ImmunoPDT
IMMUNOPHOTODYNAMIC THERAPY

Project no. 37489

Immuno-PDT

Instrument **SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT**

Thematic Priority **LifeSciHealth**

Immunophotodynamic therapy of cancer: concepts and applications.

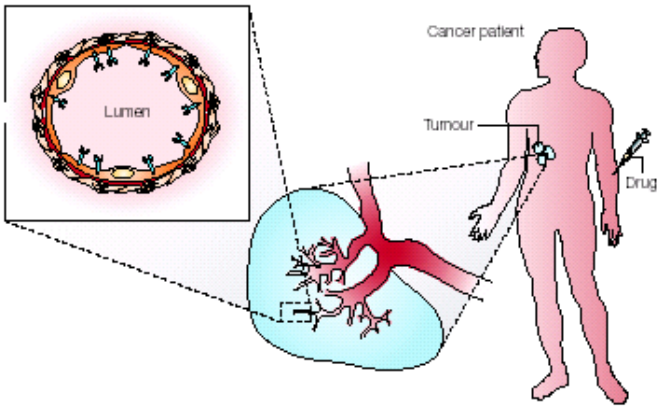
Final Activity Report

Period covered: from 1st October 2006 to 30th September 2009
Date of preparation: 5th November 2009

Start date of project: 1st October 2006 Duration: 36 months

Project coordinator name Chiara Falciani
Project coordinator organisation name: Philogen

Photodynamic therapy of cancer, i.e. the generation of reactive oxygen species in the tumor environment which follows the irradiation of suitable photosensitizing molecules, is an attractive modality for the selective ablation of inoperable superficial neoplastic lesions, such as certain head and neck, gastrointestinal, urogenital and gynecological tumors. It is likely that the scope and efficacy of photodynamic therapy could be enhanced by:
- the availability of novel efficient photosensitizers which absorb at in the red / near infrared region of the spectrum, where light penetration of tissues is maximal
- the use of antibody-photosensitizer conjugates or conceptually related innovative delivery systems, which concentrate the photosensitizing molecules at suitable neoplastic sites. The tumor neo-vasculature appears to be an attractive target, since the selective delivery of photosensitizers may occlude the tumor blood vessels, thus causing an avalanche of tumor cell deaths.



Legend: *The tumor neovasculature is the most accessible structure for agents coming from the bloodstream. Its selective occlusion by means of immunophotodynamic procedures can trigger an avalanche of tumor cell deaths.*

In this Project, we have put together a network of academic research groups and companies, for the development of antibody-based targeted photodynamic therapy modalities. The planned research activity started with the synthesis of novel photosensitizing molecules suitable for conjugation to antibodies, and with the identification of novel human monoclonal antibodies, capable of a selective targeting of the tumor neovasculature for immuno-PDT applications. Following an extensive in vitro characterization of the most promising antibody-photosensitizer conjugates (including an innovative delivery concept in which photosensitizers are non-covalently bound to the antibody), the therapeutic potential of the best antibody-photosensitizer conjugates have been tested in rodent models of cancer and have displayed an impressive tumor ablation performance. The L19, F16 and F8 antibodies have been moved to GMP manufacture, thus paving the way for future clinical applications.

We made substantial progress in terms of:

i. The synthesis of novel infrared photosensitizers with sufficiently water solubility (i.e., not sticky to unwanted cells and tissues), which absorb in the near-infrared and red-shifted light spectrum and which efficiently generate singlet oxygen and/or other reactive oxygen species.
ii. Isolation and validation (in vitro and in vivo) of novel human antibodies to accessible tumor-associated antigens.
iii. Investigation of a novel method for the conjugation of the antibody and photosensitizer molecules.
iv. Evaluation of the conjugates in vitro e in vivo. Our novel PDT agents have been extensively tested *in vitro*, in order to ascertain whether bound photosensitizers can retain singlet oxygen production activity upon irradiation.
v. The agents have been tested in rodent models of cancer and now will open novel therapeutic opportunities for the selective treatment of superficial tumors in accessible body cavities

Most importantly, there is a reasonable expectation of a medical benefit for cancer patients stemming both directly and indirectly from this Project:
- directly, since immuno-PDT procedures promise to be invaluable for the selective ablation of inoperable superficial neoplastic lesions, such as certain skin, head and neck, gastrointestinal, urogenital and gynecological tumors. Indeed, the discovery that squamous cell carcinoma of the skin (the second most common skin cancer) strongly reacts with the most advanced antibodies studied in the ImmunoPDT project (F8 F16, L19) paves the way for Immuno-PDT applications in this cancer type, where multiple lesions often recur in certain patients (e.g., transplant patients) and where repeated surgical excision remains an undesirable and disfigurating medical procedure.
- indirectly, since the knowledge generated by the validation of novel antibodies for vascular targeting applications is likely to have an impact in other forms of immunotherapy, including the use of full IgGs and antibody-cytokine fusions for cancer therapy [Neri & Bicknell, 2005].
We believe that our Project and our Network have contributed to the scientific and the technical objectives of the *Combatting Cancer* area.

Furthermore, in addition to our regular Meetings, we have fulfilled our Dissemination Activities duties by organizing an Experimental Course on Antibody Phage Technology. The Course has been advertised in *Nature*, has been over-subscribed, and has brought together participants from over 20 countries.
The Project also has resulted in:
40 publications in peer-reviewed scientific Journals
10 manuscripts in press
87 invited lectures at international congresses
8 patent applications.

ImmunoPDT
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http://immunopdt.net/

Project Objectives and Execution

The present project had as objectives the synthesis and conjugation of novel infrared and red-shifted photosensitizers to the most promising antibodies against vascular tumor antigens obtained by human antibody technology, the immunohistochemical characterization, the biodistribution and imaging targeting in vivo, in order to select the best antibody-photosensitizer conjugates to be taken forward into clinical trials as a final objective.

The structure of the Project was clearly defined:

- In the first year, synthesis of novel photosensitizers and identification of the most suitable antibodies for Immuno-PDT applications [fall back position: we already had a palette of photosensitizers and of antibodies - Refs: Pini A. et al., J Biol Chem. 1998; 273: 21769-76, Birchler M. et al., Nat Biotechnol. 1999; 17:984-8, Fabbrini et al., Int J Cancer 2005, 118: 1805-181- which were used as a benchmark for the new molecules, and which were coupled together]
- In the second year, we continued to synthesise novel photosensitizers, investigated innovative conjugation strategies and performed an *in vitro* characterization of antibody-photosensitizer conjugates.
- In the third year, the therapeutic activity of the most promising antibody-photosensitizer conjugates were tested in rodent models of cancer, in comparison to antibodies of irrelevant specificity and unconjugated photosensitizers.

WP1 - Selection and identification of human monoclonal antibodies suitable for Immuno-PDT applications. Months: 0-12. Participants: Philogen (8), ETHZ (12), CBA (24), Imperial (12).

WP objectives. The main goals of this Workpackage were the isolation and characterization of novel vascular targeting agents for vascular tumor targeting applications, as well as the comparative evaluation of different targets and antibodies, thus providing a ranking of different targeting agents which can be considered for immunophotodynamic applications.

The activities performed within this Workpackage can mainly be grouped in three categories:

(a) isolation of novel human monoclonal antibodies to vascular tumor targets
(b) immunohistochemical characterization of the monoclonal antibodies
(c) development of innovative antibody formats, which may display superior biodistribution properties and may thus be suitable for immunophotodynamic applications

WP achievements. 13 antibodies have been selected and characterized instead of the expected 5.

a) Novel antibodies (Philogen and ETHZ)
   (i) EDA domain of fibronectin
   (ii) A2 domain of tenascin-C
   (iii) MG50
   (iv) Carbonic Anhydrase IX
   (v) GW112
b) Immunohistochemical studies (Philogen and ETHZ)
c) Novel antibody formats
   (i) Small EDB binders, based on mutants of the globular SH3 domain of Fyn (Philogen, ETHZ)
   (ii) Uteroglobin - Antibody fusion protein (CBA)
   (iii) HuBC-1 scFv (IMPERIAL)
   (iv) Recombinant antibodies modified with non-covalent albumin binders (ETHZ, Philogen)

WP2 - Synthesis of novel photosensitizers (PSs) for Immuno-PDT applications. Months: 0-12. Participants (person-months per participant): UOH (24), Photobiotics (12), Philogen (7), Trojantec (12).

WP objectives. The synthesis of novel PSs represented an important backbone of the Project. The new PS were selected among those which absorb in the 600-800 nm region, as tissue penetration is efficient in this frequency range. The new PS also contain one or more charged groups (e.g., carboxylic acid or quaternised amino groups), as they contribute to

photosensitizer solubility and they minimize non-specific stickiness and killing of non-target cells.

WP achievements. 10 photosensitizers were synthesized and characterised instead of the expected 5.

WP 3 - Selection of antibodies (and/or single domain binding proteins) which bind to photosensitizers for bispecific Immuno-PDT applications (non-covalent photosensitizer coupling). Months: 1-12. Participants (person-months per participant): ETHZ (12), Philogen (8).

WP objectives. In addition to the classical methods for antibody-photosensitizer conjugation, based on the covalent modification of aminoacid residues (lysines or cysteines) or of oligosaccharidic moieties of immunoglobulines, we decided to investigate a novel method of coupling photosensitizers to antibodies, based on the non-covalent but stable interaction of photosensitizing molecules with specific antibody fragments or suitable single domain binders.

The first step towards the implementation of this experimental methodology has been the modification of suitable proteins with photosensitizer derivatives (to be used as antigen in antibody phage selections), followed by the biopanning of phage display libraries.

WP achievements. For selection of antibodies against the photosensitizer, the compound "SnChlorin-e_6" was coupled to Bovine Serum Albumin (BSA) and Glutathione S-Transferase (GST), used as carrier protein. The conjugates "SnChlorin-e_6/BSA" and "SnChlorin-e_6/GST" were used for selections in turn, coated on plastic. Furthermore, free BSA and GST were co-incubated with the phage library.

After 4 rounds of panning 8 specific binders were selected from the ETH2-Gold library. Sequencing revealed that the same scFv clone was selected.

WP4 – Synthesis of novel photosensitizers for Immuno-PDT applications. Months: 13-24. Participants (person-months): UOH (24)

WP objectives The synthesis of novel photosensitizers with optimal tissue penetration and solubility in order to minimize non-specific stickiness and killing of non-target cells.

Devise suitable chemistries for the coupling of photosensitizers to antibodies. In this respect, thiol reactive photosensitizer derivatives were particularly attractive, as they may enable the site-specific modification of cysteine-tagged antibodies or antibody equivalents, thus leading to chemically-defined pharmaceutical products.

WP achievements. Eight PS have been synthesized instead of the expected five.

WP5 – Evaluation of antibodies by biodistribution experiments and/or imaging experiments. Months: 13-24. Participants (person-months): ETHZ (10), CBA (15), Imperial (6), CUB (12).

Workpackage objectives. Ultimately, the tumor targeting potential of a monoclonal antibody had to be assessed experimentally by quantitative biodistribution analysis with radiolabeled protein preparations, or using near-infrared imaging methodologies.

A number of antibodies have been discovered or reformatted to make them appropriate for immuno-PDT. Before resources were invested in making photo-immunoconjugates, it was important to show that these antibodies target tumours effectively with low cross-reactivity with normal tissues. This could be done by radio-labelled biodistribution experiments, which allow quantitative measurements of tumour and tissue uptake, or by whole animal imaging studies which provide real-time, dynamic analyses.

WP achievements. Targeting characterization by biodistribution analysis was performed for fifteen instead of three antibodies.

- Biodistribution studies with anti-EDA antibodies (ETHZ, first report)
- Biodistribution studies with anti-EDB SH3 binders (ETHZ, first report)
- Biodistribution studies with antibodies against urokinase-type plasminogen activator (uPA), matrix metalloproteinases 1A, 2 and 3 (MMP1, MMP2 and MMP3; -Figure 1-), carbonic anhydrase IX, periostin and peroxidasin (also called MG50; -Figure 2-) (ETHZ, second report)

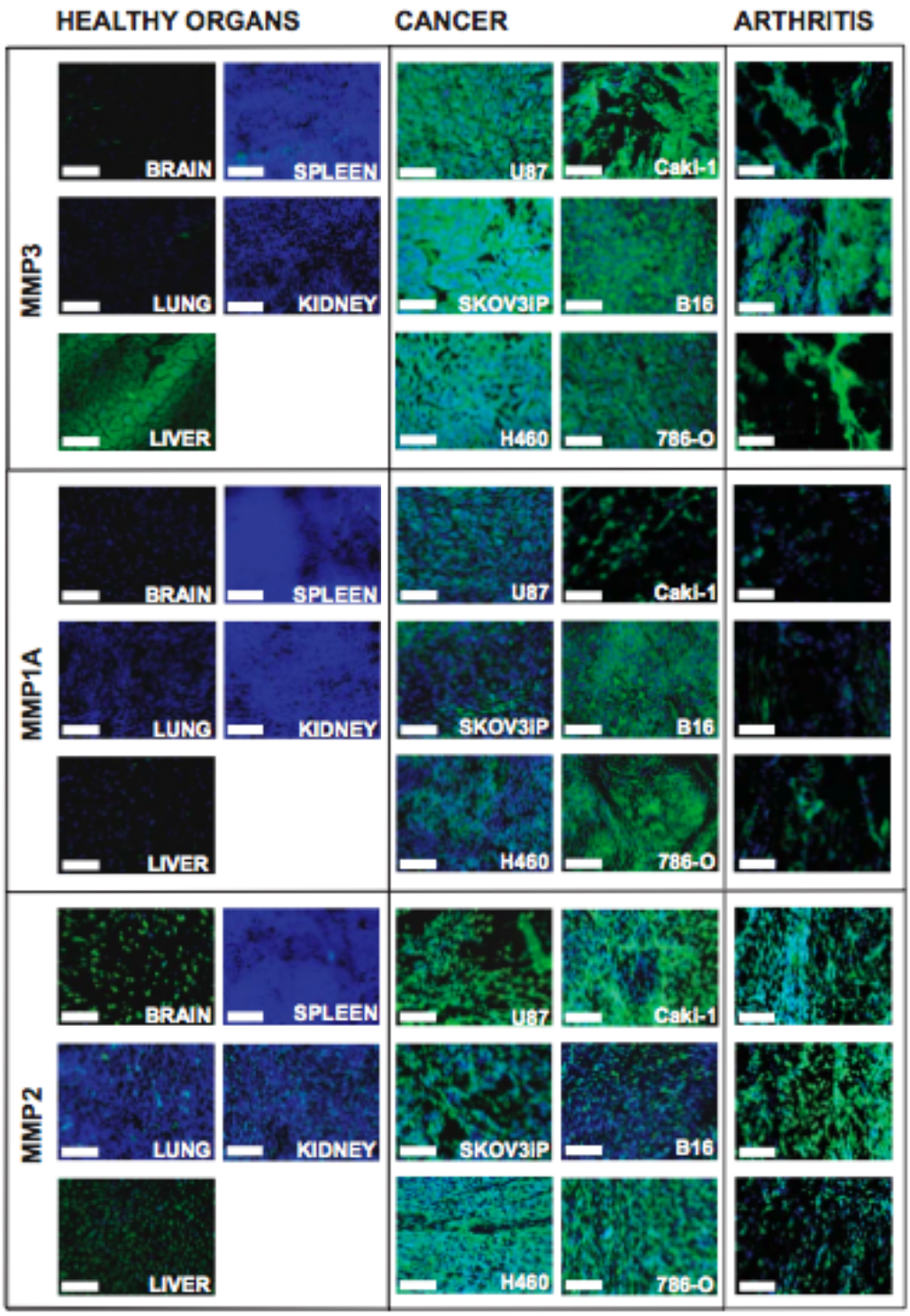

FIGURE 1. *Immunofluorescence findings with the **oBar11**, **3A**, **12C1** specific to the murine catalytic domains of MMP-1A, MMP-2 and MMP-3.*

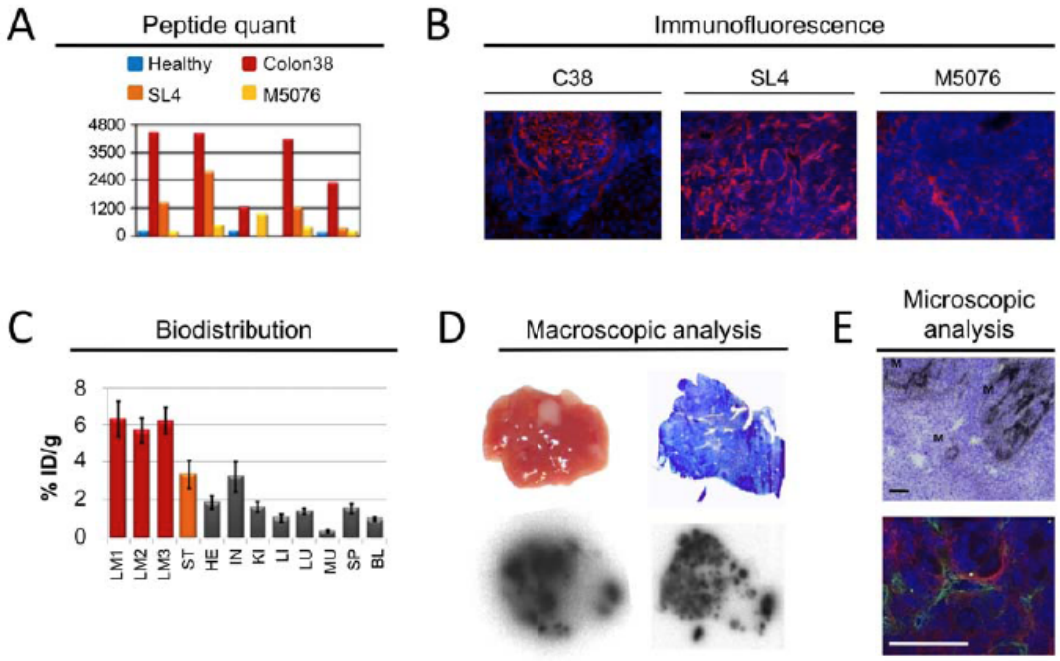

FIGURE 2. *Identification of periostin as marker of liver metastasis and biodistribution of an antiperiostin antibody.* ***(A)*** *Relative average signal intensities for multiple individual tryptic peptides belonging to periostin are displayed. Periostin exhibits an up-regulation in two of the three liver metastasis models analyzed.* ***(B)*** *Immunofluorescence analyses for all three metastases models are shown. Periostin is displayed in red, nuclei in blue. Metastases are characterized by a more intense blue staining which reflects the higher cellular density.* ***(C)*** *In vivo tumor targeting results (expressed as %ID/g +/- standard errors) using a radiolabeled anti-periostin antibody in mice bearing Colon38 metastases are shown. LM: liver metastasis; ST: spleen tumor; HE: heart; IN: intestine; KI: kidney; LI: liver; LU: lung; MU: muscle; SP: spleen; BL: blood).* ***(D)*** *Macroscopic autoradiographic analysis of the anti-periostin antibody localization in liver metastases. A photograph of a metastatic liver lobe is presented alongside with the corresponding phosphor image autoradiogram. In addition, 20µm-thick slides were stained by hematoxylin (blue panels) and submitted to phosphorimager analysis.* ***(E)*** *Microscopic analysis of anti-periostin antibody localization on metastatic lesions. The panel presents microautoradiograms of 20µm-thick sections, counterstained with hematoxylin. Sections from the same specimens were also processed by immunofluorescence, staining for nuclei (blue), CD31 as blood vessel marker (green), and for the in vivo localized anti-periostin monoclonal antibody (red). Scale bars = 100µm. Metastases are marked (M = metastases).*

- Biodistribution studies with HuBC-1 (Imperial).
- Biodistribution studies with uteroglobin derivatives (CBA, first report)
- Characterization and biodistribution studies with C6 mAb and with L19-SIP (CBA, second report).
- Generation and characterization of the mAb C6 specific for an oncofetal FN epitope within the type III repeat 8 which is cryptic when the EDB is deleted and unmasked when the EDB is inserted (CBA, second report).
- Biodistribution experiments using the monoclonal C6 specific for the new fibronectin oncofetal epitope in tumour bearing mice (CBA, second report).
- Biodistribution studies of radioiodinated antibodies to EDB injected intra-tumor (CBA, second report).
- Microvascular biodistribution of L19-SIP in angiogenesis targeting (CUB)
- Biodistribution of microvascular targeting using L19-SIP (CUB)
- In vivo characterization of vascular targeting strategies using F8-SIP antibody agains the Extradomain A of fibronectin (CUB)
- F8-SIP binds specifically to tumor microvessels in a time and blood flow dependent manner (CUB)
- Antiangiogenic treatment induces increased vascular accumulation (CUB)

- F8-SIP is suitable to directly visualize tumor angiogenesis in vivo (CUB)
- F8-SIP mediated photodynamic therapy leads to microcirculatory breakdown and a subsequent inhibition of tumor growth –Figure 3- (CUB)

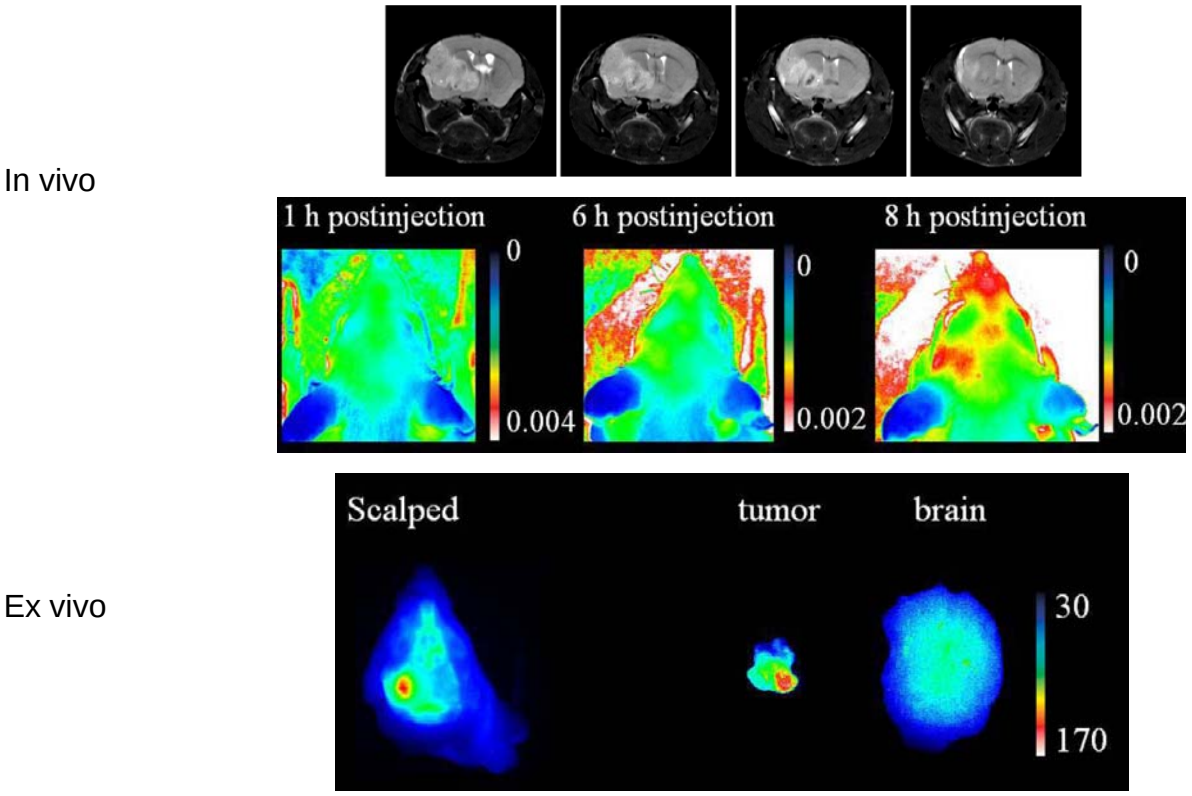

Figure 3. *Upper row: MRI images demonstrating the intracerebral growth of glioma. Middle row: In vivo NIRF imaging showing a positive fluorescence signal in MRI corresponding tumor area 8 hours after injection of the antibody. Lower row: Ex vivo imaging of tumor angiogenesis showing the high tumor specificity of the F8-SIP.*

WP6 - Evaluation of antibody-photosensitizer conjugates in in vitro models. Months: 13-24. Participants (person-months): Philogen (23), CBA (12), ETHZ (12), Imperial (18).
WP objectives. Antibody-photosensitizer conjugates for immuno-PDT applications must not only be able to selectively localize at the tumor site (WP5), with negligible stickiness to normal tissues, but also efficiently generate toxic oxygen species upon irradiation. Model photolysis experiments with cell lines which over-express the antigen of interest and with red blood cells represent a rich source of information about antibody-photosensitizer conjugates which deserve to be studied in animal models of cancer (WP7).
Workpackages 1, 2, 4 & 5 delivered an number of promising single-chain Fvs antibody fragments and photosensitisers. It was essential to show that these two components can be brought together using the wide range of coupling technologies to form effective antibody-photosensitiser photo-immunoconjugates (PICs). These PICs must demonstrate a retention of photophysical properties and gain the ability to target cells which would hopefully translate into the specific destruction of tumours in an animal model.

WP achievements. Seven Ab-PS conjugates have been characterized instead of the expected three.

- HuBC-1 scfv-PPa was characterized for photokilling activity and photohemolysis (Imperial, first report).
- In vitro PD activity of PB11 (novel photosensitisers based on meso-tetraethyl porphyrin technology) coupled to a scFv (Imperial, Photobiotics, first report).
- In vitro PDT of PB12 (novel photosensitisers based on meso-tetraethyl porphyrin technology) coupled to a tumour-specific (Imperial, Photobiotics, first report).

- Cell kill of non-targeted PEGylated PPa derivative IS-1305 (Imperial, Photobiotics, first report).
- PPa-PEG1 coupled to C6.5 scFv (Imperia, second report)
- Photocytotoxicity for SIP(L19)-PS3, SIP(L19)-PS5, SIP(L19)-PS6 (ETHZ, Philogen second report)
- Conjugation of L19 – uteroglobin fusion proteins to the photosensitizer indocyanine green (CBA, Hull, second report).
- Conjugation of ICG to L19-UG fusions proteins –Figure 4- (CBA, second report).
- Conjugation of ICG to L19-TNFalpha fusion protein (CBA, second report).

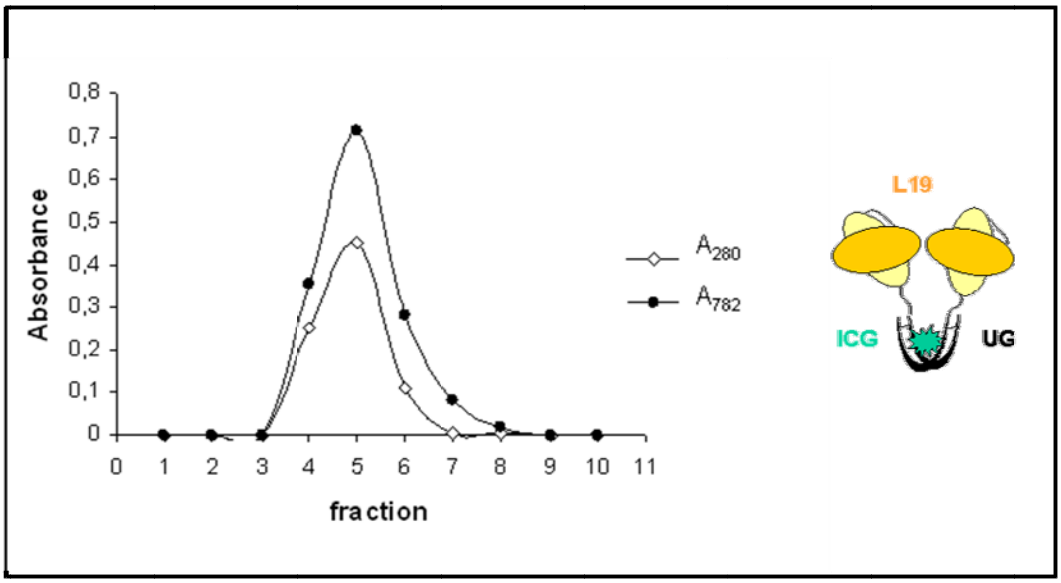

FIGURE 4. *Result of the conjugation of L19-hUG to ICG. On the X-assis the collected fractions eluted from the PD10 columns are reported and on the Y-assis the absorbance of the fractions at λ=280 nm (maximunm absorbtion peak for L19-hUG) and λ=782 (maximunm absorbtion peak forICG). On the right a schematic representation of ICG conjugated to L19-UG fusion protein.*

The binding of photosensitizers to antibody-uteroglobin is really noteworthy and it shows the importance of antibody-uteroglobin fusions proteins as essentially "ready to go and to be used" tools.

WP7 – Evaluation of the therapeutic potential of the 3 top antibody-photosensitizer candidates Months: 25-36. Participants (person-months) Philogen(21), Imperial (12), CBA (24), ETHZ (24).

WP objectives Photodynamic therapy with the most promising antibody-photosensitizer conjugates (and with free photosensitizer and an antibody-photosensitizer conjugate of irrelevant specificity used as negative control) was performed in vivo.

WP achievements. Seven conjugates have been characterized in tumor xenografts instead of the expected three.

- Treatment of F9 murine teratocarcinoma: intratumoral injection of TriPyPhNCSMe.3Cl (PS) conjugated to UG formats of L19 both divalent and tetravalent (CBA).
- In vivo experiments using L19-UG and L19-UG-L19 conjugate to PS –Figure 5- (CBA).

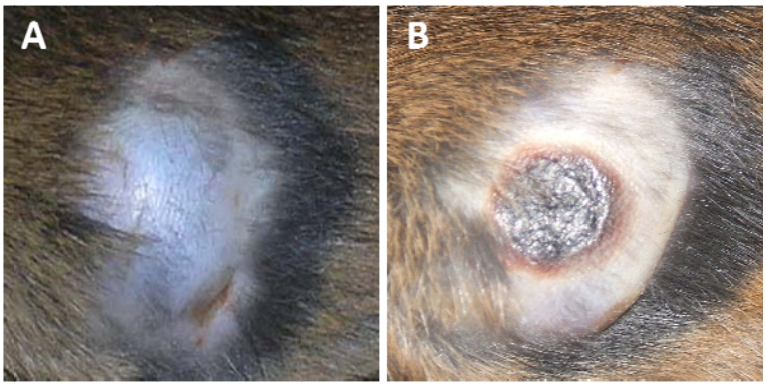

FIGURE 5. *Treatment of 129 sV mice implanted with murine F9 mouse embryonal teratocarcinoma cells, with L19-mUG conjugated to the photosensitizer TriPyPhNCSMe.3Cl and irradiated with a total dose of light of about 150 J/cm^2. Tumor before* ***A.*** *and 48 hours post* ***B.*** *light irradiation.*

- In vivo experiments using L19-TNFalpha conjugate to PS (CBA).
- In vivo experiments using L19-TNFalpha conjugate to ICG (CBA).
- Therapeutic performance of SIP(L19)-PS4 for the immuno-photoablation of superficial tumors –Figure 6- (ETHZ, Philogen)

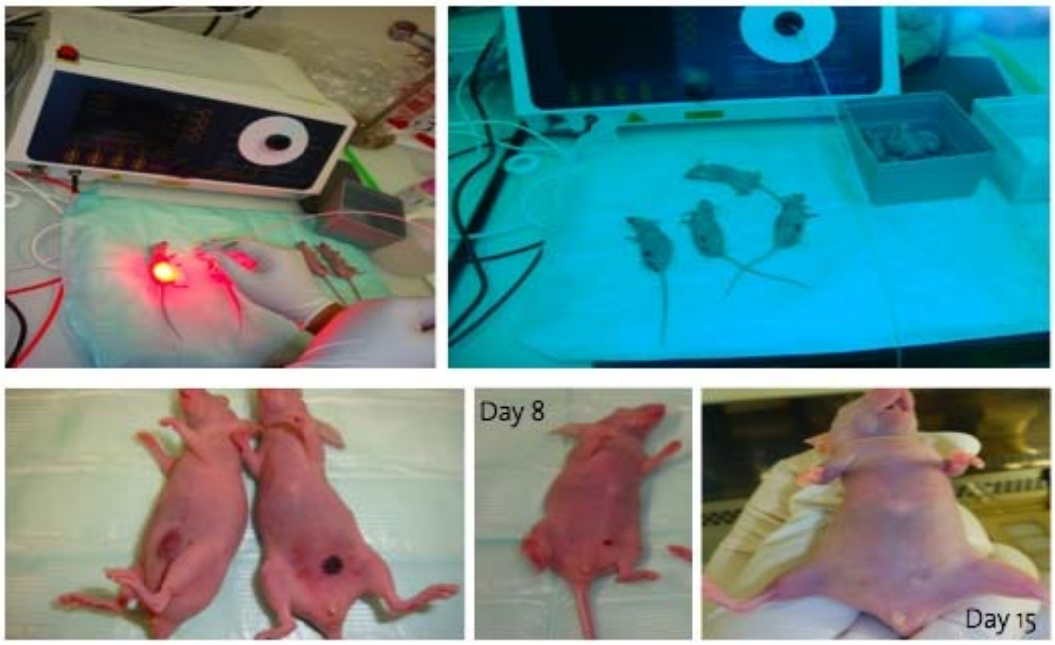

FIGURE 6. *Immunophotodynamic therapy results observed in F9-bearing tumors. Please note the complete eradication observed in mice treated with SIP(L19)-PS4, where tumors are converted into a necrotic mass which disappears by day 15.*

- C6.5-PPa-PEG1 –Figure 7- (Imperial)

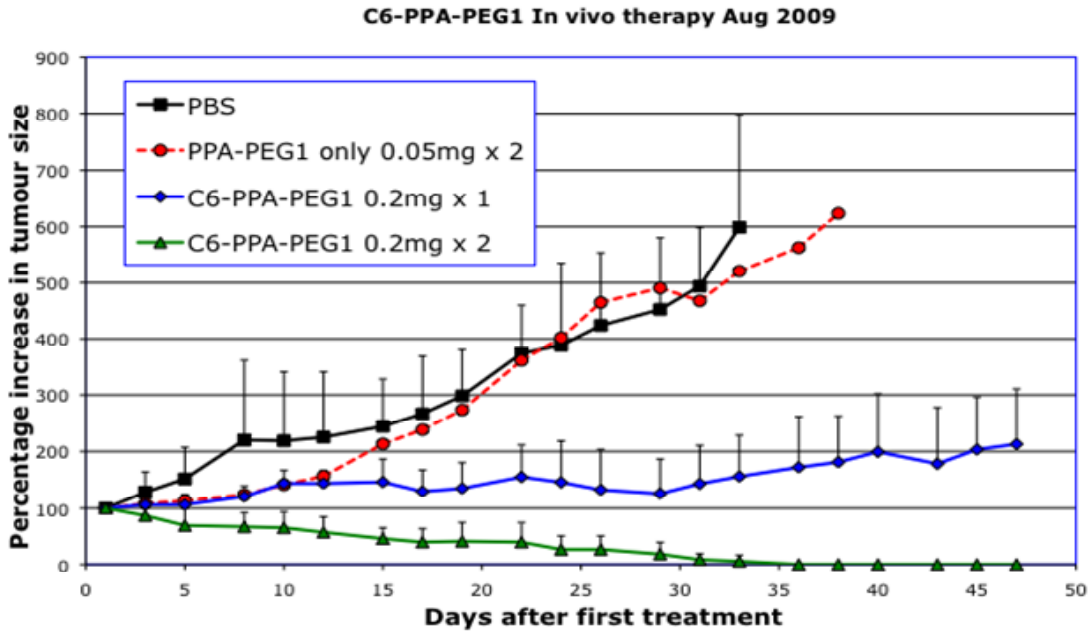

FIGURE 7. *Tumour therapy of C6.5-PPa-PEG1 PIC in an SKOV3 human subcutaneous tumour xenograft model. C6.5scFv-PPa-PEG1 (1mg/ml, 0.2ml-0.2 mg dose) was injected once (day-1) or twice (day-1 and -4). A 0.5W 670nm laser was irradiated for 10 minutes under anaesthetic directly onto the tumour. Tumour response was followed by measuring the tumours over 7 weeks. Control samples consisted of the free photosensitiser (equivalent dose to that of the PIC) and saline treated mice).*

WP 8 - Group Meetings (Months: 1-36).
Participants (person-months) Philogen(10), all other participants (2).
PHILOGEN Coordination of the Project.
Kick-Off Meeting
First Annual Meeting
Meeting for the First Report
First Report
Second Annual Meeting
Third Annual Meeting
Second Report

The Final IPDT Meeting was held as a Satellite Symposium of the 13th Congress of the European Society for Photobiology EPS.

WP 9- Experimental course on Antibody Phage Technology

ETHZ
Experimental Course on Antibody Phage Technology
An Experimental Course on Antibody Phage Technology was held in Zurich in January 2008, as part of our Dissemination Activities duties. The Course has been advertised in *Nature*, has been over-subscribed, and has brought together participants from over 20 countries.

Picture of the participants of the 3rd Experimental Course on Antibody Phage Technology, held in Zurich on 4-8 February 2008.

DISSEMINATION AND USE
Exploitable knowledge

Exploitable product(s)	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	***Owner*** *& Other Partner(s) involved*
EDA domain of fibronectin (antibody)	*Medical*		*Two patent applications* PCT/IB2008/000965 and US60/951,765	***Philogen***
A2 domain of tenascin-C (antibody)	*Medical*		*Patent application (2008)*	***Philogen Group***
Uteroglobin - Antibody fusion protein	*Medical*		*Patent application FI 2009A000006*	***Zardi-CBA***
Antibody to a new epitope on oncofetal fibronectin	*Medical*		*Patent application FI 2008A000240*	***Borsi-Balza-Carnemolla-Castellani-Sassi-CBA***
HuBC-1 scFv	*Medical*	2011	*Patent application 2008*	***Photobiotics***
Biological materials and uses thereof	*Medical*		*GB 0904825.7 (filed 20-3-09)*	***Photobiotics***
Antibodies fused to the translocating homeodomain of Antennapedia for use in photodynamic therapy of cancer	*Medical*		*Patent application planned for 2008*	***Trojantec***
Porphyrins 2-12 and 14	*Medical*		*Patents will follow successful biological evaluation – currently in progress*	***University of Hull and Photobiotics***

DISSEMINATION OF KNOWLEDGE
Press release (General public): Several Press releases are prepared by Philogen during the reporting period, in coincidence with significant advances in its pipeline.
Media briefing (General public): Planned through Press Releases by the individual parties, and by a Media Briefing document which will be circulated by the Coordinator at the end of the IMMUNO-PDT Project.
Conference (Research): 87 presentations at international conferences.
Publications (Research): 40 publications and 10 manuscripts submitted in international peer reviewed journals.
Project web-site (General public): http://immunopdt.net/

An Experimental Course on Antibody Phage Technology was held in Zurich in January 2008, as part of our Dissemination Activities duties.

The Final IPDT Meeting was held as a Satellite Symposium of the 13th Congress of the European Society for Photobiology EPS.

LIST OF PUBLICATIONS

ETHZ

- D. Grabulovski, M. Kaspar, D. Neri (2007) "A novel, non-immunogenic Fyn SH3-derived binding protein with tumor vascular targeting properties". *J. Biol. Chem.*, 282, 3196-3204.

- C. Schliemann, D. Neri (2007) "Antibody-based targeting of the tumor vasculature" *Biochim Biophys Acta,* 1776,175-92

- J.N. Rybak, C. Rösli, M. Kaspar, A. Villa, D. Neri (2007) "The extra-domain A of fibronectin is a vascular marker of solid tumors and metastases". *Cancer Res.*, 67, 10948-10957

- A. Villa, E. Trachsel, M. Kaspar, C. Schliemann, R. Sommavilla, J.N. Rybak, C. Rösli, L. Borsi, D. Neri (2008) "A high-affinity human monoclonal antibody specific to the alternatively spliced EDA domain of fibronectin efficiently targets tumor neo-vasculature in vivo". *Int. J. Cancer*, 122, 2405-2413.

- M. Pedretti, A. Soltermann, S. Arni, W. Weder, D. Neri*, S. Hillinger (2009) "Comparative immunohistochemistry of L19 and F16 in non-small cell lung cancer and mesothelioma: two human antibodies investigated in clinical trials in patients with cancer". *Lung Cancer*, 64, 28-33.

- C. Schliemann, Palumbo A, Zuberbuhler K, Villa A, Kaspar M, Trachsel E, Klapper W, Menssen HD, Neri D. (2009) "Complete eradication of human B-cell lymphoma xenografts using rituximab in combination with the immunocytokine L19-IL2." *Blood*, 113, 2275-2283 [Cover and Editorial at pages 2121-2122].

- S. Sauer, P.A. Erba, M. Petrini, A. Menrad, L. Giovannoni, C. Grana, B. Hirsch, L. Zardi, G. Paganelli, G. Mariani, D. Neri, H. Dürkop, H.D. Menssen (2009) "Expression of the oncofetal ED-B containing fibronectin isoform in hematologic tumors enables ED-B targeted 131I-L19SIP radioimmunotherapy in Hodgkin lymphoma patients". *Blood*, 113, 2265-2274. [Cover and Editorial at pages 2121-2122].

- B.M. Tijink, L.R. Perk, M. Budde, M.S. van Walsum, G.W.M. Visser, R.W. Klet, L.M. Dinkelborg, C.R. Leemans, D. Neri, G.A.M.S. van Dongen (2009) "^{124}I-L19-SIP for immuno-PET imaging of tumour vasculature and guidance of ^{131}I-L19-SIP radioimmunotherapy". *Eur. J. Nucl. Med. Mol. Imaging*, in press.

- P. Richter, M. Tost, M. Franz, A. Altendorf-Hofmann, K. Junker, L. Borsi, D. Neri, H. Kosmehl, H. Wunderlich, A. Berndt (2009) "B and C domain containing tenascin-C: urinary markers for invasiveness of urothelial carcinoma of the urinary bladder?" *J. Cancer Res. Clin. Oncol.*, 135, 1351-1358.

- C. Rösli, B. Borgia, C. Schliemann, M. Gunthert, H. Wunderli-Allenspach, R. Giavazzi, D. Neri (2009). "Comparative analysis of the membrane proteome of closely related metastatic and non-metastatic tumor cells". *Cancer Res.*, 69, 5406-5414.

- K.H. Altmann, D. Neri. (2009) "Next-generation therapeutics". *Curr. Opin. Chem. Biol*. 213, 231-234.

- J. K.J. Ahlskog, C. Schliemann, J. Mårlind, U.Qureshi, A. Ammar, R.B. Pedley, D. Neri (2009) "Human monoclonal antibodies targeting carbonic anhydrase IX for the molecular imaging of hypoxic regions in solid tumours". *Br. J. Cancer*, 101, 645-657.

- M. Pedretti, Z. Rancic, A. Soltermann, B.A. Herzog, C. Schliemann, M. Lachat, D. Neri, P.A. Kaufmann (2009) "Comparative immunohistochemical staining of atherosclerotic plaques using F16, F8 and L19: three clinical-grade fully human antibodies. *Atherosclerosis*, in press

- C. Schliemann, A. Wiedmer, M. Pedretti, M. Szczepanowski, W. Klapper and D. Neri (2009) "Three clinical-stage tumor targeting antibodies reveal differential expression of oncofetal fibronectin and tenascin-C isoforms in human lymphoma". *Leukemia Res*., 33, 1718-1722.

- K. Schwager, M. Kaspar, F. Bootz, R. Marcolongo, E. Paresce, D. Neri*, E. Trachsel. (2009) "Preclinical characterization of Dekavil (F8-IL10), a novel clinical-stage immunocytokine which

inhibits the progression of collagen-induced arthritis". *Arthritis Res. Ther.*, 11, R142. [* = corresponding author]

- C. Schliemann, C. Rösli, H. Kamada, B. Borgia, T. Fugmann, W. Klapper, D. Neri (2009) "In vivo biotinylation of the vasculature in B-cell lymphoma identifies Bst-2 as a target for antibody-based therapy" Blood, in press.

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Philogen

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- P. Richter, M. Tost, M. Franz, A. Altendorf-Hofmann, K. Junker, L. Borsi, D. Neri, H. Kosmehl, H. Wunderlich, A. Berndt (2009) "B and C domain containing tenascin-C: urinary markers for invasiveness of urothelial carcinoma of the urinary bladder?" *J. Cancer Res. Clin. Oncol.*, 135, 1351-1358.

- C. Rösli, B. Borgia, C. Schliemann, M. Gunthert, H. Wunderli-Allenspach, R. Giavazzi, D. Neri (2009). "Comparative analysis of the membrane proteome of closely related metastatic and non-metastatic tumor cells". *Cancer Res.*, 69, 5406-5414.

- M. Pedretti, Z. Rancic, A. Soltermann, B.A. Herzog, C. Schliemann, M. Lachat, D. Neri, P.A. Kaufmann (2009) "Comparative immunohistochemical staining of atherosclerotic plaques using F16, F8 and L19: three clinical-grade fully human antibodies. *Atherosclerosis*, in press

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- C. Schliemann, C. Rösli, H. Kamada, B. Borgia, T. Fugmann, W. Klapper, D. Neri (2009) "In vivo biotinylation of the vasculature in B-cell lymphoma identifies Bst-2 as a target for antibody-based therapy" Blood, in press.

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Photobiotics and Imperial

- Bhatti M, Yahioglu G, Milgrom LR, Garcia-Maya M, Chester KA, Deonarain MP. (2008). Targeted photodynamic therapy with multiply-loaded recombinant antibody fragments. *Int. J. Cancer*, 122(5), 1155.
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CBA

- High-molecular tenascin-C as an indicator of atypical cells in oral brush biopsies. Driemel O, Dahse R, Berndt A, Pistner H, Hakim SG, Zardi L, Reichert TE and Kosmehl H. *Clin. Oral. Investig.* 11:1, 93-99, 2007.
- Expression analysis of extracellular matrix components in brush biopsies of oral lesions. Driemel O, Kosmehl H, Rosenhahn J, Berndt A, Reichert TE, Zardi L and Dahse R. *Anticancer Res.* 27:3B, 1565-70, 2007.
- Therapy-induced antitumor vaccination by targeting tumor necrosis factor alpha to tumor vessels in combination with melphalan. Mortara L, Balza E, Sassi F, Castellani P, Carnemolla B, De Lerma Barbaro A, Fossati S, Tosi G, Accolla RS, Borsi L. *Eur J Immunol*, 37:12, 3381-92, 2007.
- Internalization via Antennapedia protein transduction domain of an scFv antibody toward c-Myc protein. Avignolo C, Bagnasco L, Biasotti B, Melchiori A, Tomati V, Bauer I, Salis A, Chiossone L, Mingari MC, Orecchia P, Carnemolla B, Neri D, Zardi L, Parodi S*. FASEB J.* 2008 Apr;22(4):1237-45.
- mRNA expression and protein distribution of fibronectin splice variants and high-molecular weight tenascin-C in different phases of human fracture healing. Kilian O, Dahse R, Alt V, Zardi L, Hentschel J, Schnettler R, Kosmehl H. *Calcif Tissue Int.* 2008 Aug;83(2):101-11.
- Expression of the oncofetal ED-B-containing fibronectin isoform in hematologic tumors enables ED-B-targeted 131I-L19SIP radioimmunotherapy in Hodgkin lymphoma patients. Sauer S, Erba PA, Petrini M, Menrad A, Giovannoni L, Grana C, Hirsch B, Zardi L, Paganelli G, Mariani G, Neri D, Dürkop H, Menssen HD. *Blood.* 2009 Mar 5;113(10):2265-74.
- A novel human fibronectin cryptic sequence unmasked by the insertion of the angiogenesis-associated extra type III domain B. Balza E, Sassi F, Ventura E, Parodi A, Fossati S, Blalock W, Carnemolla B, Castellani P, Zardi L, Borsi L*. Int J Cancer.* 2009 Aug 15;125(4):751-8.
- Use of uteroglobin for the engineering of polyvalent, polyspecific fusion proteins. Ventura E, Sassi F, Fossati S, Parodi A, Blalock W, Balza E, Castellani P, Borsi L, Carnemolla B, Zardi L. *J Biol Chem.* 2009; 284 (39):26646-54.
- Impact of Tumour microenvironment on the expression of the angiogenesis associated EDB fibronectin isoforms. Coltrini D, Ronca R, Belleri M, Zardi L, Indraccolo S, Gavazzi R, and Presta M. *The J of Pathology,* Sept 2009.
- Ventura E, Parodi A, Balza E, Borsi L, Castellani P, Carnemolla B and Zardi L. Alternative splicing of the angiogenesis associated extra-domain B of fibronectin regulates the accessibility of the loop B-C of the type III repeat 8. *Submitted.*

University of Hull

- Alonso, C.M.A., Palumbo, A., Bullous, A.J., Pretto, F., Neri, D., Boyle, R.W. (2009) Site specific and stoichiometric conjugation of cationic porphyrins to anti-angiogenic monoclonal antibodies. Bioconjugate Chem. Accepted subject to minor revision.

Trojantec

- Christina A. Kousparou, Spyros Stylianou, Agamemnon A. Epenetos. Cancer Stem Cells. Forum of Clinical Oncology, 2007:6(1-2): 58-62.

LIST OF CONFERENCE COMMUNICATION

ETHZ

- Targets, targeting and proteomics. 7th Swiss Course on Medicinal Chemistry, Leysin (Switzerland), 1-6 October 2006
- Vascular tumor targeting: from the bench to the clinic. EU Workshop "Molecular targets for cancer programme", Luxembourg, 6-7 October 2006
- Vascular tumor targeting. Lecture at the University of Padova (Italy), 10 October 2006.
- Antibody-based vascular tumor targeting: from target discovery to clinical trials. HUPO 5th Annual World Congress, Long Beach (USA) 28 October – 1 November 2006
- Vascular targeting. 18th EORTC-NCI-AACR Symposium, Prague (Czech Republic) 7-10 November 2006.
- Ligand-based targeted therapies. Symposium "Milano new drugs, angiogenesis, environment and stroma: models, research and care". Milano (Italy), 10-11 November 2006
- Antibody Targeting of the extracellular matrix. Lecture at the Biozentrum of the University of Basel (Switzerland), 17 January 2007-09-13
- Vascular Targeting with Recombinant Antibody Derivatives. Keystone Symposium on Antibodies as Therapeutics, Lake Louise (Canada), 1-5 February 2007
- Vascular targeting: target identification, ligand isolation and clinical development of therapeutics. VI Laboratorio di Metodologie Sintetiche in Chimica Farmaceutica. Siena (Italy) 11-16 February 2007.
- Vascular tumor targeting. IFOM-IEOcampus FRIDAYSEMMinars, Milano (Italy) 23 March 2007.
- Engineering antibody-based fusion proteins for therapeutic applications. 2nd Singapore Biologics Manufacturing Conference (Singapore) 28-30 March 2007
- Ligand-based vascular tumor targeting: from the bench to the clinic. Seminars of the MRC Human Genetics Unit, Edinburgh (UK), 12 April 2007
- Antibody-based vascular tumor targeting: from the bench to the clinic. Cancer Immunotherapy CIMT Meeting, Würzburg (Germany), 13 April 2007
- Vascular tumor targeting. Seminar at the University of Catania (Italy), 23 April 2007.
- Antibody-based vascular tumor targeting. The Protein Engineering Summit (PEGS), Boston (USA), 14-18 May 2007
- Vascular tumor targeting. Seminar at the Institute of Clinical Chemistry, University of Zurich (Switzerland) 22 May 2007.
- Vascular tumor targeting. "The Future of Medical Sciences", Milano (Italy) 7 June 2007.
- Antibody-based vascular tumor targeting. Recombinant Antibodies IBC Conference, Berlin (Germany) 31 May – 3 June 2007
- Vascular tumor targeting: about targets, ligands and applications. CCA/V-ICI Keynote Lecture. VUMC Cancer Center Amsterdam (The Netherlands) 10 September 2007
- Vascular targeting antibody derivatives: from bench to the clinic. Lecture at the

International Meeting “Cellular and Molecular Mechanisms of Tumor Progression and Metastasis”, Kloster Seeon (Germany) 22-25 September 2007

- Vascular targeting of cancer and of chronic inflammation. International Conference on “Vascular Targeted Therapies in Oncology”, Mandelieu (France) 4-8 October 2007
- Vascular tumor targeting: from the bench to the clinic. Seminar at Nerviano Medical Sciences, Nerviano (Italy) 9 May 2008
- Chemical proteomics for the discovery of vascular markers of disease. Lecture at the 3rd Annual Biomarkers Congress, Manchester (UK) 15 May 2008
- Antibody-based vascular tumor targeting. Lecture at the Minisymposium “Drug Targeting” of the Division for Medicinal Chemistry of the Swiss Chemical Society, Basel (Switzerland), 29 May 2008
- The use of blood vessels as targets. 7th International Conference on Recombinant Antibodies, Dublin (Ireland) 24-26 June, 2008.
- Vascular targeting antibodies: from the bench to the clinic. 20th Meeting of the European Association for Cancer Research, Lyon (France) 5-8 July, 2008.
- Tumor targeting. Lecture at the Exploratory Worskshop "Molecular signaling in cardiovascular and oncological diseases: similar and shared pathways" of the European Science Foundation. Pisa (Italy) 15 July 2008
- Antibody-based vascular targeting: from the bench to the clinic. Lecture at the Departement Klinische Forschung, University of Bern (Switzerland), 1 September 2008.
- Antibody-based vascular tumor targeting: from the bench to the clinic. Lecture at the Collegium Helveticum, Forum Medizinische Wissenschaften “Onkologie”, Zurich (Switzerland) 24 September 2008
- Antibody-based vascular tumor targeting: from the bench to the clinic. Lecture at the Department of Dermatology, University of Zurich (Switzerland) 24 September 2008
- Vascular targeting antibodies for the therapy of cancer and arthritis: from the bench to the clinic. International Symposium "Recombinant Antibodies: new developments for future challenges CNIO, Madrid (Spain), 22 October 2008.
- Vascular targeting antibodies for the therapy of cancer and arthritis: from the bench to the clinic. Lecture at the Symposium “Protein Engineering and the Design of new Therapeutic Proteins”. Emeryville (USA), 6 November 2008.
- Engineering of antibody-based therapeutics and imaging agents: from the bench to the clinic. Lecture at the Biotechnet Switzerland / Swiss Biotech Association Meeting, Olten (Switzerland) 19 November 2008.
- Vascular targeting with recombinant antibody derivatives. Lecture at the 19th International IBC Conference on Antibody Engineering, San Diego (USA), 8 December 2008.
- Next-generation therapeutic antibodies. VIth Symposium of the Austrian Proteomics Platform, Seefeld (Austria) 18-21 January 2009.
- Vascular targeting antibodies for the therapy of cancer and arthritis: from the bench to the clinic. Lecture at the Center for Molecular Medicine (CeMM), Vienna (Austria) 11 May 2009.
- Vascular targeting in melanoma. 7th World Congress on Melanoma/5th Congress of the European Association of Dermato-Oncology (EADO), Vienna (Austria) 13 May 2009.
- Vascular tumor targeting: from the bench to the clinic. Lecture at the BIOTEC.ORG meeting of the Societa’ Chimica Italiana, Forte dei Marmi, Lucca (Italy) 21 May 2009
- Antibody-based vascular tumor targeting: from the bench to the clinic. Keynote Lecture at the “Targeting alpha-particle emitting radionuclides to combat cancer” (TARCC) Meeting, Nantes (France) 26 May 2009.

- Vascular tumor targeting: from the bench to the clinic. Lecture at the University College Dublin, Dublin (Ireland), 6 June 2009.
- Vascular targeting antibody derivatives: from the bench to the clinic. IBC Recombinant Antibodies Conference, Köln (Germany), 18 June 2009.
- Chemical proteomics for the discovery of vascular markers of pathology: from the bench to the clinic. 4th Annual National ItPA Conference, Milano (Italy) 25 June 2009
- Antibody-based vascular targeting: from the bench to the clinic. 3rd European Conference on Chemistry for Life Sciences (ECCLS), Frankfurt am Main (Germany) 4 September 2009
- Vascular targeting antibody-photosensitizer conjugates for the therapy of cancer and other angiogenesis-related diseases European Society of Photobiology, Wroclaw (Poland) 7 September 2009.
- Ligand-based vascular targeting: from the bench to the clinic. Switzerland-Japan Biomolecular Chemistry Symposium, Tokyo (Japan) 12 September 2009.
- Translating proteomics to the clinic. Lecture at the HUPO Congress, Toronto (Canada) 27 September 2009.
- Vascular tumor targeting: from the bench to the clinic. Keynote lecture at the ISOBM Conference, Amsterdam (The Netherlands), 29 September 2009.

Philogen

- "Produzione Pilota GMP di Farmaci Biotechnologici da cellule Umane" Convegno AFI - Sviluppo Farmaci Biotechnologici Mar 7- 8, 2007 Pavia – Italy.
- "Removal of Viruses and Process Related Contaminants from Antibody Constructs. A Case Study" European Downstream Forum May 8 - 9, 2007, Goettingen – Germany.
- 3d. Workshop on Molecular Targets for Cancer. Bergen Norway July 11-12, 2008.
- "Dall' Invenzione alla Sperimentazione Clinica: la Storia di un Farmaco Biotecnologico Italiano". Dalla Pre-clinica alla ricerca Clinica di Fase I - IRR meeting. Dec 10 - 11, 2008 Milano – Italy.
- "Purification of a therapeutic antibody-cytokine fusion protein. A case study". Biotech: Innovation in Process - ISPE meeting Apr 2, 2009. Milano – Italy.
- "Immunoconjugates for cancer therapy from invention to clinical investigation: the history of an antibody-cytokine fusion protein in oncology" VIIth Meeting Network Italiano per la Bioterapia dei Tumori NIBIT October 1st-3rd , 2009. Siena – Italy.
- "cGMP production of therapeutic antibody-cytokine fusion proteins" PEGS Europe, Protein Engineering Summit. 6th-8th October, 2009. Hannover – Germany.

University of Hull

- Selective targeting of photodynamic sensitisers to tumour tissue. R.W. Boyle, J. Greenman, N. Pesa, K. Smith, N. Cochon, R. Leconte, J.E. Van Lier, D. Hunting. Invited Keynote Lecture 1st Meeting of European Platform for Photomedicine, 24th – 27th March, 2008, Dubrovnik, Croatia.
- Strategies for the antibody targeting of PDT sensitisers. C.M. Alonso. R.W. Boyle, A. Bullous. International Tetrapyrrolic Society meeting, September 2007, Lund, Sweden
- Immuno-PDT Symposium, European Photobiology Society Conference, Wroclaw, Poland, Sept 2009.
- International Tetrapyrrolic Society Conference, Trinity College, Dublin, Sept 2008.
- Plenary Lecturer – European Platform for Photodynamic Medicine, Dubrovnik, Croatia, March 2008.

- International Tetrapyrrolic Society Conference, Lund, Sweden, Aug 2007.

Photobiotics

- Tumour targeted PDT. 1st Meeting of European Platform for Photomedicine, 24th – 27th March, 2008, Dubrovnik, Croatia.
- Application of Monoclonal Antibodies in Clinical Oncology. Cyprus, June 2007 Conference presentation.
- Tumour targeted PDT. 6th European Biophysics conference, Imperial College London, July 2007, "Novel photosensitisers for PDT".

Trojantec

- The 2nd UK Cancer Stem Cell Meeting. Institute of Cell and Molecular Science Barts and the London School of Medicine and Dentistry - 20th November, 2007.
- Antibodies-Europe Conference. Vienna, Austria 7-8 November 2007.
- "The Notch meeting". Athens, Greece 23-27 September 2007.
- The 24th International Conference on the Advances in the Application of Monoclonal Antibodies in Clinical Oncology and Satellite Symposium on Cancer Stem Cells June 2007, Limassol, Cyprus.

Imperial

- Application of Monoclonal Antibodies in Clinical Oncology, Cyprus, June 2007 Conference presentation "Tumour targeted PDT"
- 1st European platform for photodynamic medicine, Dubrovnik, Croatia, March, 2008, conference presentation, "Tumour targeted PDT".
- 6th European Biophysics conference, Imperial College London, July 2007, "Novel photosensitisers for PDT".
- Dr Mahendra Deonarain. Application of Monoclonal Antibodies in Clinical Oncology, Cyprus, June 2009 Conference presentation "Tumour targeted PDT with PPa photo-immunoconjugates"
- Miss Ioanna Stamati. American Chemical Society Meeting (2009), Salt Lake City, Utah, USA.
- Dr Mahendra Deonarain & Dr Gokhan Yahioglu, European Society for Photobiology (2009), Wroclaw, Poland. Targeted PDT.

CBA

- Siena March 2007 Francesca Sassi – Elisa Ventura Biacore Technics
- Bologna 16/3/07 Luciano Zardi: Incontro Presso Istituto Rizzoli di Bologna con il Prof. Piero Picci e Dott. Massimo Serra, per una possibile collaborazione nell'ambito "Alleanza contro il Cancro"
- Sestri Levante 8-9/6/2007 Luciano Zardi: Congress "Diplome interuniversitaire de pratiques chirurgicales en cancerologie" tittle of the seminar : "AC humains recombinants dans le diagnostic e le traitement des cancer"
- Lectures Jan-June 2008 (total 20h) – University of Genoa, Facoltà di Farmacia, Luciano Zardi: Title "Human Recombinant Antibodies"
- Padova, Italy FORUM – Ricerca e Innovazione. Title: "Il Biotech Italiano" 15-18/5/09,
- Abano Terme, Padova, Italy: "3rd European Conference on Tumour Angiogenesis and Anti-angiogenesis Therapy" Title: "Vascular Tumor Targeting: from bench to the clinic" 5-8/11/08

- Congress “Diplome interuniversitaire de pratiques chirurgicales en cancerologie” Pietra Ligure, Savona, Italy. Title of the seminar: “Recombinant antibodies - diagnosis and therapy of cancer”
- Visit at Prof. Boyle’s Department, University of Hull, UK, from 15/07/2009 to 31/07/2009 – Elisa Ventura
- “Use of uteroglobin for the engineering of polyvalent, polyspecific fusion proteins useful for photodynamic therapy”. Wroclaw, Poland. 13th Congress of European Society for Photobiology and the 2nd Conference of the European Platform for Photodynamic Medicine, 6-8/09/2009.
- University of Genoa, Facoltà di Farmacia, Title “Human Recombinant Antibodies” Lectures Jan-June 2009 (total 20h)

Courses

1. Experimental Course on Antibody Phage Technology.