In addition, we recently provided an overview of the public resources available to help in the selection of chemical probes – rather than relying on the literature, general search engines and vendor catalogues that are biased in various ways and lack annotation – and we also included advice on how to navigate the various resources to make an informed choice of chemical probes (Antolin et al. 2019).

11.5 Concluding Remarks and Future Outlook

I hope this personal view has provided an insight into how findings in basic research on proteostasis networks can provide chemical probes and drug candidates for use in oncology research and cancer treatment. I also very much hope that the HSF1 pathway inhibitor will fulfil its potential in showing clinical activity in ovarian cancer – the hard-to-treat disease that cruelly took Sue Lindquist from us way too early.

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Conflict of Interest PW is Chief Executive, President and an employee of the Institute of Cancer Research (ICR) which has a commercial interest in a range of drug targets, including HSP90 and HSF1. The ICR operates a Rewards to Inventors scheme whereby employees of the ICR may receive financial benefit following commercial licensing of a project. PW received research funding rel-

evant to this article from Vernalis, Astex, AstraZeneca, BACIT and Sixth Element Capital/CRT Pioneer Fund. PW contributed to intellectual property on HSP90 inhibitors that was licenced to Vernalis and Novartis and on HSF1 that was licenced to Sixth Element Capital/CRT Pioneer Fund. PW has been a consultant to Novartis, a consultant/scientific advisory board member for NextechInvest, Storm Therapeutics, Astex Pharmaceuticals and CV6, and holds stock in Chroma Therapeutics, NextInvest and Storm Therapeutics. He is also a Non-Executive Director of Storm Therapeutics and the Royal Marsden NHS Trust and a Board Director of the non-profit Chemical Probes Portal.

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