Sensitizing hypoxic tumor cells against irradiation

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4 R`s in radiotherapy

**Repair**
- Endonuclease
- Excision
- Polymerase
- Ligase

**Redistribution**
- Surviving fraction vs. radiation dose
- Early S, G1, G2, M
- Late S

**Repopulation**
- Number of viable tumor cells vs. radiation treatments
- Rapid repopulation
- Slow repopulation

**Reoxygenation**
Oxygenation - hypoxia

Venous end

Arterial end

O$_2$

Normoxic = sensitive

Hypoxic = resistant (>70 µm)

Anoxic = no cell proliferation
Hypoxia

Programmed cell death/apoptosis

Cell growth/differentiation

Neaoangiogenesis/vascularisation

Genomic instability

Tumor progression/metastasis

Adaptation to reoxygenation

Adaptation to hypoxia

Poor prognosis

Resistance to therapy

Sutherland et al. 1998
Hypoxia in tumor therapy (I)

Radiotherapy

Chemotherapy

Alkylating agents
- Cyclophosphamide: 6.3
- Carboplatin: 2.4
- Melphalan: 2.2

Antibiotics, Vinca Alkaloids
- Adriamycin: 2.2
- Mitomycin C: 0.3
- Vincristine: 2.1

Antimetabolite
- 5-FU: 2.3

OER = Oxygen Enhancement Ratio

Molls Vaupel Nieder Anscher The Impact of Tumor Biology on Cancer Treatment and Multidisciplinary Strategies. Radiation Oncology, Springer Verlag Heidelberg, 2000
Reduced survival, proliferation and cytotoxicity of T cells

Reduced cytokine production (e.g. IFN \( \gamma \))

Immunosuppression by adenosine

Tumor associated macrophages supporting tumor growth (Increase in the TNF activity and altered NF-kappa B expression)

Inhibition of lymphokine activated killer (LAK) cells

Increase in regulatory T (Treg) cells

HIF1 reduces angiostatin-based gene therapy

Hypoxia-induced acidosis reduces antiangiogenetic cancer gene therapy
Definition: tumor microenvironment

“Network” of
different components,
multiple functions and
complex interactions within a tumor

e.g.  tumor microcirculation
vascular permeability
hypoxia (< 1% O₂)
acidosis
nutrient deficiencies
energy depletion
stroma - tumor interactions
tumor - immune system interactions  

Vaupel 2010
Radiosensitization via the microenvironment (I)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Mechanism</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Disruption of tubulin cytoskeleton of ECs</td>
<td>Siemann IJRO 2005; Hokland IJH 2007</td>
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<td>Anti-angiogenesis</td>
<td>Inhibition of neoangiogenesis</td>
<td>Vaupel Molls 2009</td>
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<tr>
<td>bevacizumab (Avastin)</td>
<td>Vascular (pseudo-)normalization (?)</td>
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<tr>
<td>cediranib (AZD2171)</td>
<td>Problem: redundancy of receptors</td>
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<tr>
<td>cetuximab (Erbitux)</td>
<td>Increased tumor blood flow (?)</td>
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<td>panitumumab (Vectoibix)</td>
<td>Vascular normalization (?)</td>
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<td>gefitinib (Iressa)</td>
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<td>erlotinib (Tarceva)</td>
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Radiosensitization via the microenvironment (II)

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<tr>
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<td>YC-1</td>
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<td>TAS106</td>
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<td>PX-478</td>
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<td><strong>Anti-mTOR</strong></td>
<td><strong>Radiosensitizing EC damage (?)</strong></td>
<td>Eshleman Can Res 2002; Shinohara Onco 2005; Murphy Can Res 2009</td>
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<tr>
<td>Rapamycin</td>
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<tr>
<td>Temsirolimus (CC1779)</td>
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<td>Everolimus (RAD001)</td>
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<td>Deforolimus (AP23573)</td>
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<td>HIF protease inhibitors:</td>
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<tr>
<td>Nelfinavir, Amprenavir</td>
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<td>Palomid 529/ Wortmannin</td>
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<td>PI-103</td>
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<td>NVP-BEZ235</td>
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## Radiosensitization of hypoxic tumor cells (I)

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Mechanism</th>
<th>References</th>
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<tr>
<td>Increased ( \text{O}_2 ) supply</td>
<td>Increased oxygen diffusion</td>
<td>Vaupel Molls 2009</td>
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<td>Increased blood flow</td>
<td>Increased oxygen supply</td>
<td>Vaupel Molls 2009</td>
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<tr>
<td>Correction of anemia</td>
<td>Increased oxygen supply</td>
<td>Vaupel Molls 2009; Grau &amp; Overgaard 1998; Thomas 2002; Thews 1998; Stuben 2002; Hoskin 2009</td>
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<td>RBC-transfusion EPO</td>
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<tr>
<td>High LET</td>
<td>Targeted irradiation of hypoxic areas</td>
<td>Kaanders Semin Rad Oncol 2004; Horsman Can Res 2006</td>
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<tr>
<td>low-dose</td>
<td>Improved delivery of chemotherapy</td>
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Radiosensitization of hypoxic tumor cells (II)

<table>
<thead>
<tr>
<th>Agents</th>
<th>Mechanism</th>
<th>References</th>
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<tbody>
<tr>
<td>Diethyl maleate</td>
<td>Depletion of glutathione (GSH)</td>
<td>Bump Science 1982</td>
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<td></td>
<td></td>
<td>Bump Pharmacol Ther 1990</td>
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<tr>
<td>Methylxanthine caffeine</td>
<td>Inhibition of ATM and ATR kinases PI3K/Akt pathway G1 DNA damage</td>
<td>Sarkaria Can Res, 1999; Foukas JBC 2002; Mayo PNAS 2001; Sinn Mol Can Ther 2010</td>
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<tr>
<td>Nitric oxide</td>
<td>Fixation of DNA damage by mimicking oxygen</td>
<td>De Ridder Nitric Oxide 2008</td>
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<tr>
<td>NO/iNOS</td>
<td></td>
<td>De Ridder Bull Cancer 2008</td>
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<tr>
<td>Nicotinamide = Vit B₃,</td>
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<tr>
<td>analogue</td>
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<td>Tirapazamine</td>
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<td>Dexamethasone</td>
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<td>Nimorazole</td>
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<td>Misonidazole</td>
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<td>Taxanes</td>
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<tr>
<td>Hsp90 inhibitors</td>
<td>Ubiquitination and degradation of oncogenic client proteins EGFR endocytosis</td>
<td>George Blood 2005; Bergstrom JNM 2008; Camphausen Tofilon CCR 2007; Lang Can Ther 2007; Dote Can Res 2006</td>
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</table>
# Hsp90 inhibition

## Hsp90 (heat shock protein 90)
- Molecular chaperone for client proteins with tumorigenic activity
  - (e.g. Raf-1, Akt, Src, ErbB2 (Her2), EGFR, p53, HIF, VEGF, IL-6, IGFR, p-glycoprotein (MDR2), survivin, telomerase, cyclin-dependent kinase 4)

## Hsp90 inhibition
- Bind to the ATPase domain of Hsp90
- Induce proteasomal degradation of client proteins
- Abrogate cell cycle checkpoint activation
- Enhance cell death (apoptosis, mitotic catastrophe)
- Suppress angiogenesis
- Inhibit DNA damage repair
- Radiosensitization

# Hsp90 inhibitors (radiosensitizers)

## Hsp90 inhibitors addressing the ATPase domain of Hsp90

**Geldanamycin** (*Streptomyces hygroscopicus*) (hepatotoxic, no clinical application)

**Radicicol** (hepatotoxic, no clinical application)

**17-AAG** (17-N (allylamino)-17-demethoxy-Geldanamycin) (less toxic, water insoluble)

**17-DMAG** (17-N (dimethylethylamino)-17-demethoxy-Geldanamycin) (water soluble)

**NVP-AUY922** (Novartis) (water soluble, more efficient) (?)

**Pyrazoles** (purine scaffold derivatives) (?)

## Hsp90 inhibitors addressing other sites of Hsp90 (new)

**HDAC inhibitors** (inactivating acetylation of Hsp90)

**BIIB021** (CNF2024; good bioavailability)

**Novobiocin** (blocks the C-terminus of Hsp90)
Lung carcinoma (NSCLC/ SCLC)

**Incidence**
Most frequently occurring cancer worldwide (12.4% of all cancer diseases)

5-year survival <10%

**Mortality**
Leading cause of cancer-related death
Worldwide: 1.3 million deaths/year

**Histological types**
Small cell lung cancer (SCLC): 20%
Non-small cell lung cancer (NSCLC): 80%

**Cell lines**
H1339
EPLC-272H

**Therapy**
Surgery, radiochemotherapy

*Huber Der Internist 2006; Ginsberg, Radiol Clin N Am 2007*
Radiosensitizing effects of Hsp90 inhibitors on lung cancer cell lines

SCLC: H1339
NSCLC: EPLC-272H

Mechanism of radiosensitization

cell cycle analysis
HIF, PAI-1, VEGF
Hsp70 (cytosol, membrane, extracellular)
Radiosensitivity

SCLC (H1339) and NSCLC (EPLC-272H)
Hsp90 inhibitor (17-AAG) radiosensitizes only SCLC (H1339)

Why?

**NSCLC (EPLC-272H)**

![Graph showing survival fraction vs. dose for NSCLC (EPLC-272H) with and without 17-AAG.]

**SCLC (H1339)**

![Graph showing survival fraction vs. dose for SCLC (H1339) with and without 17-AAG.]

Seed cells  17-AAG  IR  ~ 7 days  Fixation+staining

48h  24h
Hsp90 inhibitor (17-AAG) affects cell cycle only in SCLC (H1339; decrease in S-phase)

**EPLC-272H (24h after 17-AAG)**

- **0nM**: 0% in G2/M, 60% in S, 40% in G1, 0% in subG1
- **20nM**: 18.6% in G2/M, 14.2% in S, 6.7% in G1, 100% in subG1
- **100nM**: 12.7% in G2/M, 14.2% in S, 71.2% in G1, 0% in subG1

**H1339 (24h after 17-AAG)**

- **0nM**: 0% in G2/M, 60% in S, 40% in G1, 0% in subG1
- **20nM**: 15.9% in G2/M, 14.2% in S, 5.5% in G1, 64.4% in subG1
- **100nM**: 5.5% in G2/M, 14.2% in S, 71.2% in G1, 0% in subG1

*Significant difference (p < 0.05) compared to control*

**Cell cycle analysis**

- **Seed cells**
- **17-AAG**
- **24h**

*Sinclair, Radiat Res 1968*
Hsp90 inhibitor (17-AAG) reduces the hypoxia-induced HIF-1α expression only in NSCLC (EPLC-272H)

Hypoxia does not induce HIF-1α in SCLC (H1339)
The Janus face of Hsp90 inhibition

Inhibition of oncogenic signaling, tumor growth inhibition

Ubiquitination & proteasomal degradation

HSF1 trimerization & nuclear translocation

HSF1

HS1

Hsp90

Hsp90

client

ubub

ubub

17-AAG

HS1

HS1

HS1

Hsp70

Cytoprotection, therapy resistance

Anti-apoptotic

Transcription
Effects of Hsp90 inhibitors on cytosolic, membrane, extracellular Hsp70
Hsp90 inhibitor (17-AAG) increases cytosolic Hsp70 in both tumors.
Hsp70 membrane expression remains un-affected by Hsp90 inhibitor (17-AAG)
Hsp90 inhibitor (17-AAG) significantly enhances Hsp70 secretion in normoxic (N) and hypoxic (H) SCLC (H1339)

extracellular secretion

- EPLC-272H
  - 0 nM 17-AAG
  - 100 nM 17-AAG

- H1339
  - 0 nM 17-AAG
  - 100 nM 17-AAG

* indicates significance.
Exogenous Hsp70 binds to tumor cells
... and enhances radiosensitivity of CX- cells
Summary of Hsp90 inhibition

- Decrease of radioresistant S phase
- Re-binding of secreted Hsp70 and radiosensitization
- Degradation of oncogenic client proteins
- EGFR endocytosis
- Decrease Insulin-like growth factor 1 (IGF-1R)
- Cell cycle checkpoint activation (CDK4)
- Tumor growth reduction (apoptosis, mitotic catastrophe)
- Blocking of DNA damage repair (DS-break repair)
- Suppression of angiogenesis (HIF1, VEGF blocking)
- Suppression of p-glycoprotein (MDR)

Tumor entities: Prostate, cervical, pancreatic, lung, HN, breast, bladder, oesophageal carcinomas, glioblastoma, melanoma
<table>
<thead>
<tr>
<th><strong>Team</strong></th>
<th><strong>Collaborations</strong></th>
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<tbody>
<tr>
<td>Armin Bareiß, TA</td>
<td>Alexzander Asea (Scott &amp; White Hospital Texas, USA)</td>
</tr>
<tr>
<td>Christine Bayer, PhD</td>
<td>Mike Atkinson (HMGU)</td>
</tr>
<tr>
<td>Brigitte Doß</td>
<td>Günther Dollinger (UBW), Anna Friedl (LMU)</td>
</tr>
<tr>
<td>Kathrin Hube, MD stud</td>
<td>Stuart Calderwood (Harvard Med School, Boston, USA)</td>
</tr>
<tr>
<td>Mathias Gehrmann, PhD</td>
<td>Antonio DeMaio (UCSD, San Diego, USA)</td>
</tr>
<tr>
<td>Kristin Kuhs, MD stud</td>
<td>Ralf Dressel, Frauke Alves, Christian Dullin (University Göttingen)</td>
</tr>
<tr>
<td>Sophie Lehnerer, MD stud</td>
<td>Udo Gaipl, Benjamin Frey (University Erlangen)</td>
</tr>
<tr>
<td>Anna Marwedel</td>
<td>Carmen Garrido (University Dijon, Marja Jäärtlä (University Copenhagen)</td>
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<tr>
<td>Ines Nachtigall, TA</td>
<td>Michaela Nathrath (HMGU)</td>
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<tr>
<td>Isabelle Riederer, MD stud</td>
<td>Graham Pockley, Gemma Foulds (University Sheffield, UK)</td>
</tr>
<tr>
<td>Daniela Schilling, PhD</td>
<td>Franz Rödel (University Frankfurt)</td>
</tr>
<tr>
<td>Thomas Schmid, PhD</td>
<td>Thomas Schmid (TUM), Günther Dollinger (UniBW Neubiberg)</td>
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<tr>
<td>Wolfgang Sievert</td>
<td>Gerd Schmitz (University Regensburg)</td>
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<tr>
<td>Stefan Stangl</td>
<td>Arne Skerra, Lars Friedrich (WZW)</td>
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<td>Arnulf Materny (University Bremen)</td>
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<td></td>
<td>George Themelis, Vasilis Ntziachristos (HMGU)</td>
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<tr>
<td></td>
<td>Axel Walch, Isabel Winkelmann (HMGU)</td>
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</table>
Projects and funding

NK cell therapy (IL-2/TKD)
- DFG  MU1238 7/2
- Helmholtz Gemeinschaft “Präsidentenfonds”
- BMBF-MOBITUM
- BMBF - BioChance
- SFB-824
- multimmune GmbH

Molecular therapy (Granzyme B)
- DFG MU1238 7/2
- Helmholtz Gemeinschaft “Präsidentenfonds”
- BMBF Spitzencluster m4
- multimmune GmbH
- TU-München
- EU-CARDIORISK
- TU-München

Lipidomic and proteomic profiling
- BMBF Spitzencluster m4
- multimmune GmbH
- TU-München

Radiation/ hypoxia
- DFG Paket Antrag
- EU-CARDIORISK
- TU-München

Definition of tumor markers in the serum
- BMBF Spitzencluster m4
- multimmune GmbH
- TU-München

Antibody therapy (cmHsp70.1)
- BMBF- MOBITUM
- BMBF - BioChance
- SFB-824
- multimmune GmbH
Hypoxia

Hsp90 inhibitors

Reoxygenation

Hsp70

Apoptosis

Ionizing radiation

NOX

ROS

HIF-α

VEGF/PAI-1

Angiogenesis/Invasion

Radioresistance

presumed induction

presumed inhibition

induction

inhibition
### Hsp90 inhibitor in SCLC and NSCLC

<table>
<thead>
<tr>
<th></th>
<th>H1339 (SCLC)</th>
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<th>EPLC-272H (NSCLC)</th>
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<tbody>
<tr>
<td><strong>Radiosensitization</strong></td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
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<tr>
<td><strong>Cell cycle: S-phase</strong></td>
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<td>↓</td>
<td>-</td>
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<tr>
<td><strong>Hypoxia-induced HIF-1α expression</strong></td>
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<td>↓↓</td>
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<td><strong>Hypoxia-induced PAI-1 levels</strong></td>
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<td><strong>Hypoxia-induced VEGF secretion</strong></td>
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<tr>
<td><strong>Intracellular Hsp70 expression</strong></td>
<td>↑</td>
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<tr>
<td><strong>Membrane Hsp70 expression</strong></td>
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<td><strong>Extracellular Hsp70</strong></td>
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<tr>
<td><strong>Differences</strong></td>
<td>S ↓, Hsp70 ↑</td>
<td>S ↓, Hsp70 ↑</td>
<td>HIF-1 ↓</td>
<td>HIF-1 ↓</td>
</tr>
</tbody>
</table>
Plasminogen activator inhibitor (PAI)

Matrix degradation
Metastasis

uPA: urokinase type plasminogen activator
PAI-1: Inhibitor of uPA
uPAR: membrane bound receptor of uPA
Potential mechanism of radio-sensitization: re-binding of extracellular Hsp70
Hsp90 inhibitor (17-AAG) reduces the hypoxia-induced PAI-1 expression/secretion
Hsp90 inhibitor (17-AAG) reduces the hypoxia-induced VEGF secretion
Hsp70 is located in the cytosol, on the membrane and extracellularly.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Hsp70+</th>
</tr>
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<tbody>
<tr>
<td>Lung cancer</td>
<td>65 %</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>0 %</td>
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</table>

Hsp70 membrane expression is enhanced by hypoxia

**CX-**

Brightfield

Hsp70

**FACS**

CX-

Hsp70 positive cells [%]

**Schilling FASEB 2009**