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Heat shock proteins 27, 40, and 70 as combinational and dual therapeutic cancer targets

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Abstract

The heat shock proteins are essential players in the development of cancer and they are prime therapeutic targets. Targeting multiple hsps in dual therapies decreases the likelihood of drug resistance compared to utilizing mono-therapies. Further, employing an hsp inhibitor in combination with another therapy has proven clinically successful. Examples of efficacious strategies include the inhibition of hsp27, which prevents protein aggregation, controlling hsp40's role as an ATPase modulator, and inhibiting hsp70 from acting as a molecular chaperone. While hsp40 therapies are just in the beginning stages, hsp27 and hsp70 therapies have been successfully used in dual inhibition treatments with hsp90 inhibitors and in combinational therapy with antineoplastic drugs. Both dual and combinatorial therapies show encouraging results when used in treating chemotherapeutically resistant diseases.

Keywords

hsp70; hsp40; hsp27; cancer; combination therapy

Heat shock proteins (hsps) are molecular chaperones that facilitate the proper folding and function of proteins. They are classified into families by their molecular weights: hsp100, 90, 70, 60, 40, and the “small hsps”.¹ In normal cells the hsps are responsible for maintaining protein homeostasis. However, during disease the function of hsps are hijacked, aiding the advancement of disease rather than appropriately regulating the cell.² This makes the hsps exciting targets in a wide array of diseases including Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, cardiovascular disease and other protein folding disorders.³ The field of hsps is growing rapidly and a deeper understanding of the heat shock response is leading to numerous therapeutic applications for hsp inhibitors. Given the large therapeutic field associated with hsps we focus on their use in cancer treatment.

In cancer, the hsps facilitate rapid cell division, metastasis, and the evasion of apoptosis.² The dependence of cancer cells upon hsp90 has been successfully exploited in therapeutics. There are currently 49 active clinical trials for hsp90 inhibitors for the treatment of 12 different types of cancer (clinicaltrials.gov). The importance of hsp90 in the proper folding and maintenance of proteins has been well studied and the success of hsp90 inhibitors in the clinic provides rational for investigating the role of other hsps in cancer.^{4, 5}

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While it is the hsp90 multi-protein complex that is ultimately responsible for the formation of a mature and active protein, hsp27, 40, and 70 all play important roles before the unfolded proteins reach hsp90. Specifically, these hsps interact with unique client proteins that cannot be targeted via hsp90 inhibitors (Figure 1A).^{6, 7} Stress induces an increased amount of unfolded proteins in the cytosol relative to unstressed conditions. The unfolded proteins require molecular chaperones for re-folding, which creates a greater dependence of stressed cells (versus unstressed cells) upon the hsps. The major molecular chaperones, hsp90 and hsp70, dissociate from the stress response regulator protein heat shock factor 1 (HSF1), and aid in refolding aggregated proteins (Figure 1B). In addition, translocation of HSF1 to the nucleus promotes the over-expression of all hsps including hsp27, 40, 70 and 90 (Figure 1B). While hsp70 and hsp90 are refolding proteins, hsp27, one of the small hsps, prevents aggregation of unfolded proteins that gather within the cell. Hsp40 is an intermediate protein that complexes with hsp70 interacting protein (HIP), and transfers the unfolded protein to hsp70. Hsp40 also binds directly to hsp70 and stimulates its ATPase activity.^{6, 8} Hsp70 then brings the unfolded protein to the hsp90 complex via heat shock organizing protein (HOP) (Figure 1A)^{9, 10}. Thus, although hsp90 has proven a successful therapeutic target, it is only the final step in this protein-folding scheme. New approaches involve targeting the other hsps (hsp27, hsp40 and hsp70) and focus on inhibiting their function in the protein-folding cycle. Described are the current approaches to cancer therapies targeted at hsp27, 40, and 70.

Targeting hsp27

Hsp27 is a member of the small heat shock proteins and it has a protective effect on cells.¹¹ Activation of HSF1 during cell stress induces up-regulation of hsp27 in many cancer types.¹² Over-expression of hsp27 is also associated with promoting drug resistance, aggressive cancers, metastasis, and poor patient outcomes.^{13,14-17} Its subsequent knock-down restores sensitivity, making development of inhibitors promising for use in combinational (in combination with other chemotherapeutic classes) and dual therapies (two hsp inhibitors). Hsp27 is a ubiquitous protein that is ATP independent and it functions as a molecular chaperone when non-covalently oligomerized.¹⁸ The oligomerization status of hsp27 controls its functionality as a molecular chaperone.¹⁹ The ability to oligomerize is reduced in *in vitro* cell based assays by phosphorylation at serine residues 15, 78, and 84.²⁰ However, *in vivo* oligomerization has been tied to cell-cell contact and is independent of phosphorylation status.²⁰ Hsp27 also regulates client proteins that are involved in the apoptotic pathway including: Akt, p53, and NF- κ B.²¹ In addition it prevents the aggregation of cytoskeletal elements including actin, which is required for the activation of matrix metalloproteinase-2 (MMP2).¹⁴ The function of hsp27 and the role that it plays in cancer were recently reviewed,²² thus, we focus on therapeutic advances that target hsp27.

Hsp27 therapies focus on three distinct approaches. The first involves developing small molecules that bind to the protein directly and inhibit its function.^{23, 24} The second utilizes protein aptamers that bind the protein and disrupt function.²⁵ The third approach employs an antisense oligonucleotide (ASO), which targets the mRNA that encodes for hsp27, thus preventing translation of the protein.

Two molecules are currently under development as small molecule hsp27 inhibitors: quercetin and RP101 (Figure 2). Quercetin is a bioflavonoid that has been widely studied for its anti-cancer properties.²⁶ It inhibits the HSF1 dependent induction of the hsps,^{27, 28} and exhibits anti-tumor effects in prostate, breast, squamous cell, ascites, and gastric cancer cell lines.²⁹⁻³⁴ In addition quercetin potentiates the effects of many first line chemotherapeutic agents including doxorubicin, cisplatin, gemcitabine, and 5-fluorouracil.³⁵⁻³⁶ Via inhibition of hsp27, quercetin reduced the viability of lung cancer cells (A549) *in vitro* (compared to

drug alone) by 40% and 30% when used in a combination treatment with either cisplatin or gemcitabine respectively.²⁴ Co-treatment of quercetin and either cisplatin or gemcitabine against A549 cells that were enhanced for “stem cell-like” properties that contribute to drug resistance, showed that cells had reduced viability by 20% and 30% respectively compared to solo treatment with cisplatin or gemcitabine.²⁴ Despite evidence showing quercetin as a chemo-sensitizer and the use of quercetin in clinical trials for the treatment of chronic pain, sarcoidosis, cystic fibrosis, hepatitis C, and prostate cancer prevention, there are no ongoing anti-cancer trials.³⁷ However, the broad application of quercetin to treat disease highlights the importance of the heat shock response, and further studies on quercetin in relation to specific hsp27 inhibition are warranted.

RP101 (also known as bromovinyldeoxyuridine, BVDU, brivudine) is a nucleoside that binds via π -stacking with Phe29 and Phe33 of hsp27 thereby inhibiting its function (Figure 2B and 3a).²³ RP101 functions as a chemosensitizing agent and prevents the development of resistance.³⁸ *In vitro* testing showed that RP101 prevented resistance of rat sarcoma (AH13r) cells to mitomycin by reducing their growth 5-fold compared to mitomycin alone.²³ Also, when combined with gemcitabine, RP101 reduced invasion of fibrosarcoma cells (HT-1080) by 30-50% compared to gemcitabine alone.²³ In the pilot study RP101 increased the survival of stage III and IV pancreatic cancer patients by 8.5 months compared to controls. RP101 recently finished a phase II clinical trial for the treatment of pancreatic cancer in combination with gemcitabine.³⁹ However, overdosing caused an increase of the toxic side effects of gemcitabine and thus the combination provided a 25% increase in survival only for patients that had a body surface area (BSA) 1.85m^2 compared with gemcitabine combined with placebo.²³ There were no side effects caused by RP101, and more accurate dosing would likely improve the survival rates for all patients regardless of size.²³ Development of second-generation candidates of RP101 are ongoing.³⁸

The second approach to targeting hsp27 utilizes peptide aptamers that bind to the protein and disrupt its function (Figure 3b). Protein aptamers are small amino acid sequences that are designed to bind to a specific protein domain.⁴⁰ The aptamer is designed to outcompete the protein that would bind to that domain, thus inhibiting its function. Currently, two lead peptide aptamers are under investigation: PA11 and PA50. Similar to the small molecule inhibitors of hsp27, peptide aptamers are not effective on their own but are used to sensitize cancers to other therapies. PA11 increased the radio-sensitivity of head and neck squamous cell carcinoma cells (SQ20B) by 47%. PA11 also increased cell death by 15%, 15%, and 20% when used in combination with drugs cisplatin, doxorubicin, or staurosporine respectively, versus treatment with drug alone.²⁵ When tested *in vivo* PA11 reduced SQ20B xenograft growth by 80% after radiation treatment compared to control.²⁵ PA11 prevents hsp27's oligomerization, which leads to hsp27's inability to inhibit early stage protein aggregation and induces proteotoxic stress that ends in cell death.²⁵

PA50 has a different mechanism than PA11, inhibiting hsp27 dimerization, while having little effect on its ability to oligomerize. By inhibiting dimerization, PA50 disrupts hsp27's ability to participate in cell-signaling events thereby interfering with processes essential for cell survival. Similar to PA11, PA50 increases radio-sensitivity of SQ20B by 32% (versus control). PA50 also increased cell death by 20%, 50%, and 25% when used in combination with drugs cisplatin, doxorubicin, or staurosporine respectively compared to drug alone.²⁵ When tested *in vivo* PA50 reduced SQ20B xenograft growth by 50%.²⁵ Although PA50 was more effective *in vitro*, it lacked the potency that PA11 showed *in vivo*. These data suggest that hsp27 oligomerization is a key component of tumor growth. The preclinical success of peptide aptamers suggests this avenue of cancer therapy has potential.

The third approach involves hsp27 antisense oligonucleotide, OGX-427, which acts to decrease the expression of hsp27 (Figure 3c).⁴¹ OGX-427 targets the human hsp27 translation initiation site (5'-GGGACGCGGCTCGGTCAT-3') and prevents the translation of hsp27 mRNA, thereby decreasing the expression of the protein compared to untreated cells.⁴¹ In combination with chloroquine OGX-427 decreases prostate cancer xenograft (PC-3) tumor volume by ~2-fold after 7 weeks compared to chloroquine alone.⁴² A combination of gemcitabine and OGX-427 reduced the average tumor size by 50% in a mouse model of pancreatic cancer (Mia-PaCa-2) compared to treatment with gemcitabine alone.⁴³ In the phase I trial on metastatic bladder cancer preliminary results show that of the 15 patients treated with OGX-427, 33% had complete responses with no pathologic evidence of disease (as observed in post-surgical tissue following 4 doses of OGX-427).⁴⁴ In a phase II trial for castrate resistant prostate cancer (CRPC) where OGX-427 was used in combination with prednisone, 71% of patients were progression-free at 12 weeks, compared to 33% of patients treated with prednisone alone.⁴⁵ The combination of OGX-427 with abiraterone, a successful CRPC drug, is in phase II clinical trials. The current clinical results encourage the use of hsp27-targeted therapy particularly as a combinational clinical therapy.

Targeting hsp40

The function and role of hsp40 (also known as DNAJ) is poorly understood. Consisting of ~49 different isoforms and splice variants, each with a unique and often opposing function, makes targeting hsp40 and understanding its chaperone role very challenging.⁴⁶ Indeed, the large number of isoforms and functions make it significantly less “druggable” than hsp27, 70 or 90. As a family of proteins that contain a “J-domain”, hsp40s are primarily known for their regulation of hsp70's ATPase activity.⁴⁷ Current research focuses on understanding the role of hsp40 isoforms in hsp70's regulation, and their subsequent involvement in disease progression. The isoforms are broken down into three subclasses based on the level of conservation of their J-domain to that in *e. coli*.⁴⁸ In cancer, the down-regulation of subclass I and II hsp40 isoforms including the large splice variant (L) of DNAJA3, DNAJB4, and DNAJB6(L) correlate with a highly aggressive and invasive phenotype.⁴⁹ The structure and role of hsp40 in disease has been recently reviewed and we focus on the recent advances in therapeutics that target hsp40.^{46, 48, 50}

Due to the limited knowledge on hsp40 there are currently only two successful approaches taken in targeting this hsp. Both are in early stages of development. The first is an immunological approach vaccinating for the isoform DNAJB8, and the other is a small molecule targeting hsp40.^{51,52} Most cancers have a lowered expression of hsp40 isoforms, however renal cell carcinoma (RCC) cells express DNAJB8 while normal cells do not.⁵² In addition, when cancer stem cells (CSCs) (which initiate new tumors from a small number of cells) were selected out of the general population of RCCs, the over-expression of DNAJB8 was more pronounced in CSCs than in the main population of RCC cells.⁵² This indicates an importance of DNAJB8 in tumor initiation and marks it as a specific antigen for CSCs making it a good immunological target.⁵² Vaccinating a mouse model of RCC (RenCa) with DNAJB8 DNA showed that relative to control, the treated mice had a significant decrease in tumor size ($p=0.03$).⁵² Further, three of the five mice treated had no tumor growth, even when re-challenged with larger amounts of RenCa cells.⁵² This first report using DNA vaccination to target DNAJB8 for the treatment of cancer was extremely promising.

The second strategy involves a small molecule inhibitor that targets hsp40. Reported this year, the first class of molecules to bind hsp40 are phenoxy-N-arylacetamides, with the most potent compound reporting an $IC_{50} = 130$ nM in a luciferase binding assay (Figure 4).⁵¹ Discovered during a screen for compounds that inhibit Hsp70's ability to fold denatured luciferase, the arylacetamides were investigated for both hsp70 and hsp40 binding.⁵¹ A

thermal degradation assay and regulation assay involving both hsp40 and hsp70 led to the conclusion that this class of compounds modulates hsp40's function. These molecules are promising therapeutic leads as well as exciting tools useful in understanding the function of hsp40 and targeting this complex family of proteins.

Targeting hsp70

Hsp70 refers to a family of chaperone proteins that are 70 kDa and have multiple cellular locations and expression profiles. All of the proteins share homology and contain 2 major domains, a nucleotide-binding domain (NBD) and a substrate-binding domain (SBD). The NBD contains the ATPase pocket and it binds J-domain-containing proteins, such as hsp40 that regulate hsp70's ATPase activity (Figure 5A).⁵³ The SBD contains the EEVD motif, which is responsible for binding client proteins and facilitating protein folding and transportation. Like hsp27, the stress inducible form of hsp70 is up-regulated by HSF1, and hsp70 acts to protect cells during stress.⁵⁴ Hsp70 inhibits the apoptotic pathway by binding to Bax and Apaf-1 preventing them from continuing the apoptotic cascade, and protecting cells from death.⁴ Hsp70 is up-regulated in many cancer types, and it is induced after chemotherapeutic treatments that trigger the heat shock response.⁵⁵ Although somewhat clinically successful, hsp90 therapy's downfall is that it triggers the heat shock response and causes a large increase in hsp70 expression.⁵⁶ This induction leads to cell rescue, drug resistance, and disease advancement.⁵⁷ Hsp70's induction and subsequent ability to rescue tumor cells upon hsp90 inhibitor treatment have lead to exploration of hsp70 as a therapeutic target.

One new area of research involves dual therapy using hsp90 and hsp70 inhibitors. Progress has been slow despite hsp70's critical role in protein regulation and cancer progression and tremendous efforts have produced few advances in hsp70 inhibitors. As the role of hsp70 in cancer was discussed recently,^{53, 54} we focus on hsp70 inhibitors used in combination with other therapies. Current hsp70 inhibitors and their development stage are summarized in Table 1.

Hsp70 inhibitors fall into three basic categories: small molecule inhibitors, protein aptamers, and antibody treatments. The development of small molecule inhibitors has been slow to produce a promising clinical candidate. The only hsp70 small molecule inhibitor that entered clinical trials, MKT-077, was halted due to renal toxicity.^{58, 59} Several compound classes (structures including 15-deoxyspergulin, 3'-sulfogalactolipids, and flavonoids) have demonstrated effects on hsp70 chaperone activity via *in vitro* biochemical assays and they show modest anti-cancer activity (GI₅₀ 2.4–50 μM).^{60–64} Geswiki and Wipf have mapped the binding of small molecule hsp70 ATPase modulators using NMR, and these are promising starts for the development of a drug design profile.⁶⁵ However, their functionality within cells has not been linked to hsp70 inhibition, and some likely act non-specifically within a cellular system.⁶⁶

Three new promising hsp70 inhibitors include: MAL3-101, pifithrin-μ, and VER-155008 (Figure 5B). These inhibitors are minimally effective as single agents against various cancer cell lines, however, they show tremendous promise when used as dual treatment agents. MAL3-101 decreased the IC₅₀ value of hsp90 inhibitor 17-AAG from 400 nM to 30 nM in myeloma cells (NCI-H929), and decreased tumor size ~4-fold compared to vehicle control in a mouse model of the same cancer type when co-administered with proteasome inhibitor PS-341 (bortezomib).⁶⁴ MAL3-101 also increased the cytotoxicity of another proteasome inhibitor, MG-132, in primary multiple myeloma cells by causing a 75% reduction in viability versus 44% when treated with MG-132 alone.⁶⁷

The small molecule Pifithrin- μ increases the effectiveness of known chemotherapeutic agents. When tested against various leukemia cells lines in combination with SAHA (vorinostat) or 17-AAG, pifithrin- μ reduced viability by ~30% and ~37% respectively compared to treatment with SAHA or 17-AAG alone.⁶⁸ VER-155008 was effective in treating multiple myeloma when used in conjunction with hsp90 inhibitor NVP-AUY922. Treatment with a sub-effective dose of NVP-AUY922 and VER-155008 decreased cell viability from 70% to 25% in myeloma cells (INA-6) and from 100% to 10% in myeloma cell line (MM.1S).⁶⁹ In addition, treatment of colon cancer cells (HCT-116) with VER-155008 exhibited no effect when used as a solo treatment, but dual treatment with 17-AAG caused 91% cell death (versus 15% cell death with 17-AAG alone). Utilizing a dual inhibition approach involving hsp90 inhibitor VER-82160 and hsp70 inhibitor VER-155008 caused an 80% reduction in HCT-116 cell survival compared to 21% when cells were treated with VER-82160 alone.⁶⁶ This reduction in survival was accompanied by an increase in caspase3/7 activity showing that the dual treatment is causing apoptosis.⁶⁶ VER-155008 has undergone pharmacokinetics studies in mice, but efficacy studies have yet to be reported. While the production of a viable single hsp70 inhibitor may be the ultimate goal, the difficulties that have been met throughout this endeavor warrant a change of focus. Promising data was generated when a dual therapy approach involving small molecule inhibitors of both hsp90 and hsp70 was utilized.

Aptamers targeting hsp70 were developed for use as sensitizers.⁴⁰ Agonists of both the SBD and the NBD have been designed to attenuate hsp70's function. The most potent aptamer, A17, binds to the NBD of hsp70 and disrupts the function of hsp70 in an *in vitro* biochemical assay (Figure 5A).⁷⁰ Treating HeLa cells with both A17 and cisplatin or 5FU increased cell death by 30%, and 45% respectively compared to treatment with drug alone.⁷⁰ Utilizing *in vivo* mouse models with B16F10 melanoma, showed that 88% of mice treated with a combination of A17 /cisplatin were tumor free, compared with 0% of mice treated with cisplatin alone.⁷⁰ Thus, similar to dual therapy approaches, a combinatorial strategy of an hsp70 inhibitor combined with an antineoplastic treatment was successful. The synergy of A17 was not tested with any other hsp inhibitors but the success of dual therapy strategies seen with hsp70/hsp90 small molecule inhibitors suggests that dual testing of A17 with other hsp inhibitors is the next logical step.^{62, 66, 68, 69}

The third strategy for developing hsp70 inhibitors utilizes the immune system, and is the most promising strategy developed to date. It is the only hsp70-targeted therapy currently in clinical trials (clinicaltrials.gov). Treatment using antibodies in combination with other anti-cancer therapies are already widely used, but they are limited by the lack of tumor specific markers.⁷¹ However, a recently developed monoclonal antibody, cmHsp70.1, successfully recognizes the extracellular motif, TKDNNLLGRFELSG (TKD) of membrane bound hsp70 (Figure 5C).⁷² The expression of membrane hsp70 is unique to tumor cells, thus making the TKD motif an excellent tumor specific biomarker.⁷² In addition, most cancer therapeutics increase the membrane concentration of hsp70 on the tumor cells, which makes targeting them with the antibody relatively straightforward, and highlights the use of this therapy in combinational strategies. Treatment of a colon cancer mouse model (CT26) with cmHsp70.1 alone led to a significantly decreased tumor weight and volume, and to a 20% increased overall survival after 20 days.⁷² CmHsp70.1 made it through a safety and efficacy phase I trial and it is currently in a phase II clinical trials for non-small cell lung cancer in combination with radiochemotherapy.⁷³

Conclusion

While there have been developments in the inhibition of individual hsps, dual inhibition and combination therapies are the future for the hsp field. Targeting multiple hsps will likely

shut down the heat shock response and associated rescue mechanisms, leading to low chemotherapeutic resistance. Dual therapy is established for combined hsp90 and 70 inhibitors and recent investigations show that hsp27 inhibitors potentiate the effects of hsp90 inhibitors in breast cancer.⁷⁴ The interconnectedness of the hsps in the protein folding pathway supports the idea that dual inhibition will increase drug effectiveness compared to solo treatments. The positive clinical results of hsp27 inhibitors RP101 and OGX-427 provide lead structures appropriate for further development. Utilizing these compounds as part of combinational has shown tremendous success. Hsp40 is a newly targeted hsp. There are some promising leads including immunological targeting with vaccines and one class of small molecule inhibitors, but there is much work to be done before the introduction of a clinical hsp40 inhibitor. Much of this difficulty is due to the immense complexity and conflicting physiological roles of the hsp40 isoforms and splice variants. Like hsp27, the early success of the hsp70-targeted immunotherapy in the clinic indicates that hsp70 inhibitors show promise. However, hsp70 is still lacking in small molecule clinical candidates and the small molecules discussed are in the early stages of development. There is much room to grow and improve the status of hsp70 inhibitors, with the most promising data coming from studies utilizing hsp70 inhibitors in dual therapies with hsp90 inhibitors. Similar to the successful strategies taken with treating HIV, the most efficacious approach will likely be combination and dual therapies, where multiple combinations of hsp inhibitors and other chemotherapy agents will be the most effective at treating drug-resistant cancers.

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Figure 1. Hsp over-expression during cellular stress

A) Hsp27 binds to the unfolded proteins that gather in the cytosol during stress. The unfolded proteins are then transferred down the protein-folding pathway, eventually reaching hsp90. The unfolded proteins are then folded by Hsp90 with the assistance of many co-chaperones, including Aha1, CDC37, and p23.

B) Unfolded proteins accumulate during stress and require excess amounts of the hsps to maintain cellular integrity. HSF1 triggers the heat shock response, which is responsible for the up-regulation of the hsps and the rescue of cells otherwise sentenced to death. The inhibition of hsp27, 40, or 70 would cause a major disruption in the rescue pathway by preventing proper protein folding. Improper folding and protein aggregation induces cell death.

Figure 2. Hsp27 small molecule inhibitors

A). Structure of the flavonoid quercetin B) Structure of nucleoside RP101.

Figure 3. Three strategies

A) Small molecule inhibitors and B) peptide aptamers both bind directly to hsp27 protein and disrupt its function. C) Antisense oligonucleotide OGX-427 binds to the sequence of mRNA that corresponds to hsp27 and prevents the expression of hsp27 protein.

Figure 4. Hsp40 inhibitor

Most potent hsp40 inhibitor: phenoxy-N-arylacetamide derivative ($IC_{50} = 0.13\mu M$ in luciferase re-folding assay).

Figure 5. Targeting hsp70

A) Hsp70 contains two major domains the nucleotide binding domain (NBD) and the substrate binding domain (SBD). The aptamer A17 and VER155008 bind to the NBD, pifithrin-μ binds to the SBD, and the antibody cmhsp70.1 binds to a specific extracellular motif known as TKD. B) Selected small molecule hsp70 inhibitors. VER 155008, MAL3-101, and pifithrin-μ present promising leads for developing hsp70 inhibitors to be used in combinational therapies. C) The antibody cmHsp70.1 induces antibody dependent apoptosis. CmHsp70.1 recognizes the TKD motif of hsp70 that is exposed on the surface of tumor cells and elicits an immune response that causes tumor specific apoptosis.

Table 1**Hsp70 therapies**

Current status of hsp70 targeted therapies to date. Nucleotide binding domain (NBD) substrate binding domain (SBD), unknown (UND), cmHsp70.0mAb epitope sequence TKDNNLLGRFELSG (TKD)

Agent	Target Site	Development Level	Disease Target
Pifithrin- μ ⁶⁶	SBD	<i>in vivo</i> mouse model	Cancer
Aptamers ⁶⁸	NBD/SBD	<i>in vivo</i> mouse model	Cancer
ADD70 ⁶⁸	SBD	<i>in vivo</i> mouse model	Cancer
15-DSG ⁶⁰	NBD	Clinical phase II/III (ineffective)	Cancer/Immunosuppressant
MAL3-101 ⁶⁵	UND	<i>in vivo</i> mouse model	Cancer
VER155008 ⁶³	NBD	<i>in vitro</i>	Cancer
Apoptozole ⁵⁹	UND	<i>in vitro</i>	UND
MKT-077 ⁵⁷	NBD	Clinical phase I (toxic)	Cancer/Alzheimer's
115-7 c ⁶³	NBD	<i>in vitro</i>	UND
Sulfogalactolipids ⁶²	NBD	<i>in vitro</i>	Cancer
cmHsp70.1 mAb ⁷⁰	TKD	Clinical phase I/II (ongoing)	Cancer
Azure C ⁵¹	UND	<i>in vitro</i>	UND
Methylene Blue ⁶¹	UND	<i>in vitro</i> /Clinical phase III	Cancer/Alzheimer's
Myricetin ⁵⁸	UND	<i>in vitro</i>	UND
MAL2-11B ⁶⁵	UND	<i>in vitro</i>	SV40