Investigation of biological mechanisms of radiation-induced circulatory diseases

Nov 25 2008

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Clinical and epidemiological phenomena

			Therapeutic doses	Doses <2 Gy
A	Coronary arteries	Increased incidence of coronary artery disease	+	+
B	Peripheral arteries	Increased incidence of hypoxic events	+	?
C	Myocardial cap-illary network	Myocardial perfusion defects	+	?
D	Myocardium	reduced systolic function valve defects pericarditis	+/-	

Answers needed

- Dose and time dependence
- Target structures
- Volume effect
- Pathogenesis
- Individual risk
- Preventive of therapeutic strategies

Agenda

- Analysis of published experimental data
  - -Coronary arteries
  - -Peripheral arteries
  - -Myocardial capillary network
  - -Endothelial cells in vitro

- Ongoing EU studies
  - -Concept and preliminary results from NOTE
  - -Concept of Cardiorisk

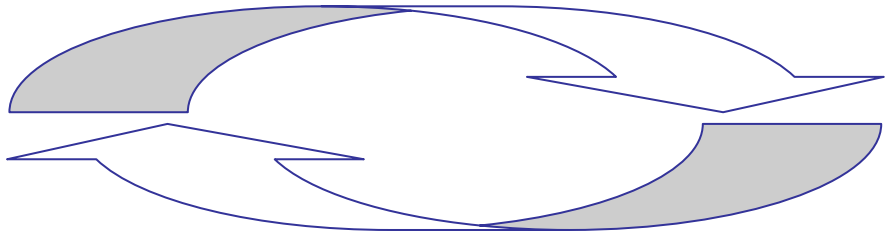
- Conclusions

- Challenges, Outlook

Published experimental data relevant to pathogenesis

A Peripheral and coronary arteries	F. Stewart et al 2006, Hoving et al 2008: mouse carotid artery: ≥ 8 Gy in ApoE -/- cause accelerated atheroclerosis of inflammatory phenotype
B Myocardium	L.F. Fajardo, Lauk/Schultz-Hector et al: extensive experimental data after ≥ 10 Gy: decrease of microvascular density leading to myocardial function loss
C Endothelial cells in vitro	Radiation induction of inflammatory cytokines between 2 hours and 1 week after Doses of 0.5-8 Gy Prothrombotic effect at 5 h to 16 m after 4-15 Gy

A Hypothetic pathogenetic pathway of coronary artery disease interaction with radiation



Shear stress

NFkB-induction

Adhesion molecules

Leukocyte migration

Monocytes → foam cells

Response to circulating LDL

Sec. Cytokine induction

Release of O-radicals and proteases from macrophages

Clonal Smc proliferation

Smc genetic instability microsatellites

Plaque formation

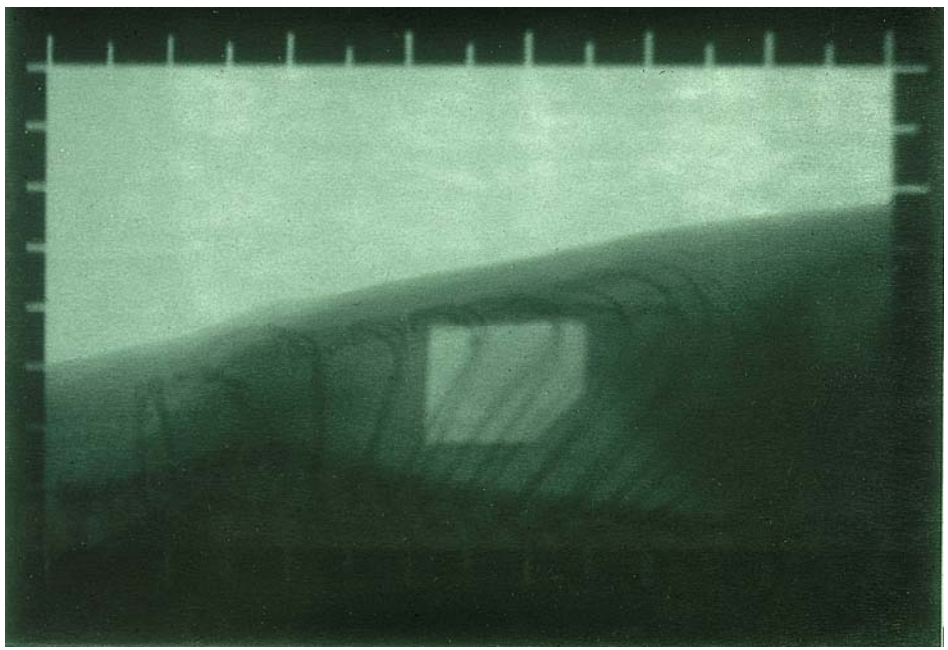
Plaque instability

hours

decades

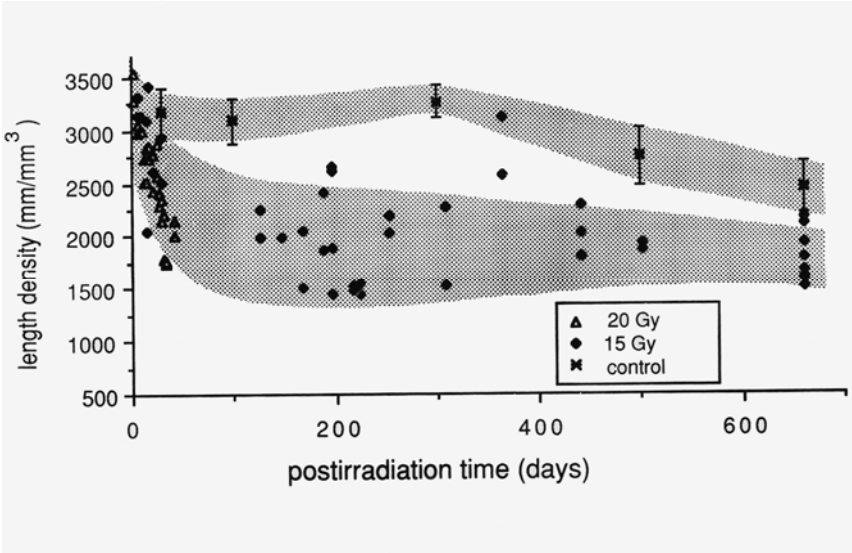
B Local heart irradiation in rats

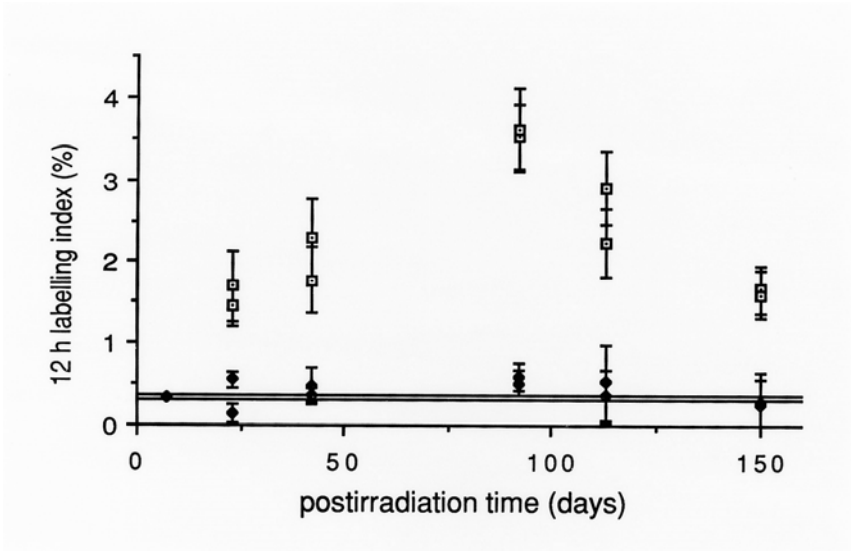
- Local heart irradiation with single doses of 10-40 Gy as well as with fractionated doses
- Individual treatment planning in order to minimize lung dose



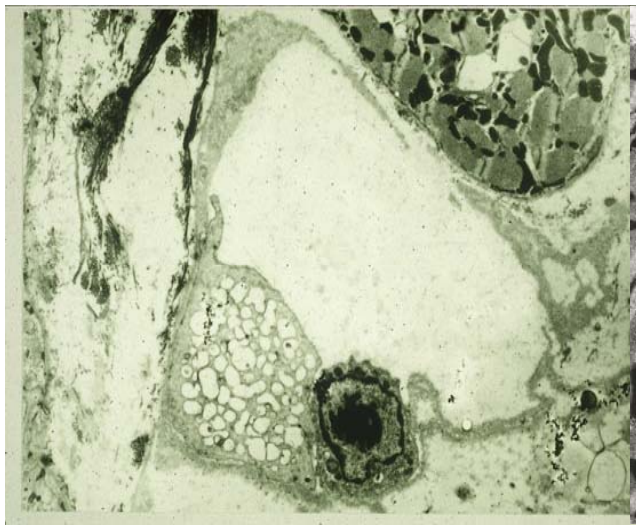
- Double exposure radiograph of rat thorax and radiation field

B Myocardial capillary density loss preceded by endothelial cell proliferation after local heart irradiation in rats

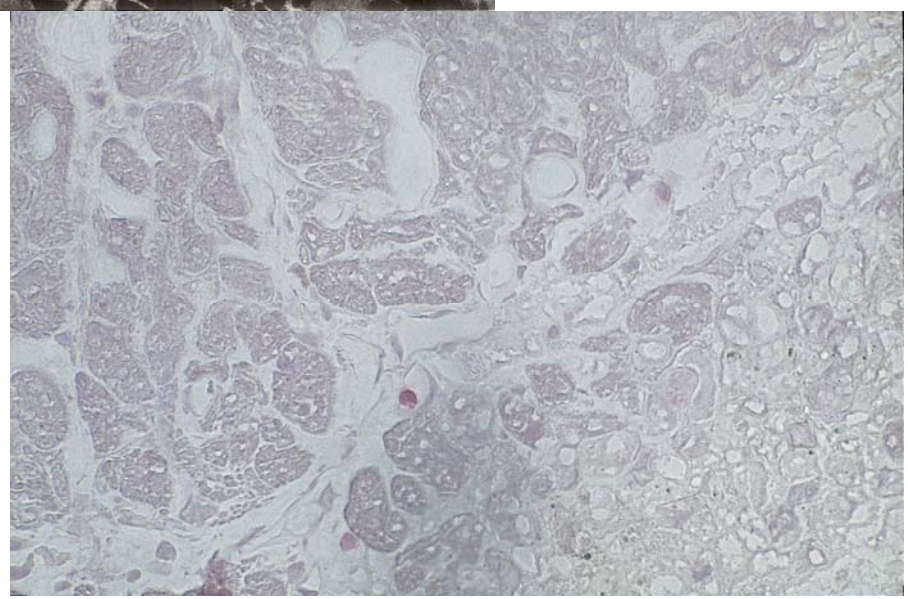




B Myocardial perfusion III: Signs of endothelial activation



- adhesion of CD8 positive lymphocytes
- endothelial cell swelling, blebbing



B Myocardial perfusion IV: Pathogenetic pathway after therapeutic doses

Endothelial cell activation (cytokine release)

Endothelial cell proliferation (expression of DNA damage)

Endothelial cell loss (reduced capillary density and perfusion)

Myocardial damage

The initial event triggering endothelial cell proliferation is not well explained by “classic radiation DNA damage”
Up-regulation of pro-inflammatory factors could be **the** critical initiating event.

Part II - Outline of talk

Universitätsklinikum Leipzig
Anstalt öffentlichen Rechts

- Radiation-induced heart disease ?
  - pericarditis
  - myocardial damage
  - cardiovascular disease (CVD)
- Experimental results / evidence & mechanisms ?
  - high dose experiments
  - ongoing research in European projects:
    - **NOTE** (FP6) *[09/2006 – 08/2010]*
    - **CARDIORISK** (FP7) *[02/2008 – 01/2011]*
- Implications for radiation protection ?

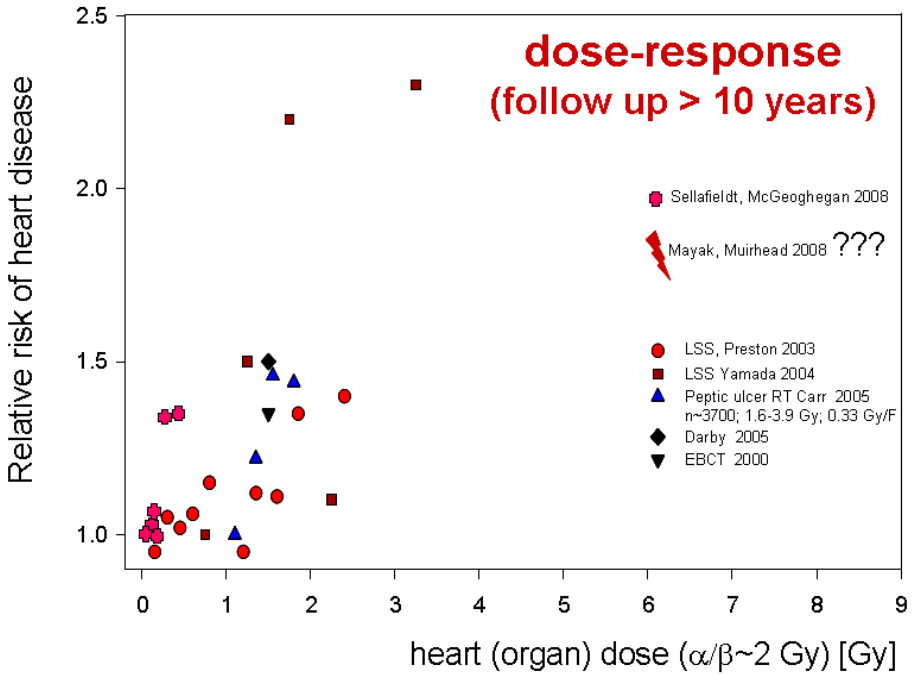
NOTE – TOWARDS A NEW PARADIGM

CARDIORISK

SEVENTH FRAMEWORK PROGRAMME
CARDIORISK is a collaborative Project funded by the European Commission under the 7th Framework Prgramm

- Investigation of biological mechanisms of radiation induced circulatory diseases -

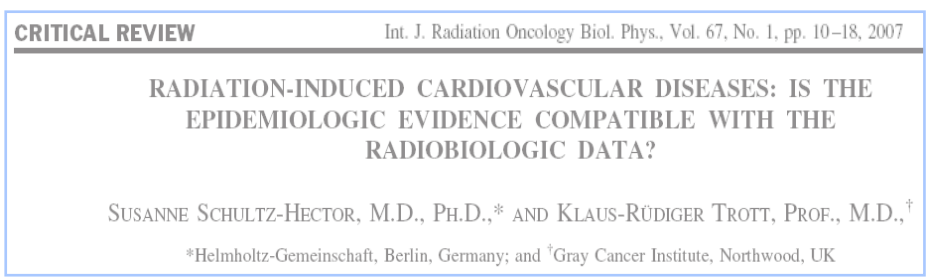
Circulatory diseases – Experimental evidence & Mechanisms ?



completed and modified after Schultz-Hector and Trott 2007

Open Questions

- **Where is the critical structure where the dose in the heart has to be calculated ?**
- **Does cardiovascular risk increase with dose after a threshold dose or without ?**
- **Does the latency depend on dose ?**
- **Does the risk depend on age at exposure ?**
- **Which circulatory / cardiovascular disease is caused by low and moderate radiation doses ?**



CRITICAL REVIEW — Int. J. Radiation Oncology Biol. Phys., Vol. 67, No. 1, pp. 10–18, 2007

RADIATION-INDUCED CARDIOVASCULAR DISEASES: IS THE EPIDEMIOLOGIC EVIDENCE COMPATIBLE WITH THE RADIOBIOLOGIC DATA?

SUSANNE SCHULTZ-HECTOR, M.D., PH.D.,* AND KLAUS-RÜDIGER TROTT, PROF., M.D.,†

*Helmholtz-Gemeinschaft, Berlin, Germany; and †Gray Cancer Institute, Northwood, UK

A Systematic Review of Epidemiological Associations between Low and Moderate Doses of Ionizing Radiation and Late Cardiovascular Effects, and Their Possible Mechanisms

RADIATION RESEARCH 169, 99–109 (2008)

M. P. Little,[a,1] E. J. Tawn,[b,2] I. Tzoulaki,[a] R. Wakeford,[c] G. Hildebrandt,[d] F. Paris,[e] S. Tapio[f,g] and P. Elliott[a]

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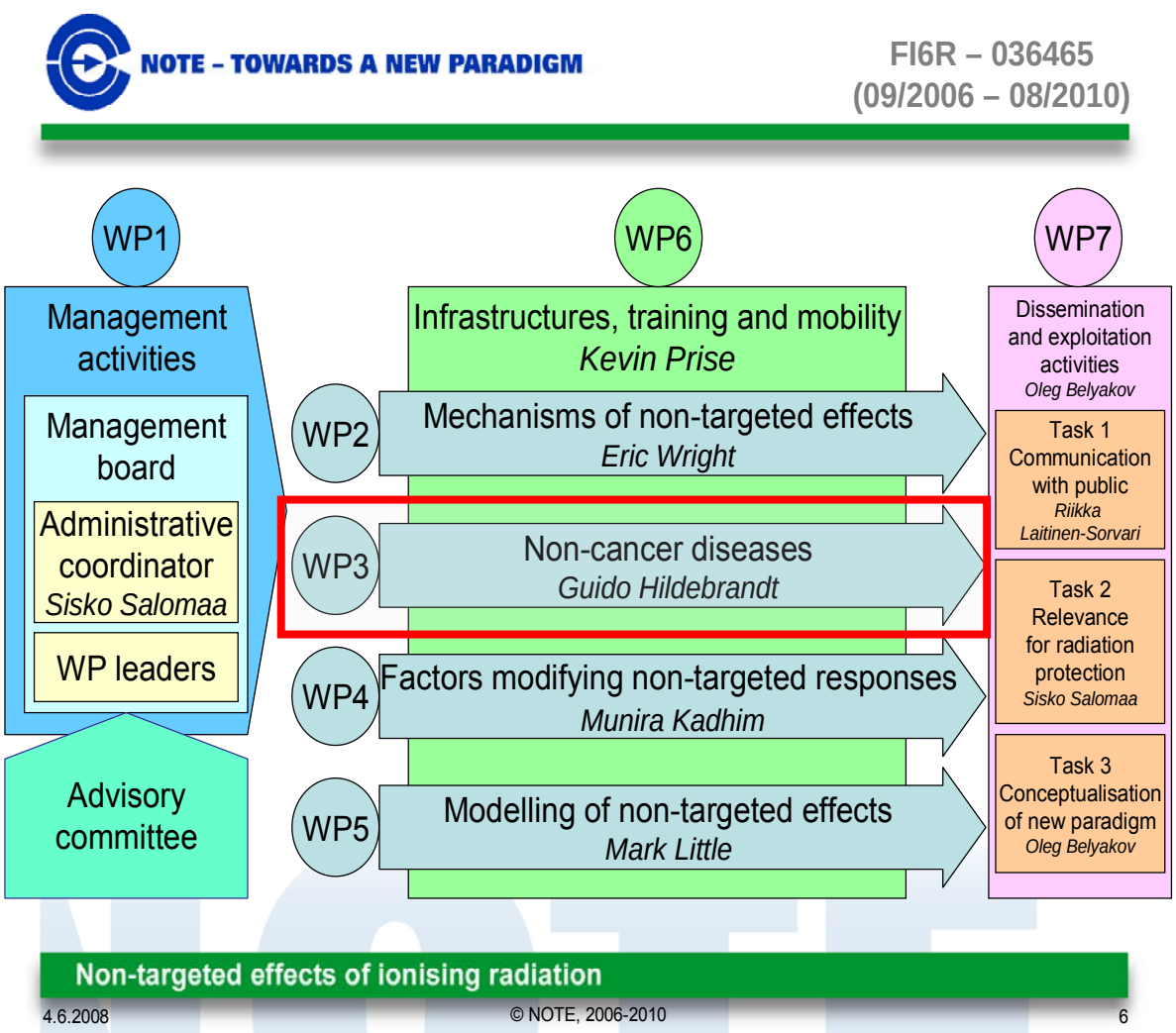
Possible Mechanisms

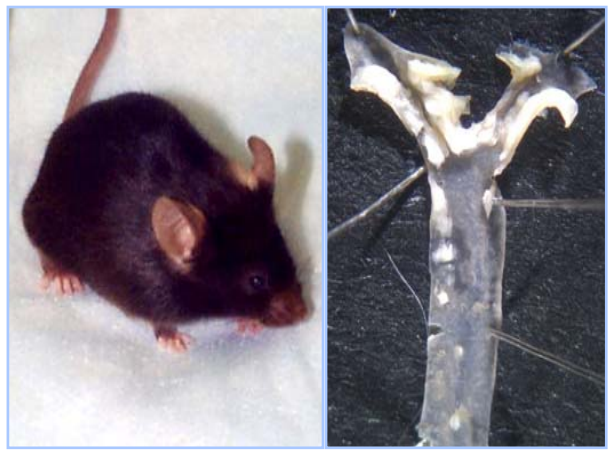
- **Inflammatory / Microvasculature theory**
  - Possible signature changes in microvasculature, e.g. fibrosis
  - Endothelial injury / dysfunction and inflammatory response
- **Possible long-term radiation effects on immune system**
- **Mutation theory**
  - Monoclonal origin of atherosclerotic plaques (G6PD)
- **Transformation of smooth muscle cells in athero-genesis pathway?**
  - Oncogene activation, LOH, and microsatellite instability

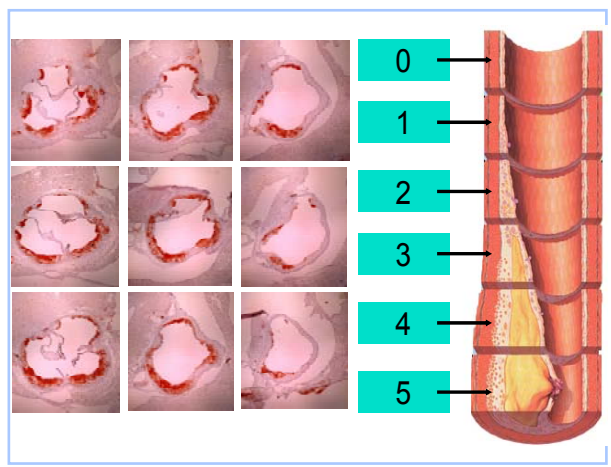
- Investigation of biological mechanisms of radiation induced circulatory diseases -

Circulatory diseases – Experimental evidence & Mechanisms ?



Models of Atherosclerosis: Apolipoprotein E -/- Mice



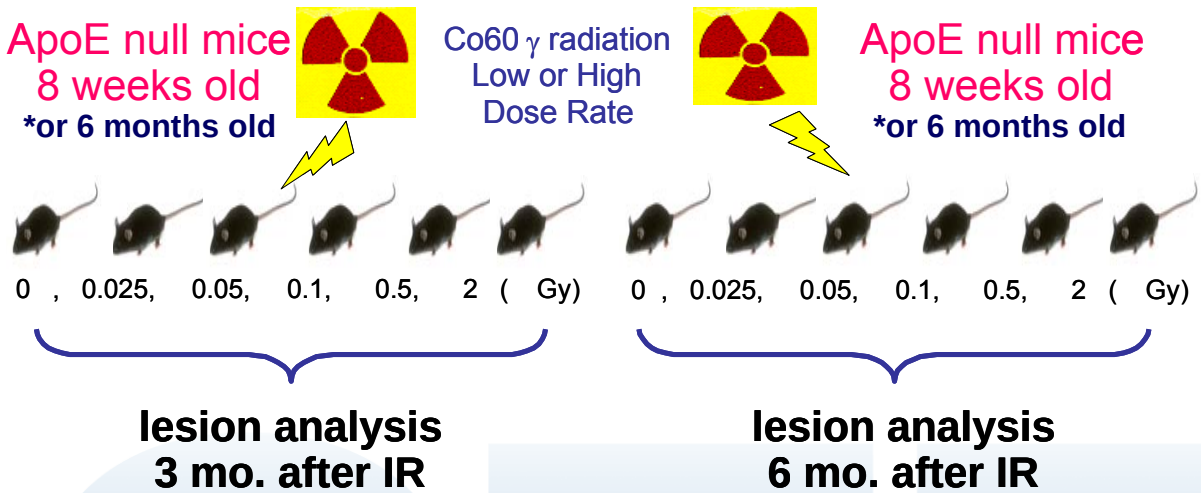


Contract-No. **FP6-036465; Integrated Project;** *Consortium:* **21 partner;** *EU budget:* **6.33 Mio. €**

3.2. Mechanisms of cardiovascular diseases induction-*in vivo.*

AECL *(R. Mitchel)* & OHIRC *(St. Whitman)*

➢ *To investigate radiation dose response on atherosclerosis progression after exposure at early and late stage disease in knockouts (ApoE-/-) [subtask 3.2.1 & 3.2.3] and in ApoE-/- TP 53+/- [subtask 3.2.3 & 3.2.4] as compared to "normals" (ApoE+/+) in vivo.*
(morphological endpoints, markers of inflammatory and stress responses)

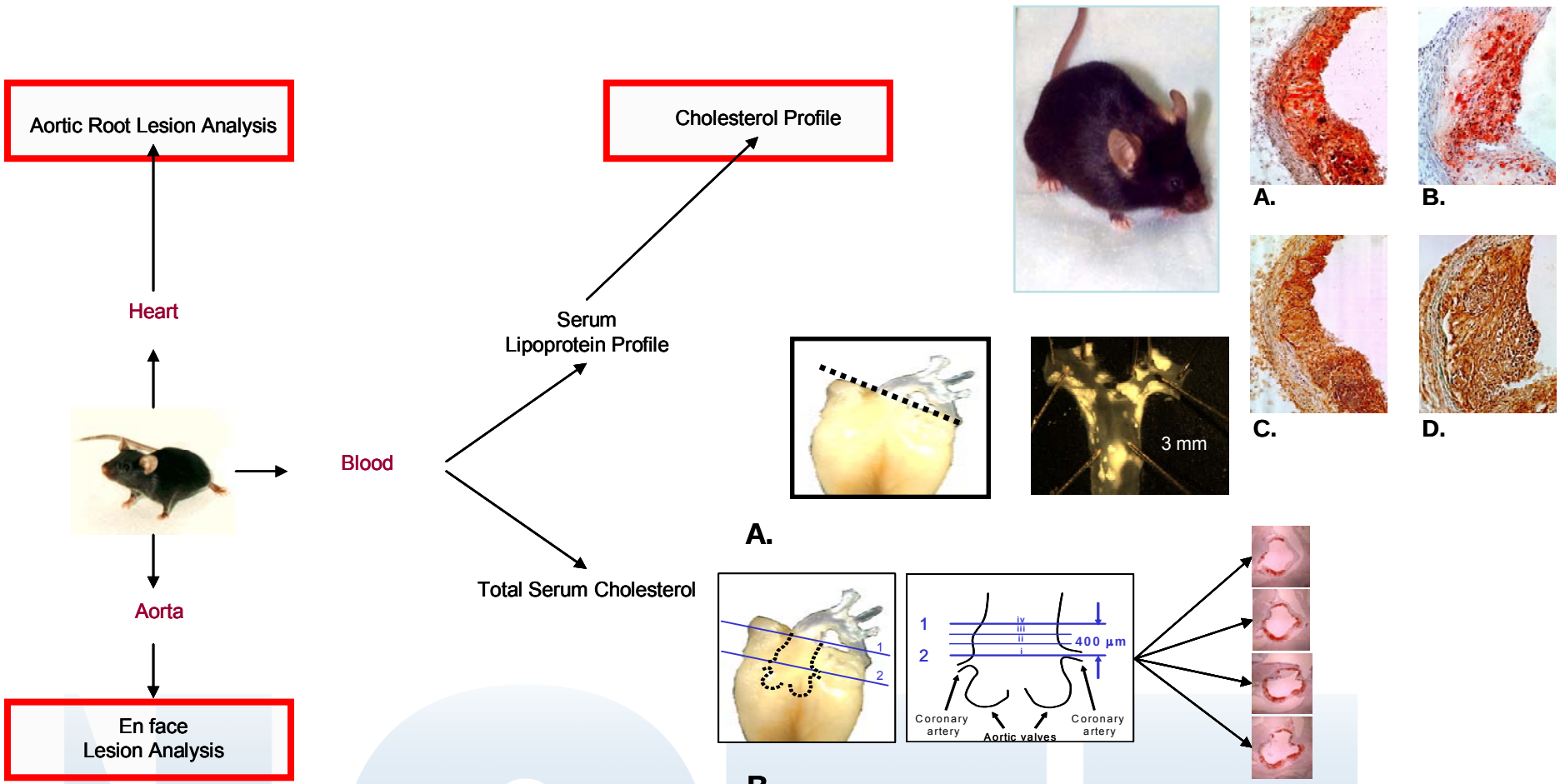


High dose rate:
0.36 Gy/min

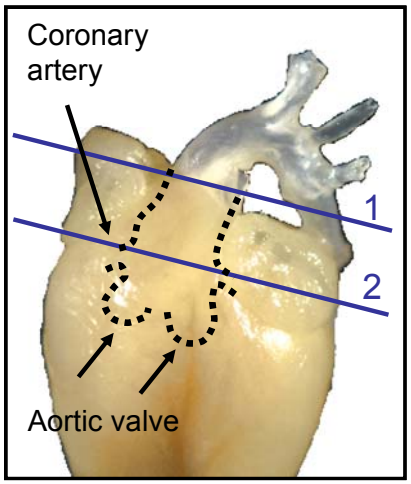
Low dose rate:
1 mGy/min,
100 mGy/d,
5 d/wk

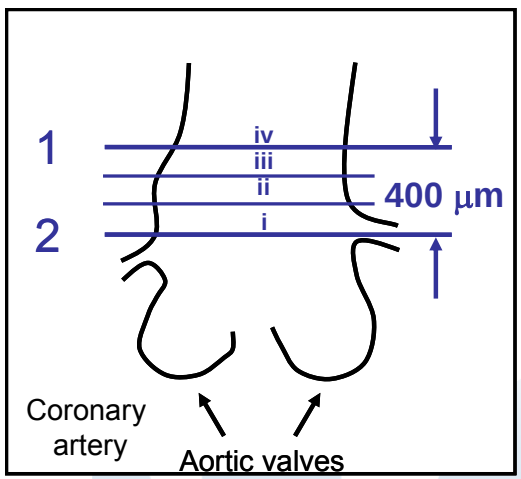
Blood and Tissue collected: Aorta, Heart, Liver, Kidney, Lung, Spleen

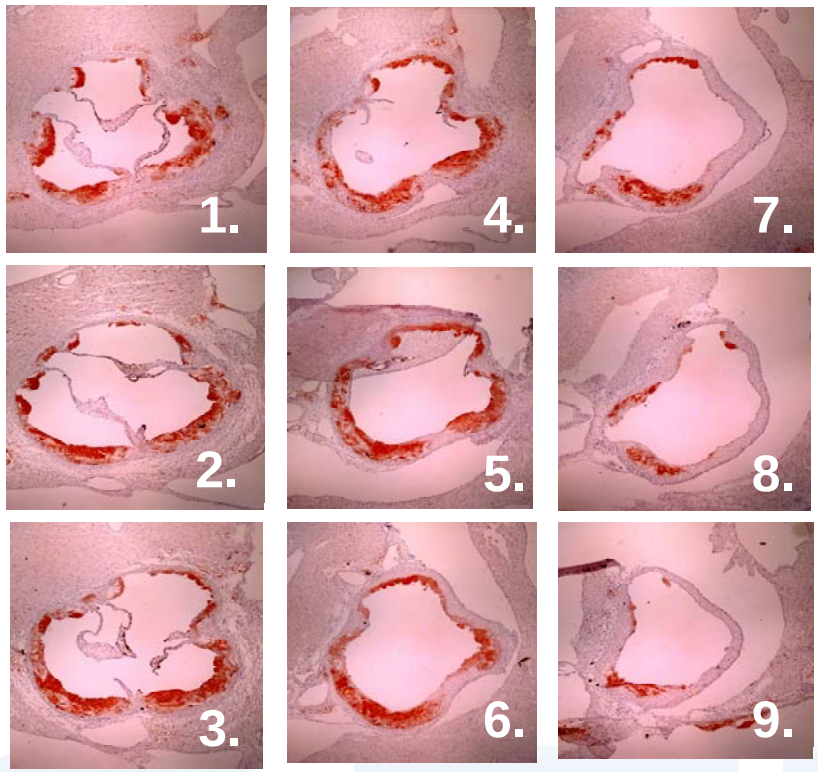
3.2. Mechanisms of cardiovascular diseases induction-*in vivo.*



Lesion size analysis: Serial Sections of the Aortic Root







- Nine sections spanning the aortic root and ascending aorta collected on one of 10 microscope slides.

  (Sudan IV staining)
- Each section is 100 μm apart from the previous one.

  (level 0 is section #5)
- ApoE null mouse (13 wks on a cholesterol-enriched diet).

3.2. Mechanisms of cardiovascular diseases induction-*in vivo.*

Lesion size analysis: Preliminary summary of results

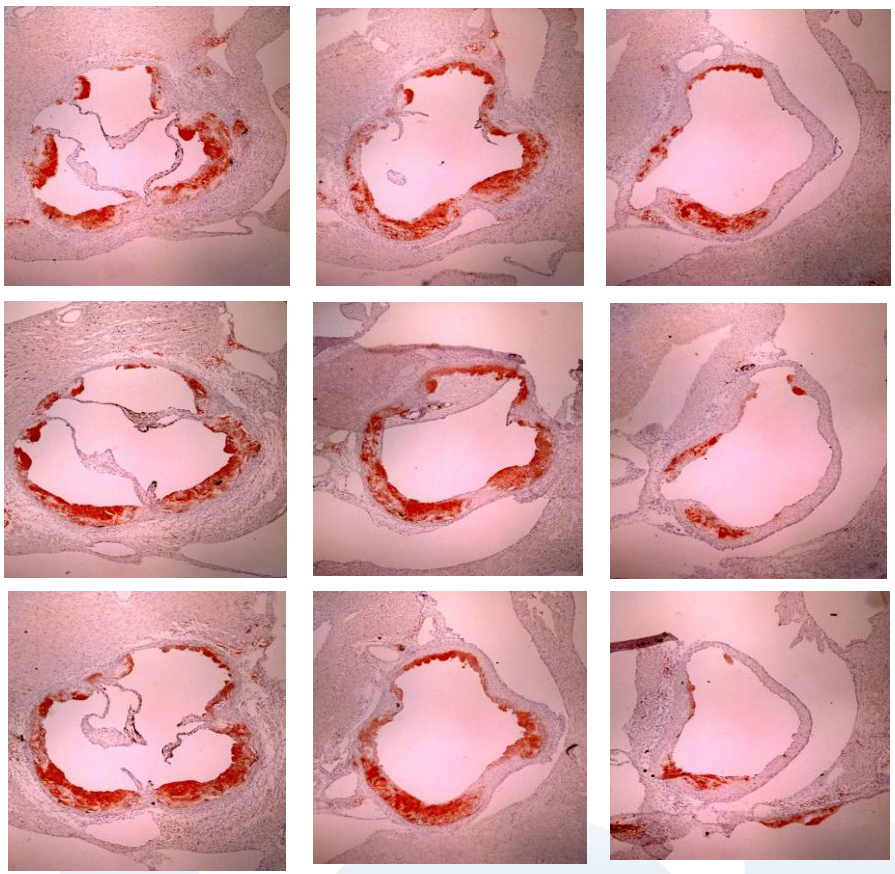
For Apo E-/- mice:

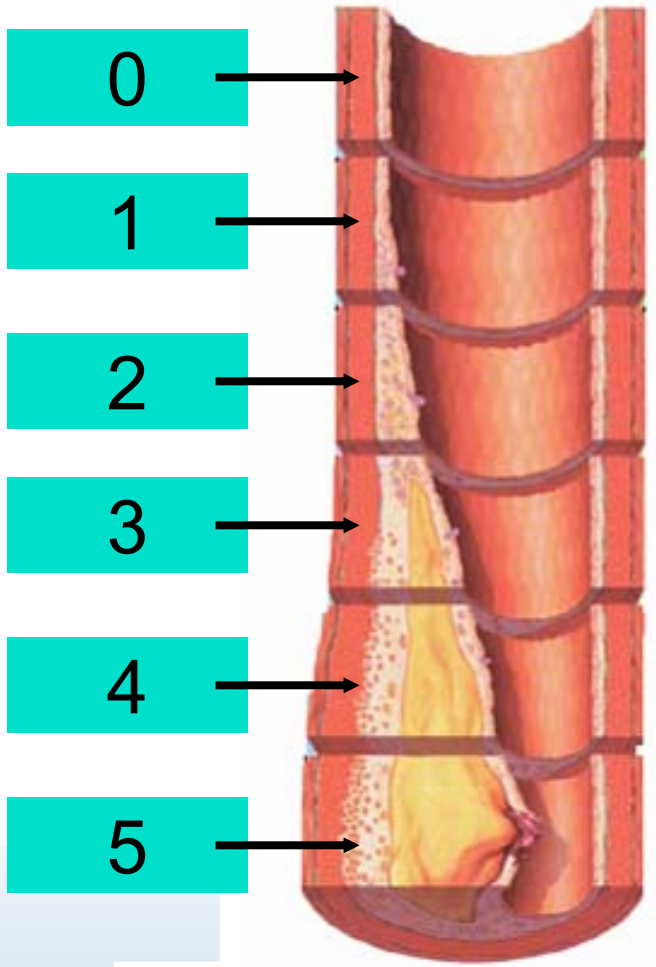
- Doses of γ-radiation between 0.025 Gy and 0.5 Gy have no significant effect on atherosclerotis size **3 months after exposure** to radiation compared to mice not exposed. 2 Gy dose significantly increased atherosclerotic lesion size in the aortic root to Low Dose Rate radiation.
- Atherosclerotic lesion size in the aortic root was significantly decreased in mice **6 months after exposure** to 0.05Gy low dose rate radiation, compared to mice not exposed to radiation. For doses of radiation of 0.025 Gy, 0.05 Gy, 0.1Gy and 2 Gy more mice should be used to test the significance of the effect.

For p53+/- ApoE-/- mice:

- Doses of γ-radiation of 0.025 Gy, 0.05 Gy, 0.1, 0.5 Gy and 0.5 Gy did not significantly affect atherosclerotic lesion size in the aortic root **6 months after exposure** to radiation, compared with mice not exposed to radiation.

Lesion stage analysis: Serial Sections of the Aortic Root





3.2.5. Extension experiments on mechanisms of cardiovascular diseases induction - *in vivo.*

AECL *(R. Mitchel, N. Priest)* **& OHIRC** *(St. Whitman)*

& ULMED *(G. Hildebrandt)* **& ICFM** *(M. Little)*

➢*Since inflammatory and thrombotic changes in endothelial cells have an important impact on the development of atherosclerotic lesions we further want to study whether low dose IR at low dose rate or high dose rate induces vascular changes in the heart of ApoE-/- mice*

- *1. Pro-thrombotic surface*
  (thrombomodulin, fibrinogen, protease-act.-Rec.1)
- *2. Inflammatory response*
  (VCAM-1, ICAM-1, E-Selectin, Thy-1)
- *3. Leukocyte extravasation*
  (granulocytes, lymphocytes, mph. type 1&2)

3.2.5. Extension experiments on mechanisms of cardiovascular diseases induction - *in vivo.*

ApoE null mice
8 weeks old

High dose rate:
0.36 Gy/min

Low dose rate:
1 mGy/min,
100 mGy/d,
5 d/wk

ApoE null mice
8 weeks old

0 , 0.025, 0.05, 0.1, 0.5, 2 (Gy)

0 , 0.025, 0.05, 0.1, 0.5, 2 (Gy)

3 mo. after IR

6 mo. after IR

Analysis of 6 blood samples and 6 hearts per time point (0, 3, 6 months after IR) with either low or high dose rate

3.2.5. Extension experiments on mechanisms of cardiovascular diseases induction - *in vivo.*

Inflammatory markers - Preliminary summary of results

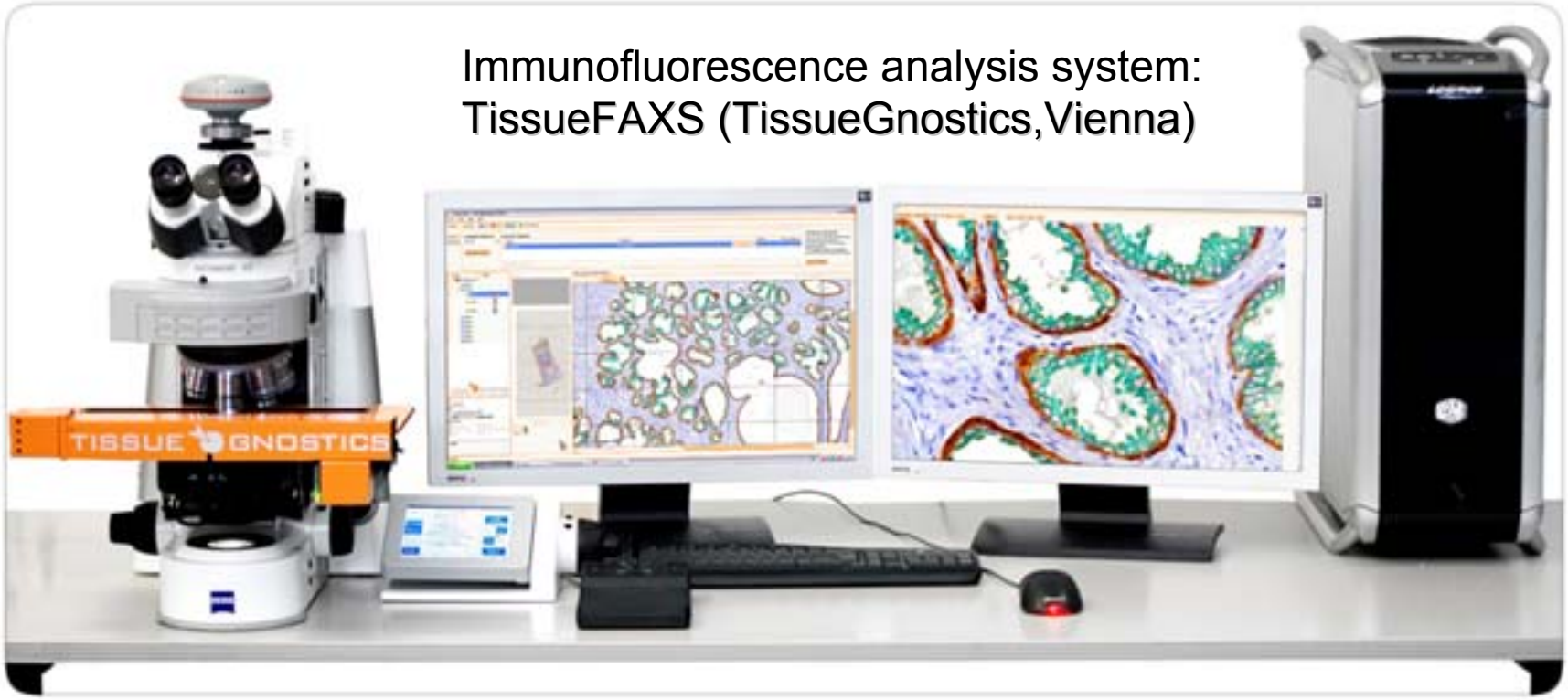
- Preliminary data indicate that irradiation at high dose rate triggers some proinflammatory response
  - increase of inflammatory cytokines
  - decrease of proinflammtory cytokines
  - acceleration of adhesion and thrombotic properties
- Relevant doses as low as 0.05 Gy in some cases
- Correlation with morphological/immunohistochemical data will clarify the significance of these findings

3.2.5. Extension experiments on mechanisms of cardiovascular diseases induction - *in vivo.*

Establishment of immunostaining for inflammatory and prothrombotic markers:

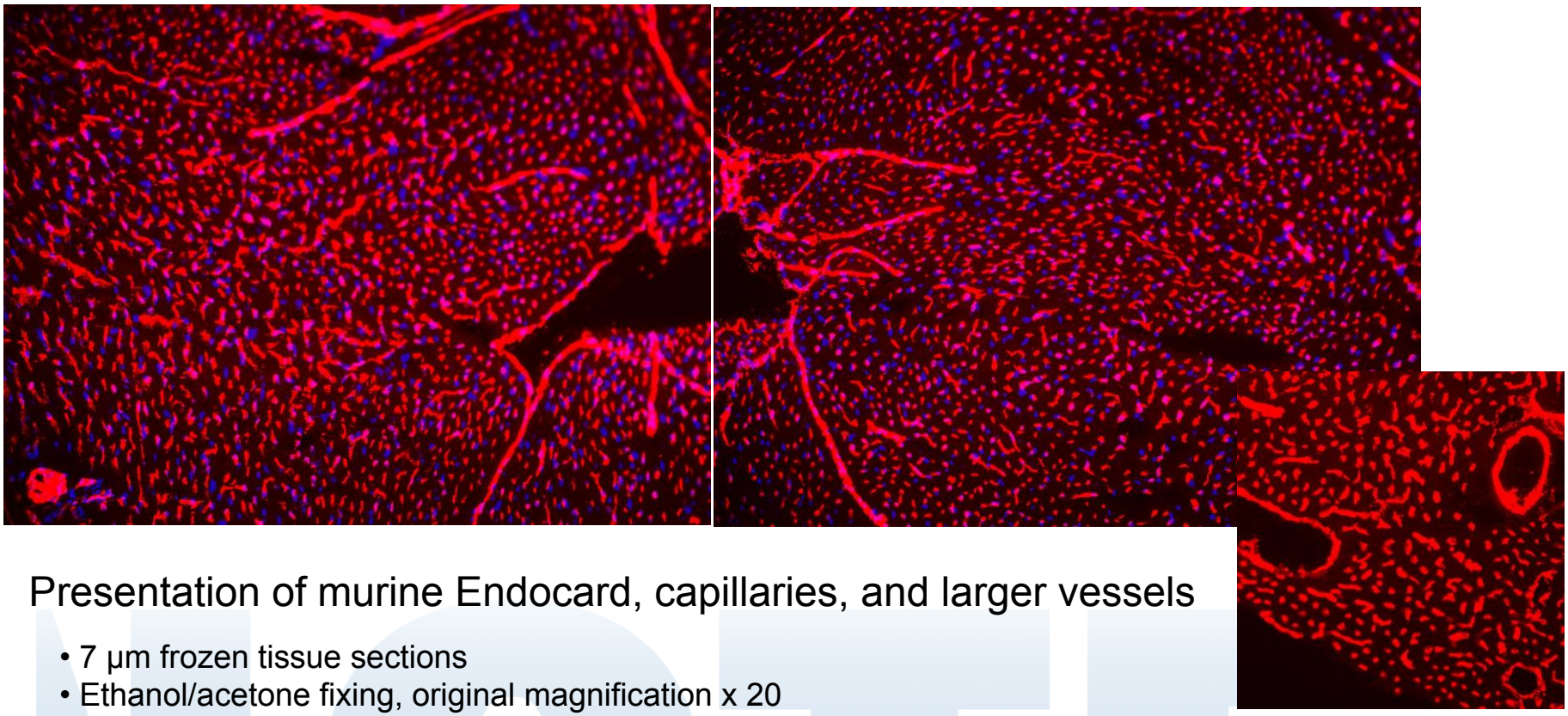
CD31, Mac-3, Thy1, vWF, ICAM, VCAM, Thromobomodullin, Fibrin

Immunofluorescence analysis system:
TissueFAXS (TissueGnostics,Vienna)



3.2.5. Extension experiments on mechanisms of cardiovascular diseases induction - *in vivo.*

CD31 (PECAM)-Cy5 / DAPI staining of murine heart

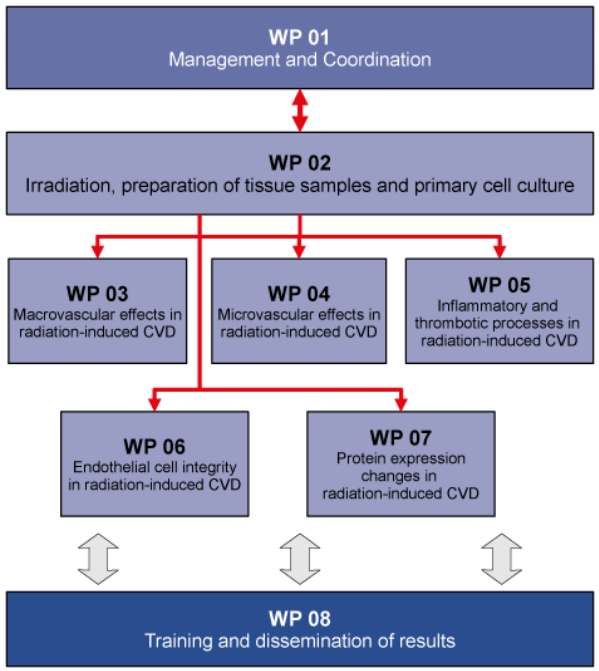


Presentation of murine Endocard, capillaries, and larger vessels

- 7 µm frozen tissue sections
- Ethanol/acetone fixing, original magnification x 20

CARDIORISK (02/2008 – 01/2011)

The mechanisms of cardiovascular risks after low radiation doses

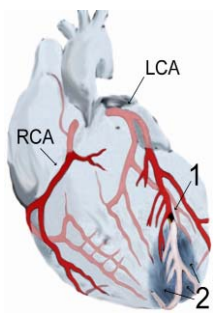


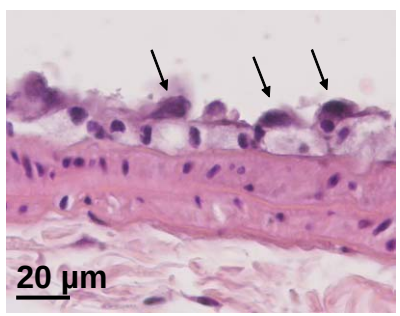
➢ Hypothesis I:

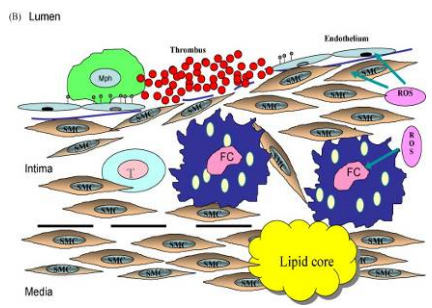
Radiation increases the frequency of myocardial infarction by directly interacting with one or more steps of the pathogenic pathway of age related coronary artery atherosclerosis.

➢ Hypothesis II:

Radiation increases lethality of myocardial infarction, which may occur due to pathologies unrelated to radiation, i.e. by reducing organ tolerance to minor acute infarctions as a result of persistent or progressive reduction of the microcirculation in the irradiated heart.







Contract-No. **FP7-211403; Collaborative Project;** *Consortium:* **11 partner;** *EU-budget:* **2.95 Mio.€**

SEVENTH FRAMEWORK PROGRAMME
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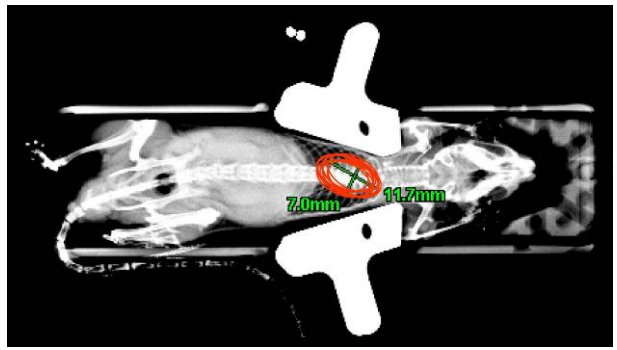
CARDIORISK (02/2008 – 01/2011)

The mechanisms of cardiovascular risks after low radiation doses

- **Work Package 2**

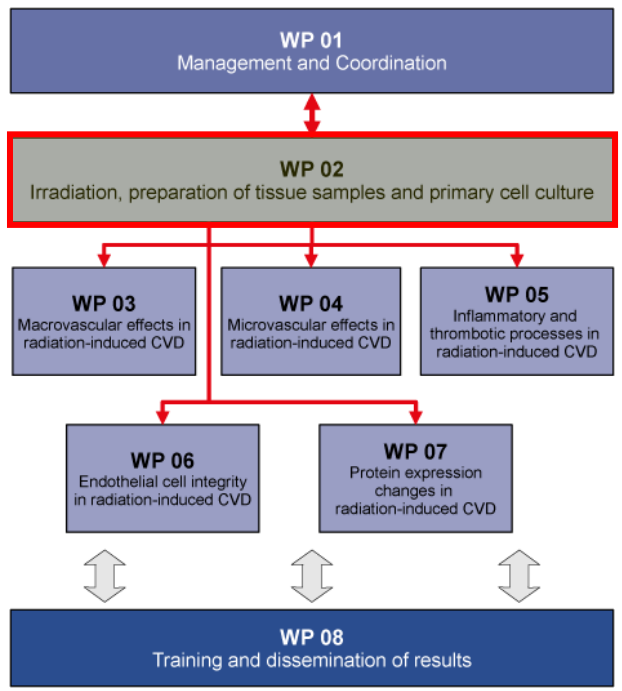
Irradiation, preparation of tissue samples and primary cell culture.

- animal breeding, housing, and irradiation:
  TUD - W. Doerr / NKI - F. Stewart
- MVHEC- / Cardiomyocyte-isolation:
  USFD - C. Kanthou / MSCCI - D. Gabrys /
  IRSN - M.C. Vozenin-Broton



➢ Objectives:

- **Local irradiation** of hearts and of two peripheral arteries of different strains of mice.
- Long-term follow up of the irradiated animals.
- Isolation of endothelial cells and cardiomyocytes at different times after local irradiation.
- Study of the different late post-radiation effects in microvascular endothelial cells.



CARDIORISK (02/2008 – 01/2011)

The mechanisms of cardiovascular risks after low radiation doses

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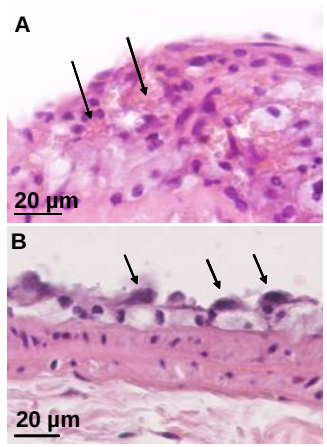
• Work Package 3

Macrovascular effects in radiation-induced CVD.

CARIM – M. Daemen / TUM – G. Multhoff, TUD - W. Doerr, NKI – F. Stewart, UL – G. Hildebrandt

➢ Objectives:

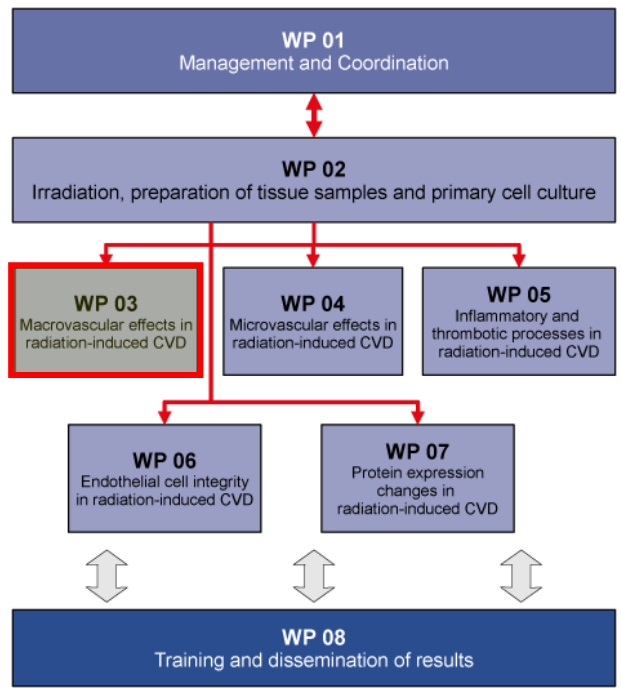
- To investigate functional and structural **macrovascular effects** in the irradiated arteria saphena by optical coherence tomography.
  - ➢ Task 1: Study of adhesive / thrombogenic properties of irradiated endothelium.
  - ➢ Task 2: In vivo optical imaging of vascular function after irradiation.
  - ➢ Task 3: Histopathology of A. saphena.



A: Erythrocyte containing MF (indicative of previous haemorrhage)

B: A-typical swollen ECs (indicative of EC disfunction)

C: Fibrin deposits



CARDIORISK (02/2008 – 01/2011)

The mechanisms of cardiovascular risks after low radiation doses

SEVENTH FRAMEWORK PROGRAMME

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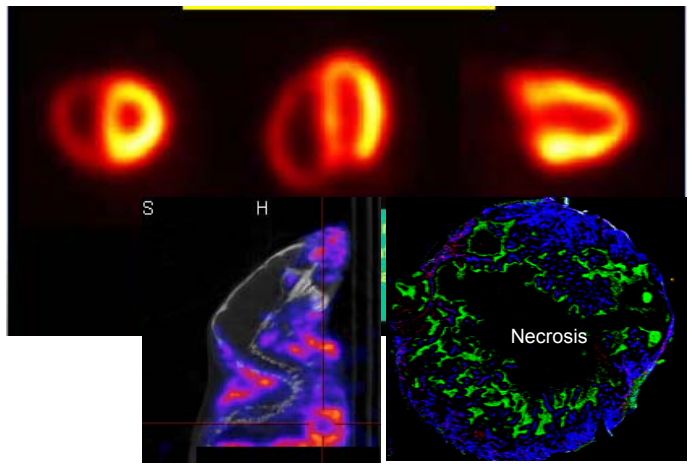
- **Work Package 4**

  Microvascular effects in radiation-induced CVD.

  NKI – F. Stewart / TUM – G. Multhoff / TUD - W. Doerr / MSCCI – D. Gabrys / CARIM – M. Daemen

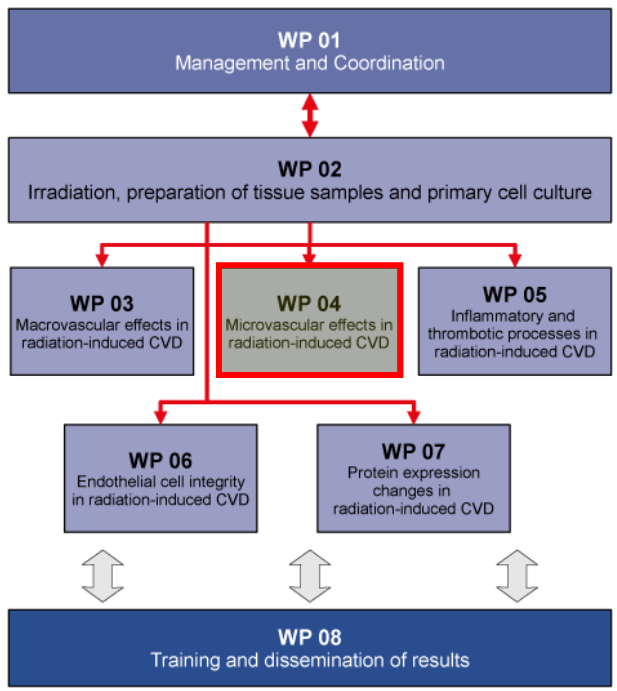


➢ Objectives:

- To study the **microvascular function** and **histopathology** after irradiation.

  ➢ Task 1: In vivo functional imaging of microvascular perfusion of irradiated hearts.

  ➢ Task 2: Morphometry of microvascular density.



CARDIORISK (02/2008 – 01/2011)

The mechanisms of cardiovascular risks after low radiation doses

- **Work Package 5**

Inflammatory and thrombotic processes in radiation-induced CVD.

UL – G. Hildebrandt / NKI – F. Stewart / IRSN – M.C. Vozenin-Broton / TUM – G. Multhoff / MSCCI – D. Gabrys / CARIM – M. Daemen

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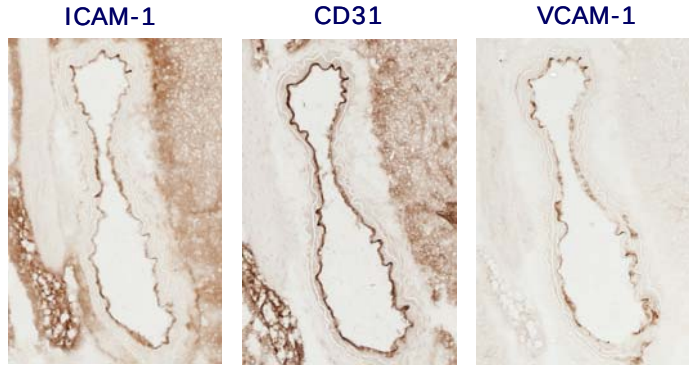
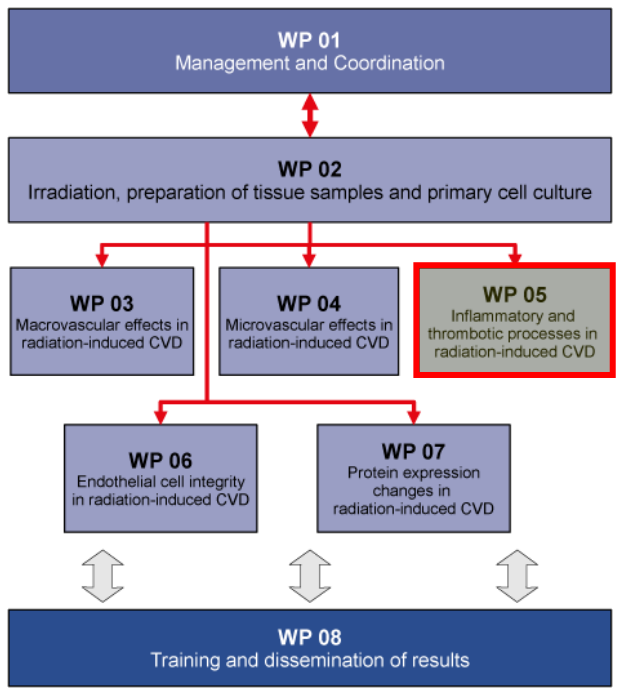
➢ **Objectives:** To determine the role of **inflammation / thrombosis in macro- and micro-vascular CVD**.

- ➢ Task 1: Investigation of A. carotis.
- ➢ Task 2: Investigation of A. saphena.
- ➢ Task 3: Investigation of stress responses in irradiated hearts.
- ➢ Task 4: Investigation of inflammatory and thrombotic responses and changes of immune function after heart irradiation.





SEVENTH FRAMEWORK PROGRAMME
CARDIORISK is a collaborative Project funded by the European Commission under the 7th Framework Programm

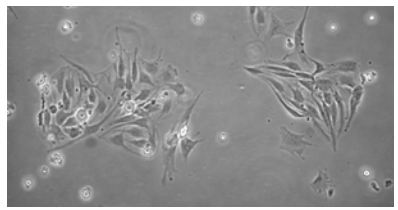
CARDIORISK (02/2008 – 01/2011)

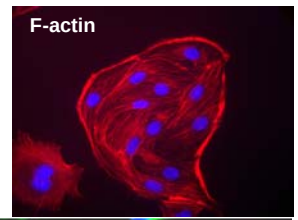
The mechanisms of cardiovascular risks after low radiation doses

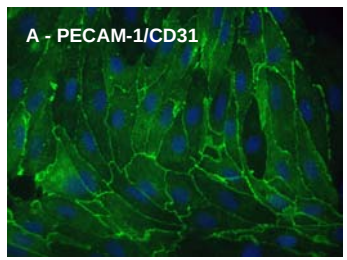
- **Work Package 6**

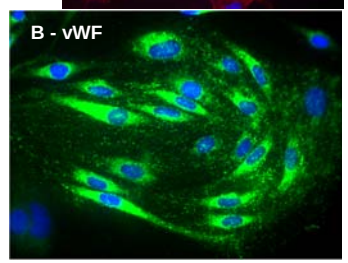
Endothelial cell integrity in radiation-induced CVD.

USFD – C. Kanthou / IRSN – M.C. Vozenin-Broton
TUM – G. Multhoff / MSCCI – D. Gabrys /
QUB – K. Prise







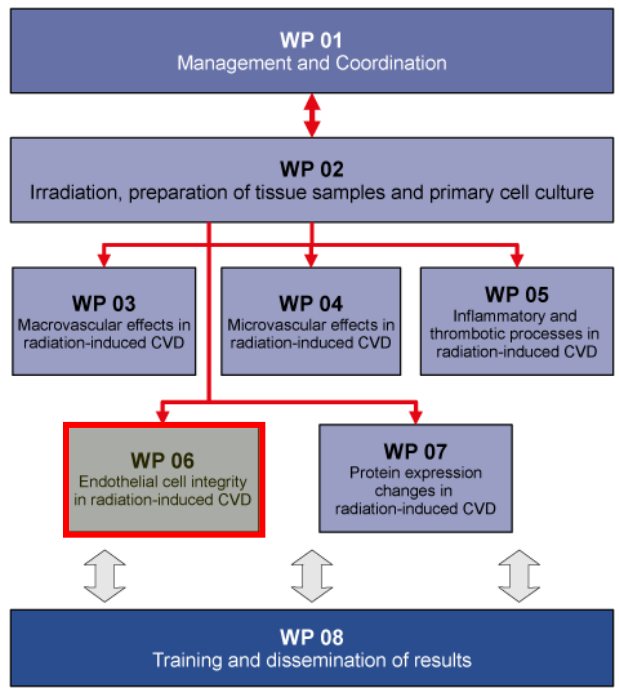


➢ Objectives:

- To investigate radiation effects on **cardiac cell integrity** in CVD by studying morphological and functional properties of **cardiomyocytes** and **endothelial cells** in vitro.

  - ➢ Task 1: Study of morphological and functional properties of cardiomyocytes.

  - ➢ Task 2: Investigation of endothelial interactions.



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The mechanisms of cardiovascular risks after low radiation doses

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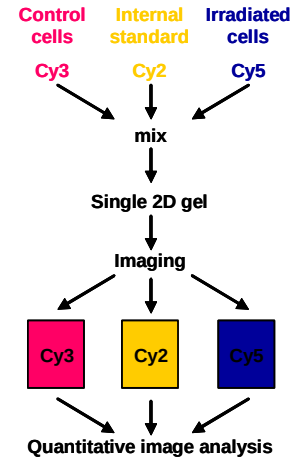
- **Work Package 7**

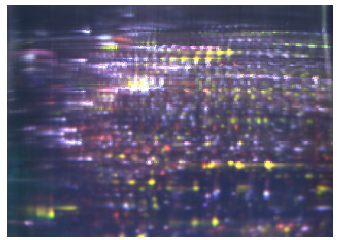
Protein expression changes in radiation-induced CVD.

HMGU – S. Tapio / STUK – D. Leszczynski / TUM – G. Multhoff

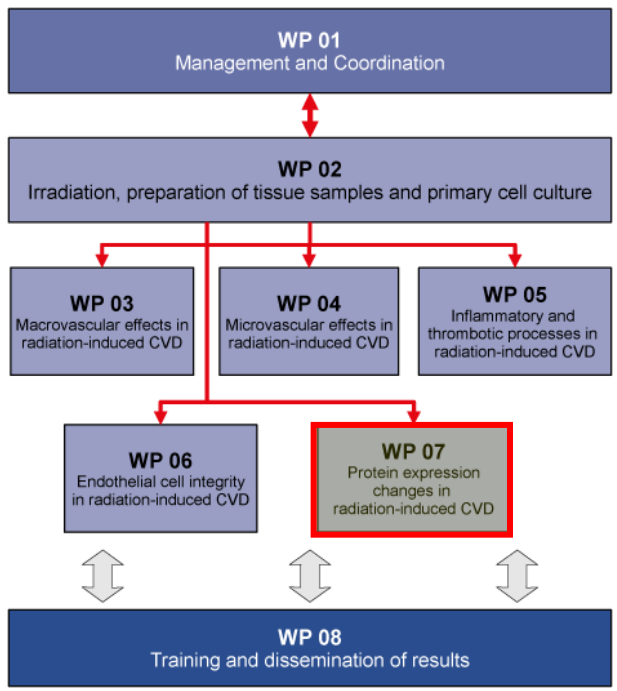
➢ Objectives:

- To evaluate the pathological changes in **protein expression** in cardiac endothelial cells after low radiation doses.
  - ➢ Task 1: Map the proteome changes in established and primary endothelial cells after low radiation doses (0.05Gy - 2Gy).
  - ➢ Task 2: Validate key components of the radiation-specific changes in the proteome in the macro- and micro-vascular models of endothelial cell irradiation.





MALDI-TOF and TOF/TOF based protein identification



CARDIORISK (02/2008 – 01/2011)

The mechanisms of cardiovascular risks after low radiation doses

http://www.cardiorisk.eu/index.php

Circulatory diseases – Implications for radiation protection ?

- **Q1:** Why do we care about the problem **?**
  - clear epidemiological evidence for doses > 0.5 Gy
  - at lower doses evidence is (to date) inconclusive
  - may have significant impact on the morbidity and mortality
  - is currently not specifically adressed by the RP system
  - public and trade unions concerns are increasing
- **ICRP position (2008):**

  „Data available do not allow for their inclusion in the estimation of detriment following low radiation doses less than 100 mSv. This agrees with the conclusion of UNSCEAR 2008 which found little evidence of any excess of risk below 1 Sv.“
- **Q2:** What do we further need to know **?**
- **Q3**: RP implications with current knowledge **?**
- **Q4:** What are we doing know **?**

Conclusions I

A **Myocardial infarction/arteriosclerosis**

- Arteriosclerosis-prone animals appear to be suiteable models to study mechanisms of radiation-induced arteriosclerosis

- In healthy wild-type animals, radiation alone does not induce arteriosclerosis within observed time spans

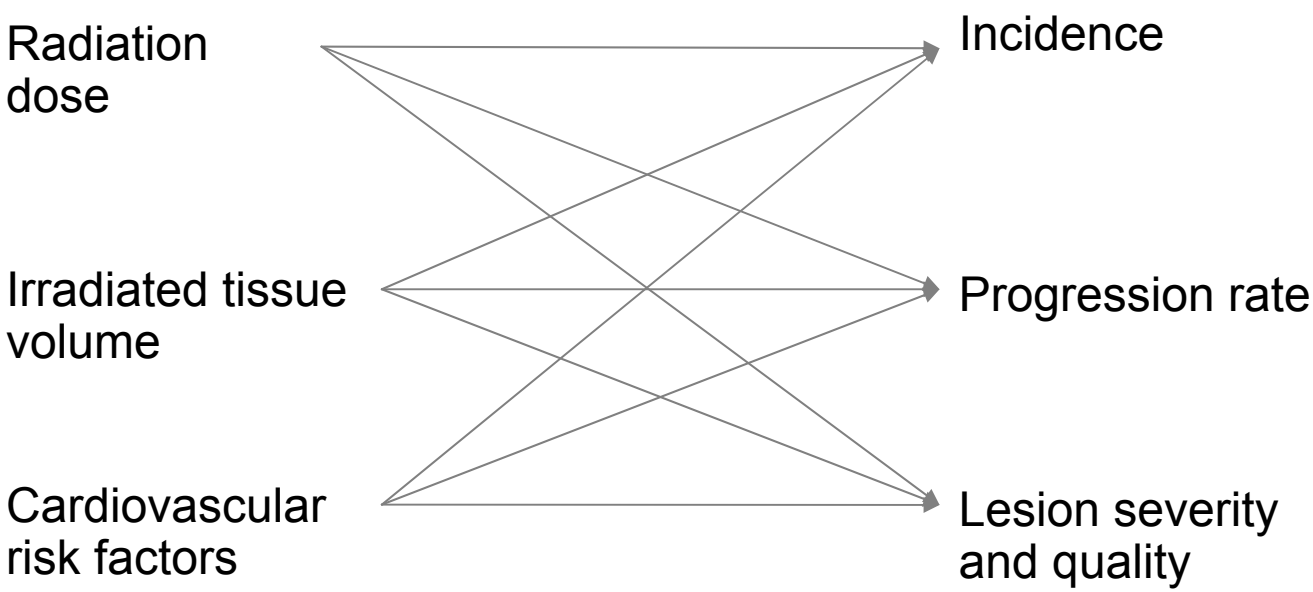
- „Inflammatory hypothesis“ is so far supported by experimental and clinical findings

B **Myocardium**

- Myocardial damage after high doses is preceded by capillary rarefication; complex interaction of tissue components

- Phenomena are best explained by proposing an interaction of DNA-damage-related effects and reversible gene expression effects

Experimental challenge I
Variables of cardiovascular radiation effects



Experimental challenge II
Use and limitations of experimental models

- Clinical/epidemiological endpoint is an increase in incidence of a multifactorial disease frequent in man and absent in wild type rodents.

- Radiation-induced cardiovascular disease cannot be distinguished clinically from other causes.

- Latent times are in the order of a decade. It is not clear, what this time-span corresponds to in laboratory animals.

- Endothelial cells play a major role. The relevance of endothelial cell cultures is very limited.

- Laboratory animals are not burdened with either genetic or life-style-associated risk factors.

- Radiation-induced cardiovascular disease is a complex disease involving several tissue/cell types. Only very limited and specific information can be obtained from cell cultures.

- The heart is surrounded by lung tissue, reacting earlier and as sensitive to radiation as the cardiovascular system.

Conclusions II

Important goal of ongoing and future experiments:

Provide a basis of sound evidence for a decision wether or not and how to include cardiovascular risk into low-dose risk assessment

Because of the particular difficulties in performing relevant and informative experiments, this will require

- long-term in vivo studies using various advanced models

- patience

- resources