# Pulmonary Neoplasia in Strain A Mice following Long-Term Tobacco Smoke Inhalation

**Rosemarie B. Lichtner**

Philip Morris International, Neuchâtel, Switzerland


**American College of Toxicology, 29th Annual Meeting,
Tucson, Arizona, USA, 9-12 November 2008**

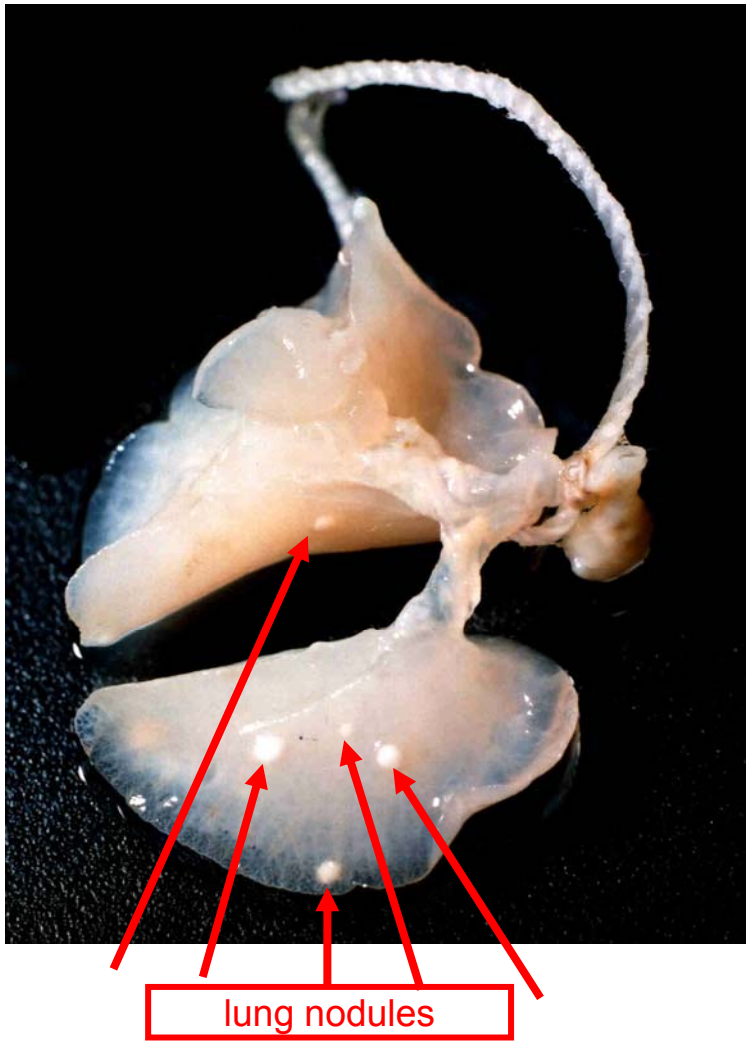
# The A/J Mouse as a Lung Tumor Model

- Philip Morris International is committed to the development of Reduced-Risk Tobacco Products. This requires a state-of-the-art scientific approach to assess the disease risk of new products

- Animal models with enhanced lung tumor formation after exposure to cigarette smoke are required to substantiate a reduced risk

- The A/J mouse has been shown to respond to cigarette smoke exposure with enhanced lung tumor formation after a recovery period of several months (Witschi et al., 1997; D'Agostini et al., 2001; Stinn et al., 2005; Curtin et al., 2004)

# Nodules in the A/J Mouse Lung




hyperplasia

↓

bronchioloalveolar adenoma

↓

bronchioloalveolar adenocarcinoma

# Objectives of A/J Mouse Lung Cancer Study

**Characterize the effects of chronic MS exposure on lung tumor response with respect to relevance for human tumors:**

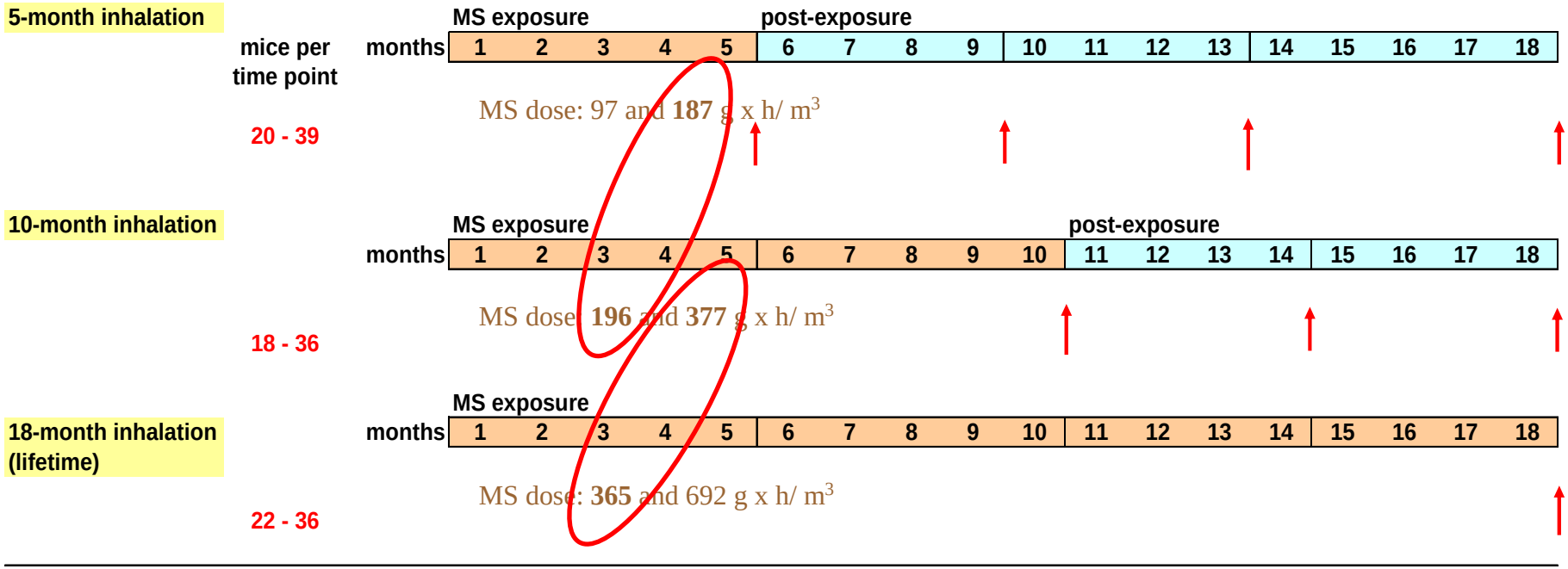
- Time course (5, 10, and 18 months exposure)
- Increasing MS concentrations (0, 150, and 300 mg total particulate matter [TPM]/$m^3$)
- Different post-exposure periods (up to 13 months)

**Endpoints:**

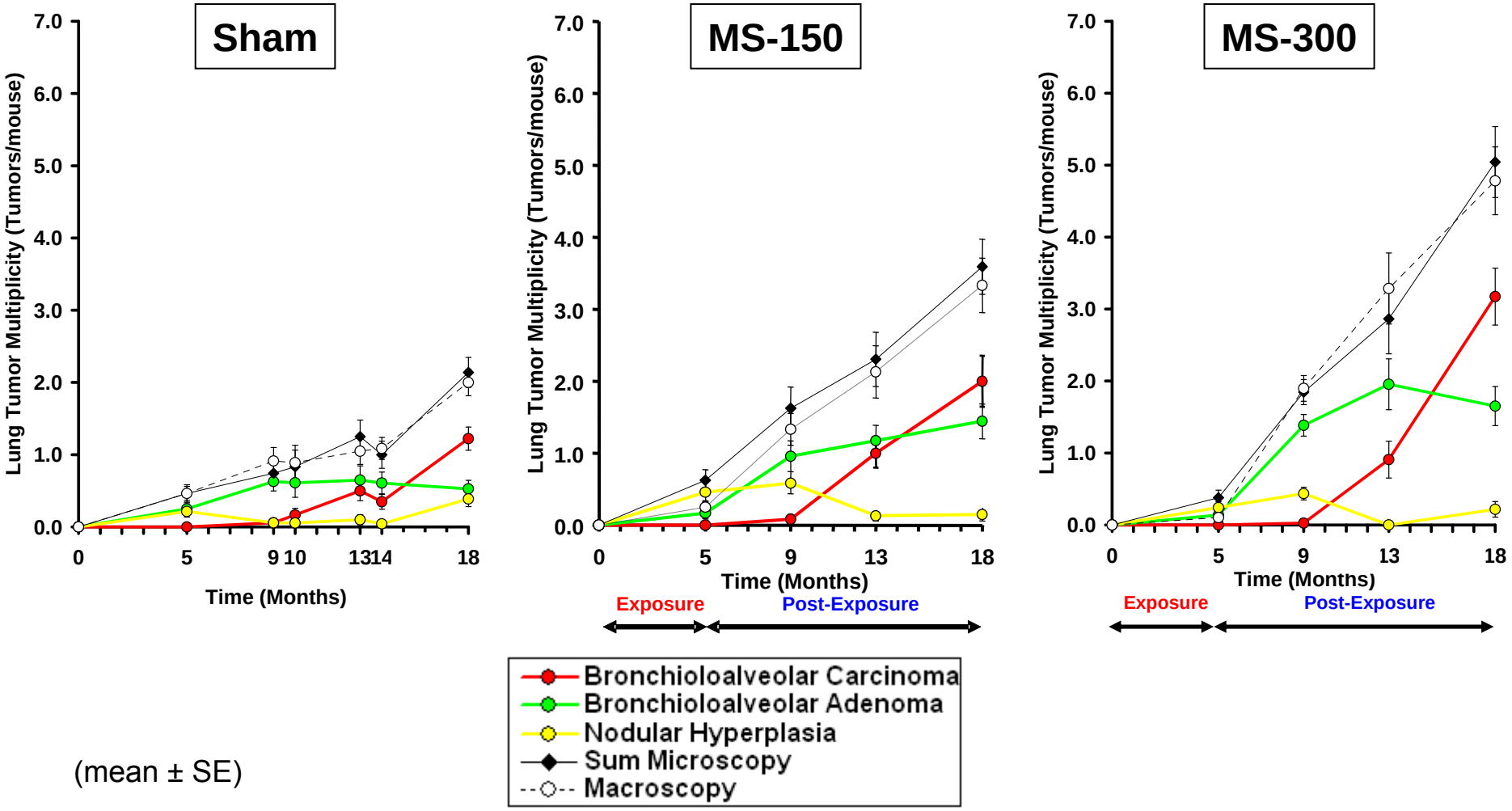
- Classical histopathology of step-serial sections to differentiate and quantify proliferative lesions and bronchiolo-alveolar adenomas and adenocarcinomas
- Gene expression analysis of tumor nodules and normal lung tissue
- *K-ras* mutation analysis in cells from lung nodules
- Analysis of bronchoalveolar lavage fluid (BALF) (see poster #33)

# Exposure Regimens of A/J Mouse Study

| | |
|---|---|
| Exposure: | 6 hours/day, 5 days/week |
| Exposure mode: | whole-body |
| MS concentrations: | 150 and 300 mg TPM/m$^3$ (MS-150 and MS-300) |

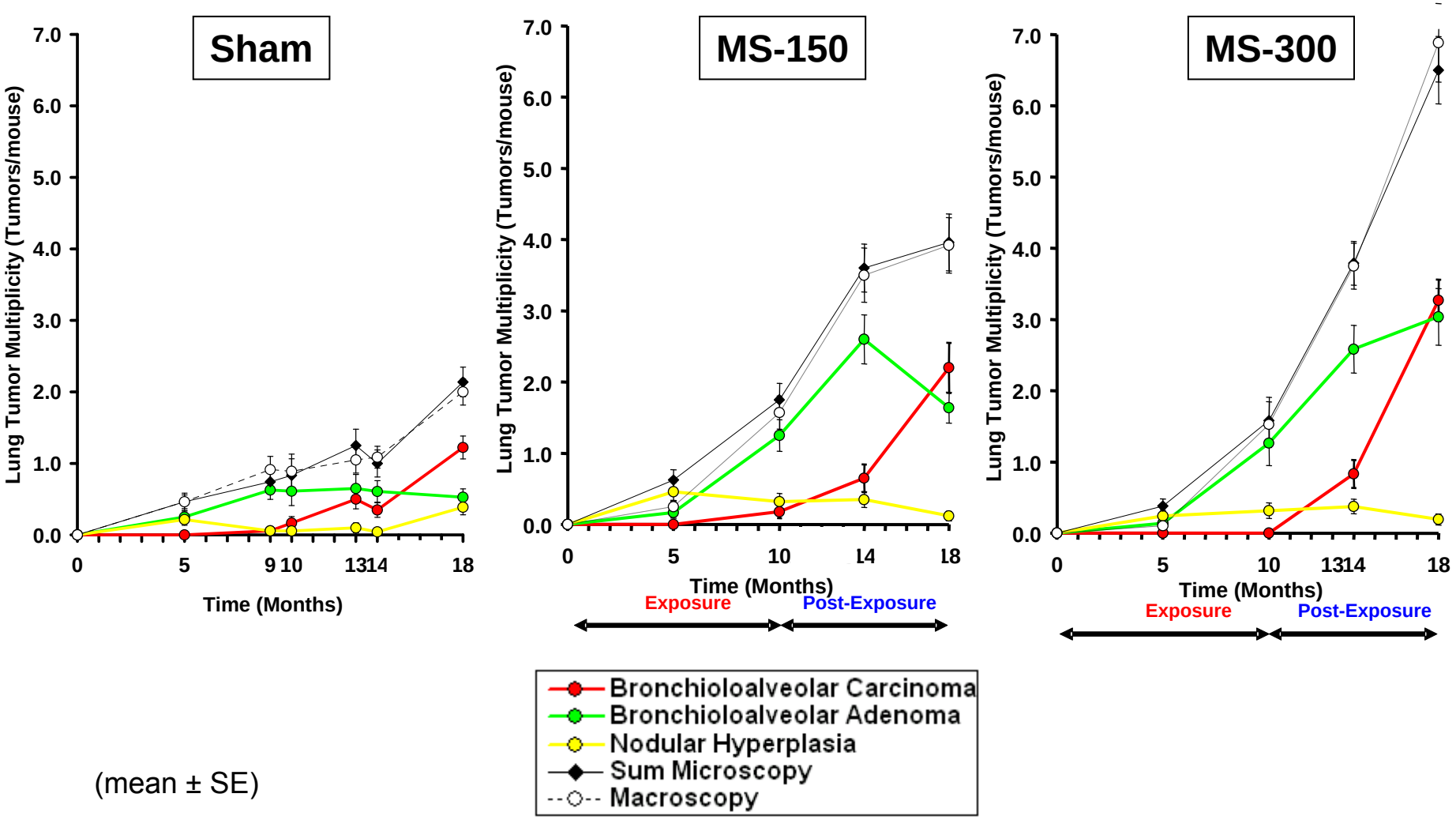


# Histopathological Evaluation of Lung Tumor Multiplicity: 5 mo Exposure + 13 mo Post-Exposure



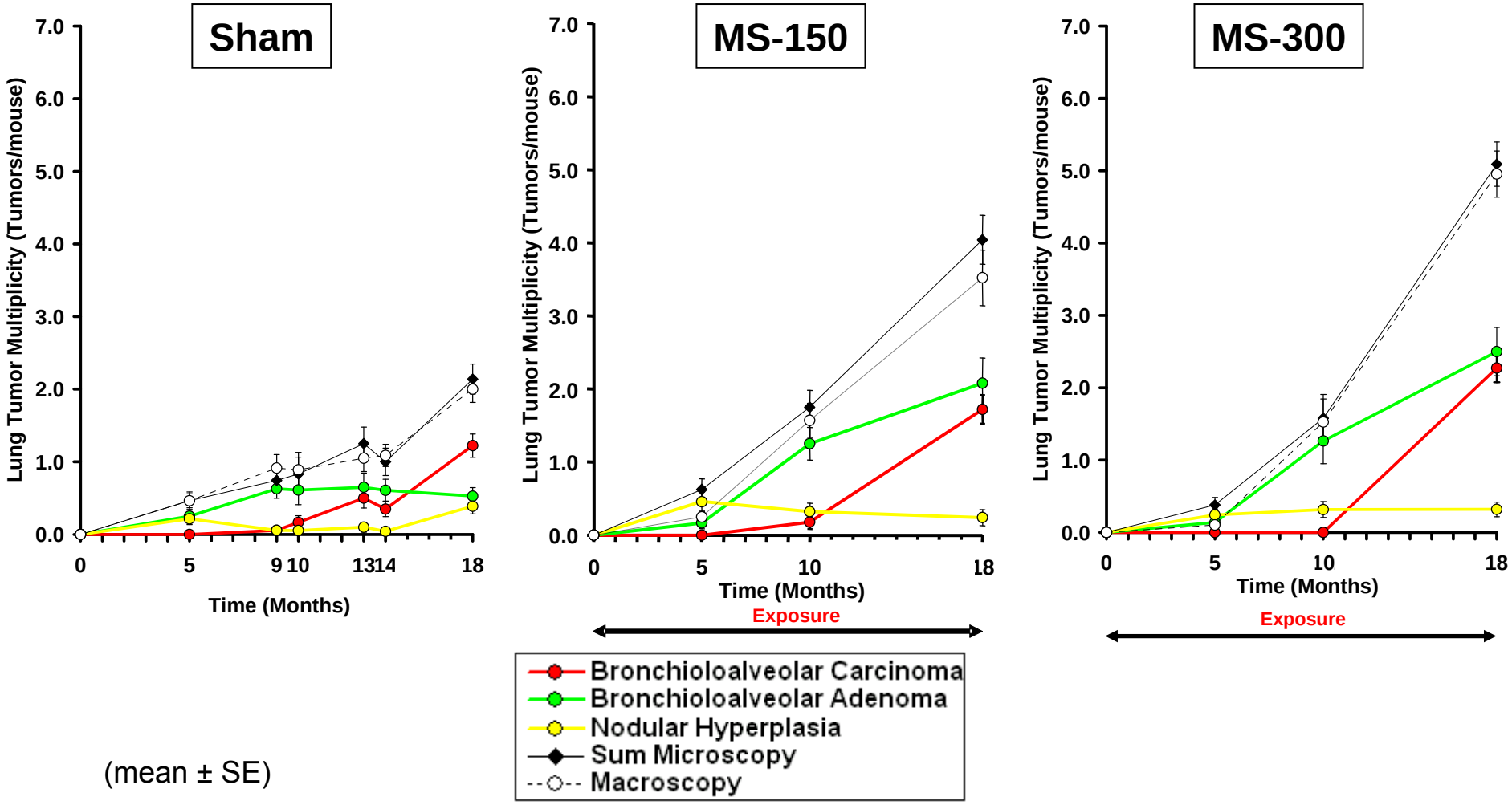

(mean ± SE)

# Histopathological Evaluation of Lung Tumor Multiplicity: 10 mo Exposure + 8 mo Post-Exposure



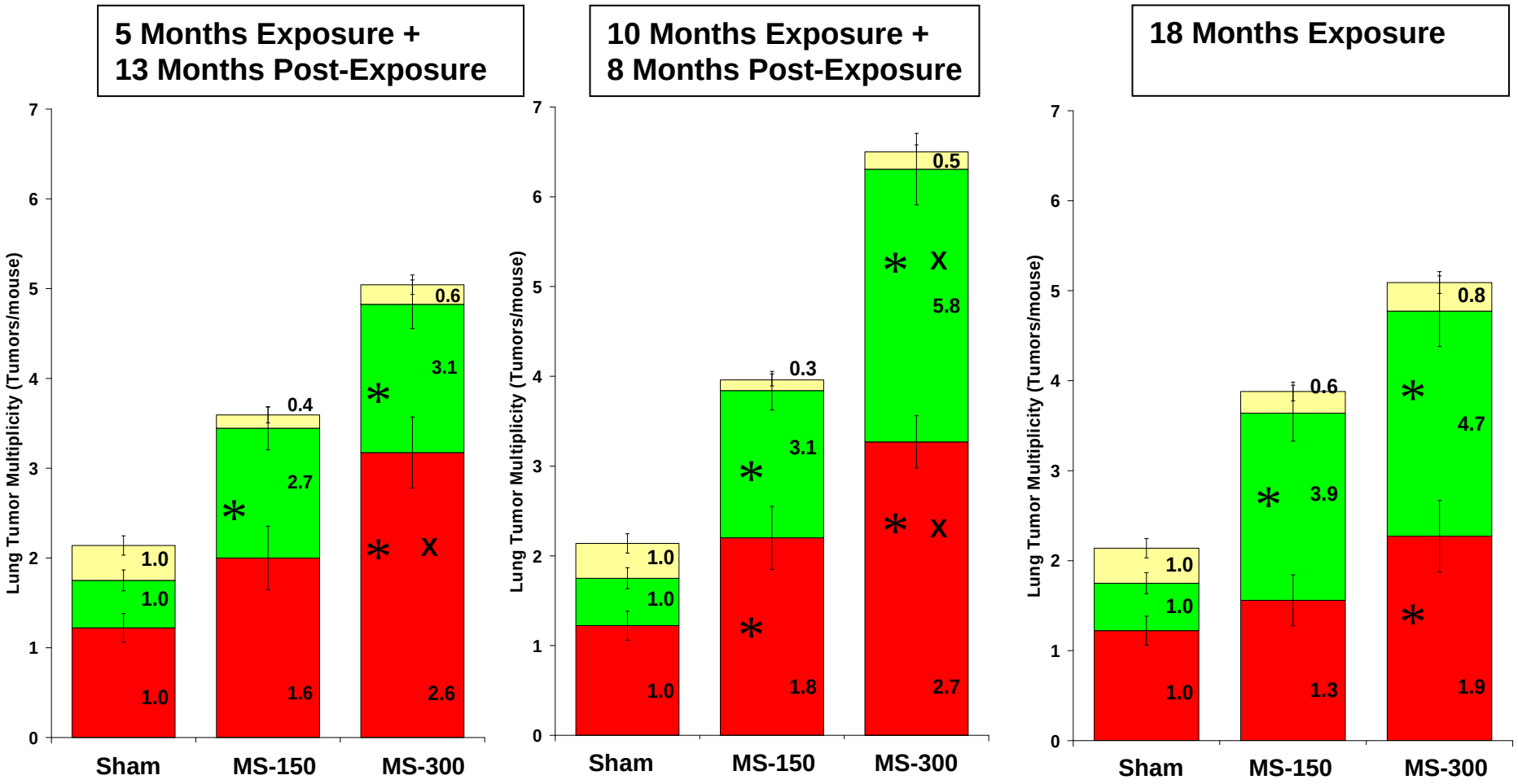

(mean ± SE)

# Histopathological Evaluation of Lung Tumor Multiplicity: 18 mo Exposure




(mean ± SE)

# Lung Tumor Multiplicity:18-Month Dissection




(mean ± SE)
* p≤0.05 compared to sham
X p≤0.05 compared to low MS

# mRNA Expression Analysis of Normal Lung Tissue and Nodules

## PROCEDURE

- Laser capture microdissection (LCM) of lung nodules and normal lung tissue
- mRNA analysis using Agilent technology

## RESULTS

- Normal lung tissue: differential gene expression pattern was induced by MS exposure

# Kinetics for Genes Coding for Antioxidant and Phase I/II Xenobiotic-Metabolizing Enzymes: Normal Lung Tissue

| gene symbol | 0.5 month | 2 months | 5 months | 5 months<br>2 days | 5 months<br>4 months | 5 months<br>13 months | 18 months |
|---|---|---|---|---|---|---|---|
| ftl2 | 1.5 | 2.1 | 2.0 | 1.3 | 1.4 | — | 1.9 |
| gclc | 6.4 | 6.7 | 5.7 | 1.1 | — | — | 4.0 |
| gclm | 3.4 | 3.7 | 4.1 | -1.2 | — | -1.4 | 2.8 |
| gpx2 | 2.8 | 3.1 | 2.5 | -1.4 | — | — | — |
| gsr | 2.5 | 2.5 | 2.1 | -1.0 | -1.3 | — | 2.0 |
| hmox1 | 2.7 | 4.5 | 4.4 | 1.6 | — | 1.5 | 4.8 |
| maff | 2.6 | 2 | 2.7 | -1.2 | — | — | 2.0 |
| nqo1 | 6.4 | 6.5 | 6.0 | -1.2 | — | — | 9.4 |
| txnrd1 | 4.1 | 3.6 | 3.6 | 1.1 | — | — | 2.4 |
| | | | | | | | |
| adh7 | 6.4 | 10.6 | 10.0 | -1.1 | — | — | 3.2 |
| aldh3A1 | 5.6 | 7.2 | 7.3 | -2.2 | — | — | 11.0 |
| akr1B8 | 4.2 | 4.3 | 4.4 | 1.6 | — | — | 3.1 |
| cyp1A1 | 64.7 | 67 | 67.4 | -5.1 | — | — | 100.0 |
| cyp1B1 | 10 | 10.6 | 8.3 | 1.5 | — | — | 44.0 |
| gsta1 | 5 | 6.3 | 5.3 | -1.7 | — | — | 2.1 |
| gsta2 | 5.7 | 7.2 | 6.5 | -1.7 | — | — | 2.3 |

← **exposure**

← **post-exposure**

Increase of 2-fold or more

# Kinetics for Genes Coding for Inflammatory Responses: Normal Lung Tissue

| gene symbol | alias | 0.5 month | 2 months | 5 months | 5 months 2 days | 5 months 4 months | 5 months 13 months | 18 months | ← exposure ← post-exposure |
|---|---|---|---|---|---|---|---|---|---|
| ccl2 | mcp-1 | 2.5 | 3.2 | 3.5 | 4.2 | 5.2 | 2.9 | 5.2 | chemokines |
| ccl3 | mip-1α | 6.7 | 7.3 | 7.8 | 8.3 | 8.9 | 3.3 | 9.7 | chemokines |
| ccl6 | mrp-1 | 3.8 | 6.9 | 6.1 | 5.7 | 3.2 | 2.2 | 6.1 | chemokines |
| ccl20 | mip-3α | 7.6 | 5.1 | 3.6 | 2.7 | 3.4 | 2.1 | 3.6 | chemokines |
| ccl5 | rantes | -1.9 | -2.0 | -2.7 | -1.6 | — | 2.2 | — | chemokines |
| cxcl1 | groα, kc | 7.8 | 9.1 | 5.6 | 6.2 | 3.8 | 2.5 | 9.6 | chemokines |
| cxcl5 | ena-78 | 64 | 59.5 | 30.1 | 7.2 | 2.1 | 16.7 | 76.7 | chemokines |
| cxcl9 | mig | 2.1 | 3.8 | 4.4 | 4.9 | — | 3.7 | 3.5 | chemokines |
| cxcl10 | IP-10 | 2.3 | 1.9 | 3.0 | 5.3 | — | 2.2 | 2.5 | chemokines |
| saa3 | | 16.4 | 17.2 | 13.6 | 15.9 | 10.5 | — | 28.9 | acute-phase response |
| orm2 | | 3.2 | 2.8 | 2.8 | 2.4 | 4.4 | 2.8 | 33.7 | acute-phase response |
| cd68 | | 2.8 | 4.7 | 5.1 | 4.0 | 3.3 | 1.8 | 4.8 | macrophage marker |
| msr | | 3.6 | 10.3 | 8.4 | 5.6 | 3.4 | — | 6.8 | macrophage marker |
| mmp12 | | 7.1 | 10.2 | 10.5 | 8.8 | 20.4 | 2.4 | 7.1 | matrix metallopeptidase |
| timp1 | | 3.4 | 2.9 | 2.5 | 2.0 | 1.8 | — | 6.9 | tissue inhibitor of metalloproteinase 1 |
| slpi | | 1.2 | 3.1 | 2.6 | 2.0 | 2.6 | 2.8 | 5.7 | secretory leukocyte protease inhibitor |
| ctsk | | 5.3 | 8.9 | 8.8 | 5.2 | 4.3 | 1.4 | 6.4 | cathepsin K |
| ctss | | 2 | 5.4 | 5.9 | 5.3 | 2.3 | — | 2.5 | cathepsin S |

≥ 2-fold increase

≥ 2-fold decrease

# mRNA Expression Analysis of Normal Lung Tissue and Nodules

## PROCEDURE

- Laser capture microdissection (LCM) of lung nodules and normal lung tissue
- mRNA analysis using Agilent technology

## RESULTS

- Normal lung tissue: differential gene expression pattern was induced by MS exposure

- Lung nodules: no differential gene expression pattern was induced by MS exposure (31 nodules, 14 normal lung tissues)

**Possible explanations**
**Technical reasons:** mainly ruled out
**Biological reasons:** High heterogeneity of nodules: e.g., independent transformation events, different tumor progression stages, and mixture of adenoma and carcinoma.

# *K-ras* Mutation analysis of Lung Nodules from MS-exposed A/J Mice

## PROCEDURE

- LCM of lung nodules from snap-frozen tissue and formalin-fixed, paraffin-embedded tissue
- Isolation of DNA, amplification with subsequent sequencing of the Exon 1 and Exon 2 fragments of the *K-ras* gene, mutation analysis of the hotspots: codons 12, 13, and 61

## RESULTS

- No MS-specific pattern was observed

# *K-ras* Mutations in LCM-derived Lung Nodules: No MS-specific Pattern

**Totals for snap-frozen tissue and formalin-fixed, paraffin-embedded tissue combined.**

| | # of Tumors and *K-ras* Mutations | | Incidence of *K-ras* mutations | # of *K-ras* Mutations in Hotspot Codons | | | # of Trans-versions |
|---|---|---|---|---|---|---|---|
| **Group** | **Tumors** | **Mutations** | **%** | **12** | **13** | **61** | **12 G→T** |
| **18 mo control** | **11** | **8** | **73** | **4** | **0** | **4** | **2** |
| **18 mo MS** | **14** | **12** | **86** | **6** | **0** | **6** | **3** |

# Summary

**A/J mice exposed to cigarette smoke: major findings**

- Significant, concentration-dependent enhancement of lung tumors, i.e., adenomas and adenocarcinomas
- No obvious shift in tumor spectrum (from adenoma to adenocarcinoma)
- Differential gene expression in normal lung tissue
  - 3 main classes: genes related to oxidative stress, xenobiotic metabolism, or inflammatory processes
- No differential gene expression in isolated lung nodules
- No MS-specific mutation pattern in exons 1 and 2 of the *K-ras* gene

# Conclusion

- Chronic exposure (18 mo) of A/J mice to cigarette smoke results in increased lung tumor formation
- Dose-dependency and good reproducibility of cigarette-smoke-dependent increased lung tumor formation in A/J mice
- The relevance of the A/J mouse model for cigarette-smoke-induced lung tumors in humans requires further validation

# Acknowledgement

Co-authors

- Ansgar Bűttner (Philip Morris Research Laboratories, Cologne, Germany)
- Stephan Gebel (Philip Morris Research Laboratories, Cologne, Germany)
- Hans-Jűrgen Haussmann (toxicology consultant, Roesrath, Germany)
- Walter Stinn (Philip Morris Research Laboratories, Cologne, Germany)
- Frans van Overveld* (Philip Morris Research Laboratories, Leuven, Belgium)

*Current address: Kessel-Lo, Belgium

Special thanks also to all colleagues at Philip Morris Research Laboratories (Leuven, Belgium and Cologne, Germany) for providing excellent support

This work was supported in part by Philip Morris USA