Heat shock proteins as an emerging therapeutic target

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Heat shock protein 90 (Hsp 90)

- Highly abundant cytoplasmic molecular « chaperone »
- Involved in the folding, stability, and activity of a large number of client proteins implicated in the oncogenic process
  - Involved in cancer cell proliferation, survival, invasion, metastasis, and angiogenesis
  - Estrogen receptor, androgen receptor, HER2, ERBB1, MEK, c-MET, AKT, MAPK (ERK), CDK, RAF, BCR/ABL, HIF1-α, hTERT
- Inhibition of HSP90:
  - Increased degradation of misfolded client proteins, in particular oncogenic proteins
  - Tumour growth inhibition
Hsp90-associated proteins

- it is believed that Hsp90 never functions in isolation in eukaryotes; it always appears to be associated with a variety of cofactors

<table>
<thead>
<tr>
<th>Cofactor</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp70</td>
<td>Hsp90 activity dependent on Hsp70 system (incl. Hsp40)</td>
</tr>
<tr>
<td>HOP</td>
<td>HOP, Heat-shock Organizing Protein, brings Hsp70 and Hsp90 together via TPR interaction domains</td>
</tr>
<tr>
<td>p23</td>
<td>modulates ATPase activity of Hsp90</td>
</tr>
<tr>
<td>HIP</td>
<td>co-chaperone of Hsp70</td>
</tr>
<tr>
<td>PPlases</td>
<td>cyclophilin-40, FKBP51, FKBP52</td>
</tr>
<tr>
<td>others</td>
<td>kinase-targeting co-chaperone Cdc37/p50</td>
</tr>
</tbody>
</table>
Hsp90 functional cycle

- Hsp90 not only assists the folding of proteins, but can also modulate the conformation/function of proteins
  - binding of steroid to Hsp90-bound steroid receptor releases the receptor in a form that can bind DNA and activate transcription

- Hsp90 is also involved in quality control: binding of denatured protein can lead either to its folding or its degradation
HSP 90 is a Valid Target for Cancer Therapy

Normal vs Cancer Cells

HSP90 tends to be overexpressed in cancer cells

Native form
- Present in normal cells
- Low ATPase
  - Low ATP/ADP affinity

Super-chaperone complex
- Present in malignant cells
- High ATPase
  - High ATP/ADP affinity

- Overexpressed mutated oncoproteins
- Micro-environmental stress
Lower gene expression of HSP90 correlates with improved survival in NSCLC

HSP Inhibitors are Selective to Cancer Cells

- 17-AAG has 100-fold higher affinity for tumor-derived Hsp90 than normal cells
- Tumor cells contain Hsp90 complexes in activated high affinity conformation

Kamal N. Nature 2003
HSP90 Inhibition
Simultaneous inhibition of multiple molecular pathways

Cancer Related HSP90 Client Proteins
Her2, Her3, c-Met, PDGFR, VEGFR, Flt-3, Abl, c-kit, Src, Akt, Raf, Mek, Erk, Stat, ER, AR, Bcl, Survivin, CDK, Cyclin

Active HSP90
Flored Protein
Chaperone Complex
Functional Client Protein
Tumor Growth

HSP-90 Inhibitor
Inactive HSP90
Mis-folded Protein
Chaperone Complex
Degraded Client Protein
Proteasome
Tumor Growth Inhibition
Hsp90 Inhibitors- The History

1962
Heat shock proteins discovered

1970
Geldanamycin purified for use as antibiotic

2000

2005

2010

1st-generation

• 17-AAG derivatives
• Weak potency, modest clinical activity, liver toxicities

2nd-generation

• Structurally unrelated to 17-AAG
• Higher potency, improved safety
Rationale for Hsp90 inhibition in NSCLC

• NSCLC with activating mutations in EGFR
  – Mutated EGFR is a sensitive client protein of Hsp90
  – Both T790M and Met amplification are susceptible to Hsp90 inhibition (Park et al, Abstract 2450 AACR Annual Meeting 2008)

• NSCLC containing wild type EGFR
  – Many NSCLC cell lines are sensitive to Hsp90 inhibitors in vitro
  – Multiple proteins important in the progression of NSCLC are client proteins of Hsp90
    • HER2
    • p-AKT
    • EML4-ALK
    • c-RAF
HSP 90 Inhibition: Potential Treatment Strategies

Hsp90-Addiction

- **High**
  - "Oncogene addicted tumors"
  - Single-Agent

- **Low**
  - "Driven by multiple pathways"
  - Combination
# HSP 90 Inhibitors in Clinical Development

<table>
<thead>
<tr>
<th>Agent</th>
<th>Sponsor</th>
<th>Administration</th>
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<tbody>
<tr>
<td>17AAG</td>
<td>NCI/Kosan</td>
<td>iv</td>
</tr>
<tr>
<td>KOS-953 (tanespimycin)</td>
<td>Kosan</td>
<td>iv</td>
</tr>
<tr>
<td>17DMAG</td>
<td>Kosan</td>
<td>Iv &amp; oral</td>
</tr>
<tr>
<td>IPI-504</td>
<td>Infinity</td>
<td>Iv</td>
</tr>
<tr>
<td>STA-9090 (Ganetespib)</td>
<td>Synta</td>
<td>iv</td>
</tr>
<tr>
<td>AUY-922</td>
<td>Novartis</td>
<td>Iv</td>
</tr>
<tr>
<td>DS-2248</td>
<td>Daiichi</td>
<td>Oral</td>
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<tr>
<td>XL-888</td>
<td>Exelexis</td>
<td>Oral</td>
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<tr>
<td>IPI-493</td>
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<td>Oral</td>
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<tr>
<td>MPC-3100</td>
<td>Myrexis</td>
<td>Oral</td>
</tr>
<tr>
<td>SNX-5422</td>
<td>Serenex</td>
<td>Oral</td>
</tr>
<tr>
<td>CNF-2024</td>
<td>Biogen Idec</td>
<td>Oral</td>
</tr>
</tbody>
</table>

Ramalingam S., ASCO 2011.
IPI-504 in advanced NSCLC

Eligibility:
- Histologically confirmed NSCLC, stage IIIb (with effusion) or IV.
- Failed prior EGFR TKI therapy.
  - No limit to the number of prior therapies.

Mutant EGFR Cohort

Enroll 10 patients

If >1 CR, PR, or SD for at least 3 months

Expand to a total of 29 patients

Wild-Type EGFR Cohort

Enroll 10 patients

If >1 CR, PR, or SD for at least 3 months

Expand to a total of 29 patients

## Efficacy

<table>
<thead>
<tr>
<th></th>
<th>Total (n=68)</th>
<th>Wild Type</th>
<th>Mutant</th>
<th>Wild Type</th>
<th>Mutant</th>
<th>No Rearr.</th>
<th>+ Rearr.</th>
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<td>EGFR Status</td>
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<tr>
<td>(n=68)</td>
<td>76</td>
<td>40 (53)</td>
<td>28 (37)</td>
<td>26 (34)</td>
<td>12 (16)</td>
<td>12 (16)</td>
<td>3 (4)</td>
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<td>KRAS Status</td>
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<tr>
<td>(n=38)</td>
<td>5 (7)</td>
<td>4 (10)</td>
<td>1 (4)</td>
<td>3 (12)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>2 (67)</td>
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<td>ALK Status</td>
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<td></td>
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</tr>
<tr>
<td>(n=15)</td>
<td>18 (24)</td>
<td>10 (25)</td>
<td>6 (21)</td>
<td>4 (15)</td>
<td>5 (42)</td>
<td>3 (25)</td>
<td>3 (100)</td>
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</table>

**Patients (n [%])**

**Objective Response Rate (All PRs) (n [%])**

**RECIST SD or better ≥3 months (n [%])**

**Median PFS (95% CI)**

IPI-504 - Response by Genotype

17-AAG Activity in EGFR mutant and ALK + NSCLC Cells

Sequist L, J Clin Oncol 2011
AUY922

- Intravenous non-geldanamycin–based HSP90 inhibitor
- Acceptable safety profile
  - The MTD has been determined to be 70 mg/m^2 i.v. once-weekly
- AUY922 has shown promising clinical activity as a single agent therapy in Non-Small Cell Lung Cancer (NSCLC) patients:
  - EGFR mutant patients with acquired resistance to EGFR TKIs
  - ALK+ patients both ALK inhibitor naive and pre-treated
  - EGFR wt/Kras wt/ALK-) NSCLC patients
- AUY922 combination with trastuzumab Ph II study in HER2+ gastric cancer is ongoing
- Clinical efficacy in HER2+ breast cancer as a single agent and in combination with trastuzumab
AUY922A2206 Study Design

AUY922 weekly 70 mg/m² 3rd-5th line non-small cell lung cancer

Stratification:
- EGFR mut 2nd/3rd line
- EGFR mut
- Kras mut
- EML4-ALK
- EGFRwt Kraswt/ALK-

End points:
Primary
- Confirmed ORR or SD at 18 weeks

Secondary
- Overall Survival
- Progression free Survival
- Safety and tolerability

Recruitment completed

Garon EB et al. Proc. ASCO 2012
### AUY922 2206 study. Patient Demographics and Disease Characteristics

<table>
<thead>
<tr>
<th></th>
<th>KRAS-mut (n=28)</th>
<th>EGFR-mut (n=35)</th>
<th>EGFR/KRAS/ALK wt (n=33)</th>
<th>ALK+ (n=22)</th>
<th>All (N=121*)</th>
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<tr>
<td>Age (median), years</td>
<td>60.0</td>
<td>63.0</td>
<td>63.0</td>
<td>53.0</td>
<td>60.0</td>
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<td>Sex (male), %</td>
<td>17 (61)</td>
<td>10 (29)</td>
<td>15 (46)</td>
<td>7 (32)</td>
<td>52 (43)</td>
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<td>WHO PS</td>
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<td>0</td>
<td>10 (36)</td>
<td>13 (37)</td>
<td>10 (30)</td>
<td>9 (41)</td>
<td>43 (36)</td>
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<td>1</td>
<td>18 (64)</td>
<td>19 (54)</td>
<td>20 (61)</td>
<td>11 (50)</td>
<td>70 (58)</td>
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<td>2</td>
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<td>3 (9)</td>
<td>3 (9)</td>
<td>2 (9)</td>
<td>8 (7)</td>
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<td>Histology</td>
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<td>Adenocarcinoma</td>
<td>23 (82)</td>
<td>32 (91)</td>
<td>27 (82)</td>
<td>20 (91)</td>
<td>105 (87)</td>
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<td>Squamous cell</td>
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<td>0 (0)</td>
<td>2 (6)</td>
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<td>Other</td>
<td>4 (14)</td>
<td>3 (9)</td>
<td>4 (12)</td>
<td>2 (9)</td>
<td>13 (11)</td>
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<td>Prior regimens</td>
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<td>1 (4)</td>
<td>3 (9)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>2</td>
<td>8 (29)</td>
<td>13 (37)</td>
<td>16 (49)</td>
<td>5 (23)</td>
<td>42 (35)</td>
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<tr>
<td>3</td>
<td>14 (50)</td>
<td>11 (31)</td>
<td>4 (12)</td>
<td>7 (32)</td>
<td>38 (31)</td>
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<tr>
<td>≥4</td>
<td>5 (18)</td>
<td>8 (23)</td>
<td>13 (39)</td>
<td>9 (41)</td>
<td>36 (30)</td>
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<td>Metastatic sites</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lung</td>
<td>12 (43)</td>
<td>22 (63)</td>
<td>17 (52)</td>
<td>11 (50)</td>
<td>64 (53)</td>
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<tr>
<td>Bone</td>
<td>7 (25)</td>
<td>15 (43)</td>
<td>14 (42)</td>
<td>11 (50)</td>
<td>49 (41)</td>
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<tr>
<td>Lymph nodes</td>
<td>8 (29)</td>
<td>9 (26)</td>
<td>12 (36)</td>
<td>5 (23)</td>
<td>34 (28)</td>
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<tr>
<td>Liver</td>
<td>4 (14)</td>
<td>10 (29)</td>
<td>8 (24)</td>
<td>4 (18)</td>
<td>27 (22)</td>
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<tr>
<td>Pleura</td>
<td>1 (4)</td>
<td>6 (17)</td>
<td>6 (18)</td>
<td>9 (41)</td>
<td>22 (18)</td>
</tr>
<tr>
<td>Brain</td>
<td>6 (21)</td>
<td>7 (20)</td>
<td>4 (12)</td>
<td>4 (18)</td>
<td>21 (17)</td>
</tr>
</tbody>
</table>

*3 patients were of unknown genotype
AUY922 Clinical Benefit in Lung Cancer
All patients

Best CT response. All patients (n= 92)

Preliminary response rate 15%, DCR 51%

Garon EB et al. Proc. ASCO 2012
AUY922 Clinical Benefit in Lung Cancer.

EGFR mutant patients

EGFR mutant (n=25)

Preliminary response rate 20%, DCR 57%

*One PR not yet confirmed, and one PR not confirmed as per RECIST

Garon EB et al. Proc. ASCO 2012
AUY922 Clinical Benefit in Lung Cancer.

ALK+ patients

ALK+ stratum (n=17)

Preliminary response rate 29%, DCR 57%

Garon EB et al. Proc. ASCO 2012
AUY922 Clinical Benefit in Lung Cancer.

EGFR wt /Kras wt/ALK negative patients

EGFR/KRAS/ALK wild type stratum (n=28)

Preliminary response rate 9%, DCR 42%

Garon EB et al. Proc. ASCO 2012
AUY922 Clinical Benefit in Lung Cancer.
Kras mutant patients

KRAS mutant stratum (n=20)

* Target lesion shrinkage in C4 scan but also new brain lesions detected

Garon EB et al. Proc. ASCO 2012
AUY922 Safety Summary

- AUY922 at 70 mg/m^2 i.v. weekly is well tolerated.

- Mild-to-moderate diarrhea (60 - 70%; Gr 3 events 5-7%) and nausea (40 – 50%; Gr 3 events 5%) are the most commonly reported AEs.

- Most common reversible visual AEs are: Night blindness, Photopsia, and Vision blurred. Frequency around 60%.
  - Mostly mild/moderate, rarely lead to dose interruption or discontinuation.
  - Severe (grade 3) events are rare 4% (13/353 patients). The severity of visual impairment increased with higher dose level.
Ganetespib (STA-9090)

• Potent Hsp90 inhibitor, structurally unrelated to first-generation ansamycin class of Hsp90 inhibitors
  – Superior activity to these agents in preclinical studies
  – Potent activity multiple NSCLC models, single agent/combo

• Promising single-agent antitumor activity seen in early clinical trials, multiple cancers

• Well-tolerated to date, >350 patients treated, 15 trials
  – Most common AE: diarrhea; generally Grade 1 and 2, manageable with supportive care

• Absence of serious liver or common ocular toxicities seen with other Hsp90i
Comparison of Ganetespib with 17-AAG

STA-9090 has better potency compared to 17AAG in Hsp90 binding and lung cancer cell growth inhibition.

(A) Hsp90 binding FP assay

17AAG 
$IC_{50}$: 21.0 nM

STA-9090 
$IC_{50}$: 3.9 nM

(B) 1536-well viability assay (A549 cells)

17AAG 
$IC_{50}$: 88.0 nM

STA-9090 
$IC_{50}$: 13.3 nM

S Ramalingam, Malta 2011
Study Design

Ganetespib 200mg/m² once weekly for 3 weeks of 4 week cycle

- Previously treated stage IIIB/IV NSCLC
- ECOG - PS 0, 1
- Documented disease progression at study entry
- Genotyping*

Stratification

A: mEGFR (n=16)
B: m K-Ras (n=17)
C: wild-type EGFR/wild-type K-Ras (n=25)
D: Adenocarcinoma only (n=37)

Stage 1   n = 14; Stage 2
(if n ≥ 2 progression-free at wk 16)

CT scans every 8 wks
Evaluable population n = 76

Primary endpoint: PFS at 16 weeks
Secondary endpoints: ORR, DCR at 8, 16 wks; PFS, OS, Time to treatment failure, Safety and Tolerability

*1 patient with unknown genotype
First Patient First Visit, Dec, 2009;
Last Patient first visit, May, 2011
All responders (4/4) have ALK-positive tumors.
6 out of 8 (75%) patients with ALK-positive tumors had tumor shrinkage in target lesions.
1 out of 8 (13%) patients with ALK-positive tumors had no change in target lesions.
Rationale for combining Hsp90 inhibitors and taxanes

• Both drugs have single agent activity in NSCLC

• Ganetespib and docetaxel have synergistic MOAs
  – Cell cycle
  – Both drugs affect microtubuli
  – Hsp90 inhibitors interfere with taxane resistance mechanisms (eg, AKT expression, anti-apoptotic signaling through VEGF)
  – Effects of Hsp 90 inhibition on microenvironment sensitize tumors to docetaxel (vasculature, blood flow, metabolic state)

• Non-overlapping toxicity profile
  – Docetaxel DLT is bone marrow toxicity
  – Ganetespib DLT is diarrhea
Interaction between Ganetespib + Docetaxel

In vitro H522

Proia et al, AACR 2010.
GALAXY Study Schema

Stage IIIB/IV NSCLC
2nd line

Docetaxel 75mg/m² q3w

Ganetespib 150 mg/m² (d1,15)

Gather biomarker data
Enrich population
Regulatory input

PFS
OS

Stage 1 (N=240)
Phase 2b
PFS endpoint

Stage 2 (N~500)
Phase 3
(OS endpoint – TBD)

Stage 2b
PFS endpoint

OS endpoint

2nd line

PFS
OS

Stage 1 (N=240)
Phase 2b
PFS endpoint

Stage 2 (N~500)
Phase 3
(OS endpoint – TBD)
HSP-90 Inhibitors in NSCLC

Summary

• Strong rationale for this class of agents in NSCLC.
• Newer Hsp90 inhibitors have an improved safety profile and lend themselves for combination approaches.
• Hsp90 inhibitors have the most promising activity in ALK+ NSCLC.
• Need for a biomarker strategy