

Contract no. 018690 Modified Final Activity Report

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Project acronym: EURO-Laminopathies

Project title: Nuclear Envelop-linked Rare Human Diseases: From Molecu-

lar Pathophysiology towards Clinical Applications

Instrument: STREP

Thematic priority: Life Science, Genomics and Biotechnology for Health

MODIFIED FINAL ACTIVITY REPORT

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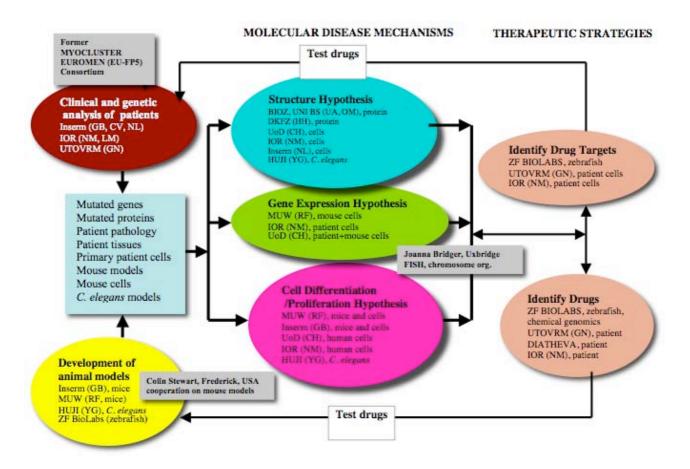
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1 PROJECT EXECUTION

1.1 Project Objectives

The EURO-Laminopathies consortium brought together clinical and basic researchers as well as company-based research, aiming at understanding the molecular basis of a heterogeneous class of human diseases linked to mutations in lamins and lamin-associated proteins (known as laminopathies). Understanding lamin function in normal tissues and its dysfunction in diseased cells is crucial for identifying new drug targets and drugs for efficient therapeutic approaches, which is another major objective of the project.

The overall concept of the project is outlined below:



Clinicians and human geneticists used the existing links to hospitals and genetic centres to expand knowledge on disease phenotypes, genes and mutations involved in laminopathies. They collected, analyzed and organized patient cells, tissues, and clinical and genetic information of patients and patient families. Using this information, several groups in the consortium generated and studied animal disease models (transgenic mice, *C. elegans*, zebrafish). They investigated potential disease mechanisms and explored possibilities how a pathological phenotype can be rescued.

Using patient cells and tissues, transgenic animals and cells derived therefrom, and isolated mutated and wildtype proteins, the EURO-Laminopathies consortium has been testing the following disease hypothesis:

- ✓ the mechanical hypothesis, predicting that disease-causing mutations interfere with the atomic structure, assembly, and stability of lamins;
- ✓ the gene expression hypothesis suggesting that the expression of mutated lamin proteins alters chromatin organization and gene expression; and
- ✓ the cell differentiation/proliferation hypothesis proposing an impairment of tissue homeostasis and regeneration in patients.

The detailed analyses of these mechanisms is expected to yield potential new drug targets and will help to identify drugs, whose potential for treatment of a particular disease phenotype can be tested in the available animal and cellular models. Finally, existing therapies for laminopathy patients were further developed by the generation and use of theranostic tools in order to improve the efficiency of treatment and reduce side effects.

1.2 Contractors

| Role | No. | Organisation | Short name | Country |
|------|-----|--|--------------|---------------|
| CO | P1 | Medizinische Universität Wien | MUW | Austria |
| CR | P2 | Biozentrum, University of Basel | BIOZ UNI BAS | Switzerland |
| CR | P3 | Institut National de la Santé et de la Recherche Médicale | INSERM | France |
| CR | P4 | The Hebrew University Jerusalem | HUJI | Israel |
| CR | P5 | German Cancer Research Center | DKFZ | Germany |
| CR | P6 | University of Durham | UOD | Great Britain |
| CR | P7 | Instituto Ortopedici Rizzoli | IOR | Italy |
| CR | P8 | Università degli Studi di Roma tor Vergata | UTOVRM | Italy |
| CR | P9 | punkt international GmbH | punkt | Austria |
| CR | P10 | ZF BioLabs Ltd | ZF BioLabs | Spain |
| CR | P11 | DIATHEVA s.r.l. | DIATHEVA | Italy |

1.3 Performed Work and Final Results

1.3.1 Clinical and genetic analysis of patients

Contractors involved: INSERM/FR/P3, IOR/IT/P7, UTOVRM/IT/P8

Laminopathies are caused by mutations in genes encoding lamin A/C (LMNA); Lamin A-binding proteins, such as emerin (EMD) and Lamina-associated polypeptide 2 alpha ($LAP2\alpha$); and lamin A-processing enzymes (ZMPSTE24).

A total of over 274 different *LMNA* mutations are currently reported in 1235 individuals, 104 different *EMD* mutations in 289 patients and 9 *ZMPSTE24* mutations in 18 subjects. We searched for the involvement of *LMNA* mutations in patients with Emery Dreifuss Muscular Dystrophy (EDMD)-like clinical presentation, lipodystrophic and/or insulin resistance syndromes atypical for the Familal Partial Lipidystrophy (FPLD) phenotype and premature aging syndromes. The *EMD* gene was also screened in patients with muscular dystrophies and *ZMPSTE24* in patients with premature aging syndromes.

For *LMNA* mutations, <u>new clinical subgroups</u> were identified, i.e. insulin resistance syndromes atypical for FPLD and severe congenital muscular dystrophies. We have also contributed to the description of fertility and obstetrical complications in women with *LMNA*-related familial partial lipodystrophy.

Regarding the <u>FPLD phenotype</u>, we reported about a patient affected by a variant of Dunnigan syndrome characterized by partial lipodystrophy, insulin resistance and hypertrophic cardiomy-opathy with valvular involvement, due to a novel C591F heterozygous substitution in exon 11 in the C-terminal domain of lamin A.

We have identified a new subgroup of laminopathies with insulin resistance and/or lipodystrophies, called "metabolic laminopathies". Affected patients exhibit non-codon 482 *LMNA* mutations (mutations p.R28W, p.L92F, p.L387V, p.S395L, p.R399H, p.L421P, p.R439C, p.H506D, p.T655fsX49) and are characterized, in the absence of obvious clinical lipoatrophy, by severe metabolic alterations and frequent muscle signs (muscular hypertrophy, myalgias, or weakness).

A cohort of nine patients carrying the reported LMNA c.1930C > T (R644C) missense mutation was studied in detail. The study revealed extreme phenotypic diversity and low penetrance with apparently unrelated symptoms consisting of lipodystrophy and insulin resistance (2 patients), proximal weakness and contractures (2 brothers), focal segmental glomerulosclerosis, motor neuropathy, arthrogryposis and dilated cardiomyopathy, scoliosis and contractures, limb girdle weakness, hepatic steatosis and insulin resistance (1 case each).

We also studied the familial form of <u>dilated cardiomyopathy (DCM)</u>, which occurs in about 20%–50% of DCM cases, in a large cohort of 73 patients with familial DCM by *LMNA* sequencing and clinical evaluations. We detected three known (E161K, R190Q, R644C) and two new (E203V, K219T) heterozygous missense mutations in families characterized by severe DCM and heart failure with conduction system disease necessitating pacemaker implantation and heart transplantation. Therefore, testing *LMNA* in such families will be recommended for the clinicians. Interestingly, four of these mutations clustered in the protein domain coil 1B, which is important for lamin B interaction and lamin A/C dimerization.

Novel mutations in *LMNA* were identified also in patients affected with <u>progeroid laminopathies</u>, such as a heterozygous mutation (c.898G>A, p.D300N) in a 31 years old patient affected with atypical Werner syndrome; an autosomal dominant mutation (c.1968G>A, p.Q656Q) in a 35 year old man presenting with progeroid phenotypes; a homozygous mutation (c.1583C>T,

p.T528M) in an Algerian child; a heterozygous mutation (c.784G>A, p.E262K) in a 25 years old girl of Italian non consanguineous healthy parents; a heterozygous missense mutation (c.11C>G, p. P4R) in a 9 year old Italian child; and a heterozygous C>T mutations (p.R189W) in an Italian female patient with hirsutism and type A insulin resistance.

Several novel *ZMPSTE24* inactivating mutations were identified in patients affected with restrictive dermopathy. In most cases the autosomal recessive transmission was confirmed by parents' status analyis. In some families a prenatal diagnosis was further performed. In a male of New Zealand origin, two mutations were identified in the *ZMPSTE24* gene: a c.794A>G missense mutation (p.N265S) and a novel microdeletion in exon 2 (c.205_206delCT) leading to premature stop (p.L69LfsX5). These analyses allowed the further delineation of the *ZMPSTE24* mutational spectrum in laminopathies and will contribute to the generation of the UMD-*ZMPSTE24* database.

Several unreported sequence variations in genes encoding other nuclear envelope proteins were identified in patients affected with candidate progeroid syndromes or nosologically identified diseases. For example, a novel heterozygous inactivating mutation was identified in *MAN1/LMD3* in a patient affected with a cutaneous-only form of Buschke-Ollendorf syndrome. A novel heterozygous mutation of this gene (c.657C>G; p.D219E) was identified in a patient affected with a Werner-like syndrome.

Moreover, 190 probands with EDMD or EDMD-like phenotypes were screened for DNA variations in the genes encoding <u>nesprin-1 (SYNE1)</u> and <u>nesprin-2 (SYNE2)</u>. Four heterozygous missense mutations were identified by this study. Nesprin-1 and -2 are multi-isomeric, spectrin-repeat proteins that bind both emerin and lamins A/C and form a network in muscle linking the nucleoskeleton to the inner nuclear membrane, the outer nuclear membrane, membraneous organelles, the sarcomere and the actin cytoskeleton. These data demonstrate that also nesprin mutations cause EDMD and strongly suggest that uncoupling nucleoskeleton and cytoskeleton networks, due to perturbed nesprin/emerin/lamin interactions, may be responsible for the EDMD phenotype.

Apart from the identifications of these novel mutations, numerous patients remain without a molecular diagnosis, suggesting the existence of other major genes responsible for these various syndromes. As a potential modifier mechanism, we found that digenism, i.e. mutation in 2 genes within the same family, could explain in part the wide clinical variability.

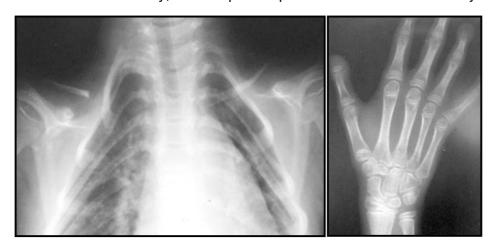


Figure 1: Skeletal anomalies (clavicular hypoplasia, clubshaped terminal phalanges and acroosteolysis in patients with progeroid svndromes, such MADA

1.3.2 Development of animal disease models

Contractors involved: MUW/AT/P1, INSERM/FR/P3, HUJI/IL/P4, ZFBioLabs/ES/P10

Mouse models, lacking lamin A/C or expressing various mutated disease-linked lamin proteins have been proven valuable tools for the analyses of the pathological phenotypes and for the identification of potentially impaired molecular mechanisms *in vivo* and *ex vivo* (in cell culture). The consortium generated and analyzed four novel lamin A/C knock-in mice expressing lamin A/C variants linked to muscular dystrophy (*Lmna* delK32, *Lmna* H222P) or progeria and MADA (tet-off progerin, *Lmna* R527H), and three transgenic animals lacking a prominent lamin A interaction partner, LAP2 α , ubiquitously or conditionally in muscle fibers and muscle stem cells. Furthermore, crosses of LAP2 α -deficient mice with mdx mice (Duchenne muscular dystrophy model) and with a mutated lamin A knock in model were generated. Besides the phenotypical analyses and behavioural analyses these mice have been and will be further tested for their cardiac function and their response to acute and chronic stress, their ability to regenerate muscle tissue, and for testing potentially deregulated pathways in primary mouse cell cultures (fibroblasts, sattelite cells, cardiomyocytes), including cell cycle regulation, differentiation, chromatin organization, and gene expression.

We have also generated transgenic *Caenorhabditis elegans* strains, each expressing GFP::Celamin with a different mutation that when present in human *LMNA* cause laminopathic disease. Each mutation was used to generate 2-5 independent lines. So far, we have tested 15 different mutations for their effects on lamin localization, lamin dynamics and changes in nuclear morphology. We have tested two of these mutations for their effect on motility, muscle morphology and interaction with heterochromatic loci. The phenotypes of the mutant lamin constructs were also compared to their effects on lamin filament assembly *in vitro*. We have also tested *C. elegans* with a deletion in emerin, LEM-2/MAN-1 or both emerin and LEM-2/MAN-1 or in *baf-1*. The muscle phenotype in these animals can serve as a model for muscle degeneration in human.

ZF Biolabs has developed a new laminopathic disease model by generating morphant zebrafish where zf-LMNA has been knocked down. Zebrafish embryos with a loss of function of lamin A have shown characteristic phenotypes with tail truncation, oedemas, growth retardation, and disorganization or absence of somites. Interestingly main tissues affected in zebrafish resembled those affected in human laminopathies. The specificity of this new model has been shown by different ways: the use of different morpholino phosphorodiamidate oligonucleotides (MOs) (designed in different regions of the zf-LMNA gene and with different strategies to obtain gene loss of function) that produced the same phenotypes, and by validating lamin A knockdown in treated embryos. Time-course analyses of the effect of MOs during the developmental stages, revealed that the latest possible time to perform screening is at 96hpf, where the expression of the zf-LMNA gene is highest and the action of MOs still detectable. Given that all laminopathies are post-natal defects and do not involve developmental problems, and 96hpf is a post-hatching stage, this time point seems to be the most appropriate for using this new model in drug testing screens.

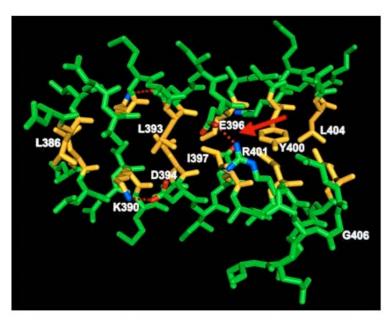
1.3.3 Molecular disease mechanisms

Using recombinantly expressed wild type and disease linked mutated lamin A/C and LAP2 α polypeptides, transgenic animals (mice, *C. elegans*, zebrafish), primary cells derived from the mouse models, and cells and tissues from laminopathy patients, the consortium investigated potential disease mechanisms and their contributions to the pathology seen in patients. In particular, the following disease hypotheses have been investigated:

- Mechanical hypothesis

Contractors involved: BIOZ UNI BAS/CH/P2, HUJI/IL/P4, DKFZ/DE/P5

Lamins belong to the intermediate filament (IF) protein family. It is well documented that the intermediate filament cytoskeleton is a primary determinant of cell and tissue plasticity. Mechanical stress causes dynamic remodelling of the IF cytoskeleton, and vice versa, remodelling of the IF cytoskeleton, for example, by introducing mutations in the IF proteins, affects cell and tissue plasticity. Similarly, it is to be expected that introducing laminopathy causing mutations in the lamin A/C polypeptide will have a more or less pronounced effect on the 3-D structure of the nuclear lamina, and hence its mechanical properties and thereby the plasticity of the nucleus and, possibly, the cell and affected tissue. Deciphering at atomic detail the structural manifestation of a given laminopathy causing molecular lesion will be key for a rational understanding of the disease sequelae and, in turn, for designing effective preventive interventions or therapeutic cures.



Interhelical salt bridge Glu396 --> Arg401

Figure 2: A highly conserved interhelical salt bridge in coil 2B of all IF proteins is a major target for disease mutations. As shown here for human vimentin, Glu396 of the one polypeptide chain forms an interhelical salt bridge with Arg401 of the second polypeptide within a parallel two-stranded α helical coiled coil (Strelkov et al. (2001) EMBO J **21**: 2055-66. In human lamin A the homologous amino acid residues implicated in this interhelical salt bridge are Glu372 and Arg377, which is the third highest hotspot in LMNA harboring laminopathy disease mutations.

20 overlapping recombinant fragments of lamins A/C and B1, B2 comprising selected parts of the coiled-coil 'rod' domain were obtained. Their stability and oligomeric state were analyzed by circular dichroism (CD), analytical ultracentrifugation (AUC) and transmission electron microscopy (TEM). To date, two human lamin A fragments (LA[305-387], LA[328-398] and LA[243-316]) were crystallized and their crystal structures solved. LA[305-387] forms a parallel left-handed coiled coil, whereas LA[328-398] forms an anti-parallel right-handed coiled coil. We are currently testing the hypothesis that the anti-parallel right-handed coiled coil may represent an atomic model for the antiparallel inter-dimer interaction occurring within lamin tetramers. In addition, the wild type lamin A fragment LA[243-316] that contains the linker 2 (L2) region and the CMT disease variant LA[243-316/R298C] have been characterised and crystallized in several crystal forms.

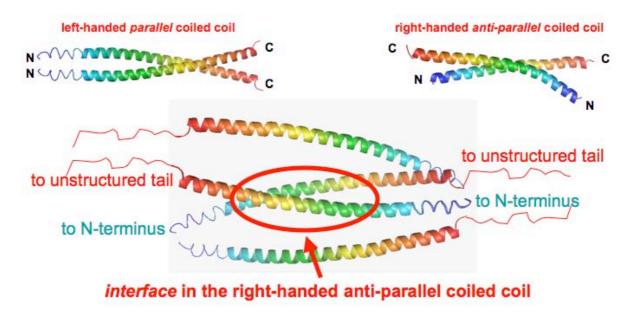


Figure 3: Combining left-handed parallel and right-handed anti-parallel crystal structures of human lamin A rod fragments to model the interface between two anti-parallel two-stranded coiled coils such as they occur in tetrameric nuclear lamin and other IF protein complexes. Whereas human lamin A coil 2B fragment LA[305-387] forms a left-handed parallel two-stranded α -helical coiled coil, human lamin A coil 2B fragment LA [328-398] forms a right-handed anti-parallel two-stranded α -helical coiled coil. Here we have used the atomic structure of the right-handed anti-parallel two-stranded α -helical coiled coil to model the interface between two parallel two-stranded α -helical coiled coils that are oriented in an antiparallel fashion relative to each other.

Lamin A is integrated into larger complexes at the nuclear periphery, called the nuclear lamina, by a huge number of specific interactions with both B-type lamins and inner nuclear membrane proteins and chromatin proteins, although the mechanistic molecular details have not been worked out yet. A potential role in the generation of distinct nucleoplasmic scaffolds for transcription factors and signaling molecules is compatible with its polymorphic behaviour, but up to now no proof for such a function has been provided. Therefore we study <u>lamin A assembly and specific interactions</u> as well as the effects of disease causing mutations on these properties.

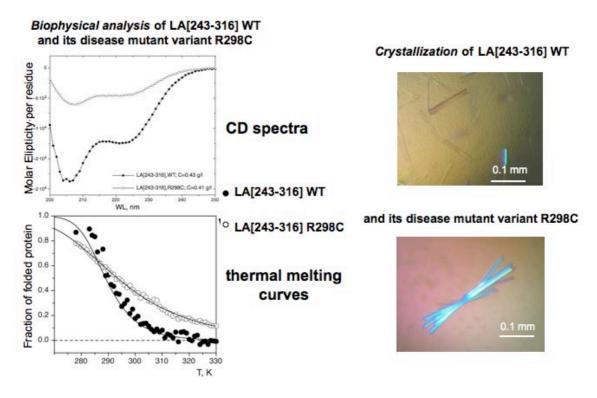


Figure 4: Biophysical and crystallographic analysis of human lamin A linker L2 construct (LA[243-316] together with the disease mutant variant LA[243-316/R298C]. CD spectra, thermal melting curves and crystals formed by the human lamin A fragments LA[243-316] WT and LA[243-316/R298C] mutant variant are shown. In both cases the fragments form parallel two-stranded α -helical coiled coils.

We have also used the *Caenorhabditis elegans* lamin as a model system to study the <u>supramolecular organization of the lamin filament</u>. Using cryo-electron tomography, we were able to show that *C. elegans* nuclear lamin forms 10 nm IF-like filaments, which are distinct from their cytoplasmic counterparts. The IF-like lamin filaments are composed of 3 and 4 tetrameric protofilaments, each of which contains two partially staggered anti-parallel head-to-tail polymers. The beaded appearance of the lamin filaments stems from paired globular tail domains, which are regularly spaced alternating between 21 and 27 nm. A mutation in an evolutionarily conserved residue that causes Hutchison-Gilford progeria syndrome in humans alters the supramolecular structure of the lamin filaments. Based on our structural analysis, we propose an assembly pathway that yields the observed 10 nm IF-like lamin filaments and paracrystalline fibers. These results also serve as a platform for understanding the effect of laminopathic mutations on lamin supramolecular organization.

- Nuclear architecture, Chromatin organization

Contractors involved: HUJI/IL/P4, UoD/GB/P6, IOR/IT/P7

Previous studies have indicated that lamin mutations may affect the organization of peripheral heterochromatin to different extents and may even lead to complete lack of heterocromatin areas. Nuclear architecture appears to be minimally affected in non-progeroid laminopathies, but it appears severely altered in progeroid syndromes. The pathogenesis of Huchinson-Gilford progeria syndrome and Mandibuloacral dysplasia has been linked to defects in heterochromatin

organization caused by accumulation of the mutated, truncated and farnesylated prelamin A (progerin) or other prelamin A forms. Within the consortium we studied the pathogenic effects of different pre-lamin A variants in detail analyzing heterochromatin-associated proteins such as HP1 and histone 3 trimethylated on lysine 9, which were found to be altered both in terms of their post-translational modifications and their intranuclear distribution patterns in laminopathic cells from progeroid laminopathies.

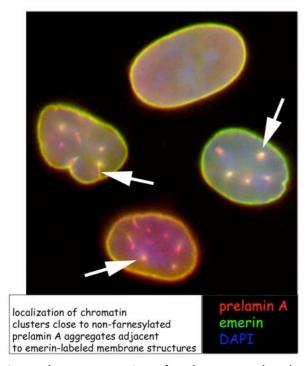


Figure 5 Changes of heterochromatin in prelamin A over-expressing cells as shown by immunofluorescnce microscopy.

We studied interactions of prelamin A with various nuclear components. We could demonstrate an interaction between prelamin A and LAP2 α , which together formed complexes with the heterochromatin protein HP1alpha. High affinity for HP1 α binding was observed particularly in cells expressing non-farnesylated prelamin A (due to mutation of the C-terminal cysteine). Consistent with these results, we demonstrated that accumulation of non-farnesylated prelamin A recruited HP1 α , LAP2 α and trimethyl-H3K9 in in-

tranuclear aggregates. As shown previously, accumulation of farnesylated prelamin A caused loss of heterochromatin and uneven distribution of heterochromatin markers. Moreover, LAP2 α was downregulated and redistributed to the nuclear periphery.. Altogether, we demonstrated that accumulation of different processing intermediates of prelamin A differentially affects chromatin organization in human fibroblasts. These effects on chromatin organization were also detected in cells treated with drugs inhibiting prelamin A processing (the farnesyltransferase inhibitor FTI-277 and the non-peptidomimetic drug N-acetyl-S-farnesyl-L-cysteine methylester (AF-CMe)), validated the use of FTIs and AFCMe as experimental models for prelamin A accumulation. More recently we also found a novel association of prelamin A with the DNA-binding protein BAF in cells from Mandibuloacral Dysplasia patients accumulating prelamin A.

Regarding the FPLD phenotype, we showed for the first time chromatin abnormalities in adipocytes, in subcutaneous adipose tissue (scAT) biopsies of 7 patients with FPLD2.

Novel aspects were also found in fibroblasts from patients carrying the newly identified <u>Nesprin mutations</u> (responsible for the EDMD phenotype). Nuclei exhibited abnormal morphology and mislocalization of emerin and SUN2, in addition to diminished nuclear envelope localization of nesprins and impaired nesprin/emerin/lamin binding. Nesprin-2 giant appears to have a role as a structural reinforcer at the NE and a protective effect in nuclei expressing mutated lamins. In fact, cultured cells expressing the 800 kDa nesprin-2 giant do not show alterations in the organization of acetylated histones, histone H1 and the active form of RNA polymerase II, which are caused by the lamin A p.S143F mutation known to produce a myopathic and progeric phenotype. While cultured dermal fibroblasts of this patient, which express low levels of nesprin-2 giant, showed severe nuclear dysmorphism, cultured keratinocytes normally expressing high levels of nesprin-2 giant, appeared normal.

Using Flourescence In Situ Hybridisation (FISH) the effect of laminopathy mutations on chromosome position and gene organisation was investigated. To date a systematic analysis of chromosome 19 has been performed on patient fibroblasts from a range of laminopathy diseases and work is still in progress on other chromosomes.

Also in *Caenorhabditis elegans* strains expressing specific disease linked missense mutation in lamin or lack specific lamin binding proteins (emerin, BAF-1, LEM2) changes in nuclear morphology and chromatin have been tested. Most importantely, in collaboration with the group of Dr. Gasser (Basel) we were able to show that low level ectopic expression of the lamin mutation Y59C causes redistribution of LacO arrays in the nucleus. Another important outcome of these studies is that in addition to their role in organizing chromatin, the lamin/LEM-domain/BAF complexes regulate also specific developmental pathways by binding to specific promoters.

- Cell proliferation / differentiation

Contractors involved: MUW/AT/P1, INSERM/FR/P3, HUJI/IL/P4, UoD/GB/P6, IOR/IT/P7

Recent studies in our consortium and in other labs have indicated a role of lamin A-LAP2 α complexes in controlling proliferation and differentiation of adult stem cells in a retinoblastoma (pRb)-dependent manner. These findings led to the formulation of the cell proliferation / differentiation hypothesis, predicting that mutations in lamin A and LAP2a can impair their cell cycle regulatory function and lead to an imbalance of self renewal, proliferation, and differentiation of adult stem cells in muscle and other affected tissues in the patients. Using LAP2α-deficient mice and tissues as well as cells derived therefrom we demonstrated that prolifeating cells in regenerating tissues (skin, muscle, colon, hematopoetic tissue) contain a small nucleoplasmic pool of lamin A, in addition to the well known peripheral lamin network at the nuclear envelope, and that this nucleoplasmic pool of lamin A is stabilized by LAP2 α . LAP2 α -deficient cells loose the nucleoplasmic pool of lamin A and, as a consequence, show a misegulation of the Rb pathway leading to hyperproliferation of progenitor cells in tissues. In view of this new concept, the role of lamin-LAP2α complexes in the regeneration of muscle by satellite cells has been and is still being studied in transgenic mice expressing muscular dystrophy-linked lamin variants (H222P and DelK32) or lacking LAP2α in different genetic backgrounds. First results from mouse primary myoblasts of the KI-LMNA models (H222P and DelK32) indicate growth retardation and delayed myoblast differentiation. LAP2 α -deficient myoblasts, in contrast, have increased proliferation rates and seem to transiently enhance the regeneration capacity of the tissue. Thus, the generated mouse models provide important tools for the detailed analyses of lamin/LAP2\alpha functions in cell cycle control at the cellular level and in tissue regeneration at the organismal level and are essential tools for future preclinical studies testing new therapies aiming at the manipulation of tissue progenitor cells in laminopathic diseases.

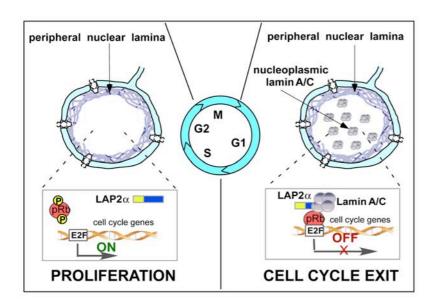


Figure 6: Model depicting the molecular mechanism how lamins may affect cell cycle progression and differentiation of tissue progenitor cells. A nucleoplasmic pool of lamins A/C in the G1 phase of cycling cells, stabilized by LAP2a, regulates pRb-mediated cell cycle exit and initition of differentiation. Diseasecausing lamin variants may affect the nucleoplasmic pool and thus, impair pRb regulation.

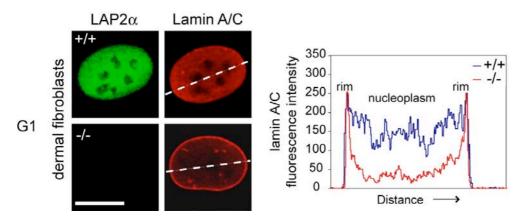


Figure 7: Nucleoplasmic lamins A/C are lost in LAP2 α -deficient fibroblasts. Left panel: immunofluorescent images of fibroblast nuclei from LAP2 $\alpha^{+/+}$ and LAP2 $\alpha^{-/-}$ mice stained for LAP2 α (greeen) and lamins A/C (red). Bar is 10 μ m. Right panel: Staining intensities of lamin A measuerd along the line shown in lef images. Note that nucleoplasmic versus rim staining is greatly reduced in LAP2 α deficient cells (red line).

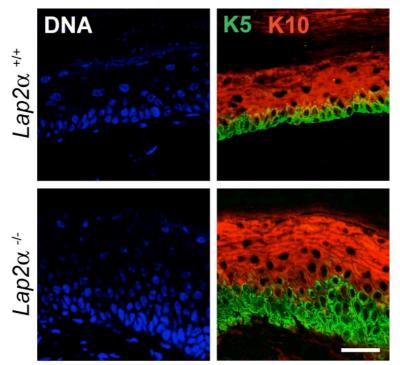


Figure 8: Paw epidermis of LAP2α-deficient mice shows hyperplasia due to hyperproliferation of early tissue progenitor cells. Immunofluorescence images of paw epidermis sections from LAP2 $\alpha^{+/+}$ and LAP2 $\alpha^{-/-}$ are shown stained for DNA (blue), keratin 5 as marker for proliferating progenitor cells (K5 green) and keratin 10 as a marker for non-proliferating differentiated cells (K10, red) are shown. Bar is 50 μm.

Most studies in human cells have been carried out in fibroblast cell lines from laminopathy patients. These studies have been useful in that they have revealed that fibroblasts harbouring lamin mutations display profound cell cycle abnormalities and that loss of lamin A or LAP2 α lead to premature entry into S-phase followed by a checkpoint arrest involving altered distribution and phosphorylation of pRb. Lamin A and LAP2 α also seem to influence cellular ageing in fibroblasts. We have found that both proteins are critical targets of oxidative stress pathways and that oxidative damage gives rise to a laminopathy like-phenotype and growth arrest. In line with these findings we find that *LMNA* mutations in patient cells result in oxidative stress that triggers premature cellular senescence. This finding clearly links laminopathy disease to normal human ageing. Additional findings from the consortium have linked cell cycle arrest and ROS damage arising from the presence of laminopathy mutations to the accumulation of pre-lamin A. This work highlights the possible use of drugs that scavenge ROS as therapeutic tools.

In addition, we have shown that the HIV protease inhibitors, currently used to treat HIV-infected patients, induce cellular prelamin A accumulation and trigger oxidative stress and premature cellular senescence, similar to the phenotypes identified in acquired lipodystrophic syndromes,

We also investigated the processing of prelamin A during differentiation of C2C12 myoblasts as well as in osteoclast and adipocyte models. Prelamin A expression and its nuclear localization changed during myoblasts differentiation and seemed to influence the expression of the muscle-specific membrane protein caveolin 3. Peripheral blood monocytes treated with various drugs causing accumulation of non-farnesylated (FTI) or farnesylated (AFCMe) prelamin A, and induced to differentiate towards the osteoclastic lineage, showed that the presence of prelamin A caused a more efficient monocyte differentiation, while bone resorption activity was low. In patients affected by FPLD2, we could demonstrate accumulation of prelamin A in subcutaneous adipose tissue. This feature was found to be associated with a reduced expression of several genes involved in adipocyte proliferation/differentiation. Moreover, the expression of *PPARG2*, *RB1*, *CCND3* and *LPL* in thigh scAT was significantly reduced in comparison with abdomen scAT.

1.3.4 Therapeutic strategies

- Identification of drug targets

Contractors involved: IOR/IT/T/P8, ZFBioLabs/ES/P10

Using the homozygous KI-*Lmna*^{H222P/H222P} mouse model as a tool we started to test potential drug targets for treating heart fibrosis, a phenotype often seen in laminopathy patients. One month oral treatment of 6 months-old female mice with the gluthathione precursor <u>N-acetyl-L-cysteine (NAC)</u>, stabilized the left ventricular dilation, hypokinesia and fibrosis and normalized the oxidative stress, glutathione depletion and the increase in soluble tumor necrosis factor alpha.

Recent studies reported the activation of cardiac <u>mitogen-activated protein kinases (MAPKs)</u> in young asymptomatic *Lmna*^{H222P/H222P} mice, and the benefits of treatment with MAPK inhibitor. Because patients may be diagnosed at a late stage of the disease, we measured the MAPKs activation in 7-month-old female *Lmna*^{H222P/H222P} mice with overt cardiac dilation and dysfunction. In contrast with the young *Lmna*^{H222P/H222} mice, 7-month-old mice did not display cardiac MAPK activation. Our study pointed out differences in the biochemical mechanisms contributing to DCM progression in symptomatic *Lmna*^{H222P/H222P} mice compared to those identified in asymptomatic young mice, suggesting that the choice of the therapeutic treatment of EDMD may depend on the stage of the cardiac disease.

As previously shown, one characteristic feature of the premature ageing laminopathies (HGPS, RD or MAD) is the accumulation of the unmaturated farnesylated prelamin A in the nucleus. We investigated the ability of a set of different drugs acting on farnesylation to rescue the nuclear disease phenotype in *vitro* and *in vivo*. We also aimed at identifying genomic biomarkers in order to monitor the effects of the experimental drugs most efficiently. We already designed an easy to handle kit for the identification of mutations in *LMNA* and are currently working on an experimental system to identify pre-lamin A in patient samples, as pre-lamin A expression levels seem to be correlated with the severity of cellular pathologies.

Using fibroblast and lymphoblastoid cell cultures from patients affected with Hutchinson-Gilford Progeria and *Zmpste24*¹⁻ mice, we could demonstrate the the joint administration of <u>statins</u> (<u>pravastatin</u>) and <u>aminobiphosphonates</u> (<u>zoledronate</u>) is beneficial against many cellular and organismal parameters of the disease. We could show that on treated human and mouse cells, nuclear morphology, chromatin distribution and DNA repair properties were strongly ameliorated by the new combined treatment. Weight and survival curves in mice were significantly improved by the treatment (lifespan was increased by about 80% in treated mice). Bone volumes and trabecular densities were almost completely recovered in these mice as well. These are a <u>preclinical proof</u> of principle that has allowed two groups in the consortium (Nicolas Levy, Giuseppe Novelli) to obtain the authorisation by the French and Italian health authorities and ethitcs committees to organise a therapeutic clinical trial for European children affected with HGPS and MADA patients. The HGPS trial is based in Marseille, la Timone children's hospital, and has started at the end of October 2008. Results are expected at the end of 2010-beginning 2011.

The MADA trial is based in Rome, Fondazione Policlinico Tor Vergata, and has started at the end of January 2009. Results are expected at the beginning of 2010.

In addition we used other model organisms to test the effects of drugs. We have previously shown that aging adult <u>C. elegans</u> cells show changes in nuclear architecture similar to HGPS fibroblasts and down regulating lamin expression in adult <u>C. elegans</u> reduces their lifespan. We used C. elegans to test the effects of FTIs on normal aging in the context of the whole animal.

We found that nuclei of adult *C. elegans*, in which lamin is downregulated, have similar phenotypes to normal aging nuclei, but at an earlier age. We further showed that treating adult *C. elegans* with the FTI gliotoxin reverses nuclear phenotypes and improves motility of aging worms. However, the average lifespan of the gliotoxin-treated animals was similar to that of untreated animals. These results suggest that lamins are involved in the process of normal aging in *C. elegans*.

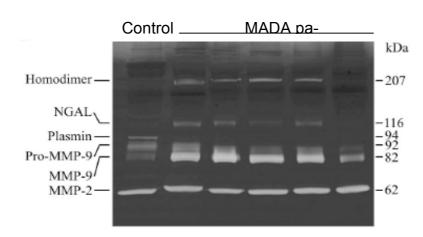
Finally we generated a new <u>zebrafish laminopathy disease model</u> by zf-*LMNA* downregulation and have started to use this model for testing drugs as a "proof of principle" for future high throughput screening aproaches. A few drugs (like valproic acid, isoniacide and fluorouracil (5-FU) from the small molecule list included in the NTP High Throughput Screening Initiative has been checked but did so far not yield any conclusive results. However, this model will be an important tool to design future high throuhput screening approaches using arge compound libraries.

Diagnostic and theranostic tools were generated in the consortium. In particular DIATHEVA developed an LMNA gene amplification kit for direct sequencing as a diagnostic tool for genetic analysys of patients and two specific antibodies against different prelamin A intermediates useful to test the efficacy of drugs acting on prelamin a processing.

- Identification of drugs

Contractors involved: IOR/IT/P7, UTOVRM/IT/P8, ZFBioLabs/ES/P10, DIATHEVA/IT/P11

Based on our studies on the molecular mechanisms of laminopathic diseases, we explored the potential of drugs known to affect disturbed pathways in diseased cells. For instance the observed altered distribution of trimethylated histone 3 (lysine 9) in progeria fibroblasts prompted us to test drugs known to affect epigenetic histone modification pathways. Indeed, treatment of patient cells with a combination of trichostatin A and mevinolin rescued the phenotype at the cellular level. Furthermore, we were testing various inhibitors of farnesyl transferase inhibitor (eg. ABT 100), which are known to rescue nuclear architecture in progeria cells and many of the phenotypes in Zmpste24-deficient animal disease models. Other studies involved the use of the PPARgamma agonist troglitazone in FPLD cells, as we have shown altered expression of the adipocyte transcription factor PPARgamma and reduced differentiation rate in FPLD fibroblasts. Finally, the identification of metallo proteinase (MMP) deregulation in MADA cells prompted us to select and test drugs acting on this enzyme in order to reduce its activity. It is expected that drugs able to inhibit MMPs could improve some clinical features of the patients. We are cur-



rently also testing drugs affecting TNFs *in vitro* and *in vivo* on a selected group of patients.

Figure 9: Gelatinolytic (MMP) activity in serum from MADA pateints, detecting four forms (207 kDa, 116 kDa, 92 kDa and 82 kDa) that may be related to MMP-9, neutrophil gelatinase B-associated lipocalin (NGAL), pro-MMP-9 and active MMP-9,

respectively. Note that in MADA serum the levels of active MMP-9 were enhanced.

2 Dissemination and Use

2.1 Exploitable Knowledge and Its Use

| Exploitable knowledge | Exploit- able re- sult(s) | Sector(s) of application | Timetable for use | IPR protection | Owners |
|------------------------------------|---------------------------------|--|--------------------|------------------------------|-------------|
| Standardized pa- | | | | A patent is possible in 2008 | |
| protocols | Methods | Medical | 2008 | Publications | P3, P8 |
| Mid-term status | | | 2007 2008 | Published | P3, P8 |
| wild torm status | | | 2008 | 1 dolloried | 1 0, 1 0 |
| Final status | | | 2009 | Published | P3, P8 |
| | | | 2007 | | |
| New animal mod- | | | 2008 | | |
| els | Methods | Industrial | 2009 | Publications | P1, P3, P10 |
| | | Scientific community | | | |
| | Disease | Pharmaceutical industry for pre-clinical | 2007 | | |
| Mid-term status | models | drug testing | 2008 | Published | P1, P3 |
| | | | | | |
| | | | 2008 | Published | |
| | | | 2009 | | |
| Final status | | | 2009 and beyond | to be pub- lished | P1, P3, P10 |
| Standardized | | | | | |
| clinical treatment protocols (SOP) | Methods | Medical | 2009 | Publications | P8, P11 |
| protocolo (CC1) | Wietriede | Modical | 2007 | T dolloations | 7 0, 7 7 7 |
| Mid-term status | | | 2008 | Published | P8, P11 |
| | | | 2008 | Patent | |
| | | | 2008 | Published | |
| | | | 2009 | | |
| Final status | | | 2009 and beyond | to be pub- lished | P3, P8 |
| Novel targets and drugs for thera- | Method | Medical | 2009 | Publications | P3, P7, P8, |

| peutic interven- tion | Small molecules | | | | P10 |
|---|--------------------|--|--------------------|----------------------|-----------------------|
| Novel pathways and functions of Lamin complexes | Methods | Molecular Biologists, Medical researchers | | | |
| Mid-term status | | | 2008 | Published | P1, P3, P4, P6, P7 |
| | | | 2009 | Published | P1, P3, P4, |
| Final situation | | | 2009 and beyond | to be pub- lished | P6, P7, P8, P10 |

Table 1

2.1.1 Brief description of Exploitable Knowledge (modified)

Standardized patient examination protocols / Methods:

P3/INSERM/Gisèle Bonne: The prospective evaluation of the primary prevention of cardiac sudden death has desmontrated the benefit of the implantation of ICD in subjects carrying *LMNA* mutation in families with cardiomuscular laminopathies. Therefore, genetic diagnosis is recommended in relatives of patients with cardiac diseases carrying the *LMNA* mutation together with a close cardiologic followup. In young relatives, the presymptomatic genetic diagnosis can be proposed at an age of 10 years on a "case to case" basis with a close genetic counselling.

P3/INSERM/Corinne Vigouroux: Recent genotype-phenotype studies have evidenced that a careful neuromuscular and cardiac examination with echocardiography and 24-h electrocardiogram monitoring is needed in patients with lipodystrophic syndromes linked to *LMNA* mutations. In addition, in patients presenting with insulin resistance syndromes, muscular and/or cardiac symptoms should be screened for mutations in *LMNA*.

P8/UTOVRM/Giuseppe Novelli: A standardized examination protocol was developed for the assessment of progeroid and lipodistrophic laminopathic clinical signs. by a medical working group at Fondazione Policlinico Tor Vergata of Rome, consisting of physicians specialized in internal medicine (bone and metabolic defects), paediatrics, radiology, and medical genetics. The protocol includes of medical history record, and detailed physical examination and genetic analysis with endocrinological, haematological, dermatological, dietologic, odontostomatologic, neurological, cardiological, and dismorphologic consulences. At genetic level, both the molecular analysis of *LMNA* and *ZMPSTE24* coding region were included in the standardized examination protocol of laminopathic patient.

New animal models / Methods and Disease models:

P1/MUW/Roland Foisner: We expanded and characterized the previously generated LAP2 α deficient mouse line. Our studies identified a phenotype of adult stem cells and early progenitor cells in regenerative tissues in LAP2 α -deficient lines. Progenitor cells in epidermis, intestine, muscle and the hematopoietic system showed hyperproliferation and decreased differentiation. Furthermore, cardiac function was affected, consistent with the previously reported *LAP2* mutation in human DCM. This mouse line, thus provides an animal model for analysing lamin-mediated adult stem cell function at organimal level. Furthermore, conditional LAP2 α knockout

mice specifically ablating LAP2 α in muscle stem cells or in muscle fibers, using the Cre-Lox strategy (Pax7-Cre and MCK-Cre mice, respectively) were generated, allowing the detailed analyses of lamin-LAP2 function in adult stem cells versus differentiated muscle fibers. Finally, new mouse models, including a double knock out (LAP2 α -deficient, dystrophin-deficient), and a double mutant (LAP2 α -deficient and $Lmna^{delK32}$ knock-in) line, were generated to study the functional relationship between LAP2 α , lamin A and dystrophin in vivo.

P3/INSERM/Gisèle Bonne: Two Knock-in mouse models reproducing *LMNA* mutations identified in patients were produced and characterized. The KI-*Lmna*^{H222P} mice reproduce an Emery-Dreifuss muscular dystrophy mutation and mimics quite well the human disease when the mice carrying the mutation at homozygous state, with the development of muscular dystrophy and dilated cardiomyopathy together with conduction defects. The KI-*Lmna*^{delK32} mice reproduce a severe congenital muscular dystrophy mutation and interestingly, reproduce quite well the severity of the mutation as homozygous mice died within 2 weeks of age from muscle maturation defects and heterozygous mice develop a dilated cardiomyopathy. This is the first knock-in mouse models for *Lmna* gene that express a phenotype at the heterozygous state.

P3/INSERM/Nicolas Lévy: Two mice models reproduicing progeria were produce: 1) a Tet-off mouse was generated at SEAT, Service d'Expérimentation Animale de Transgénèse et de Recombinaison Homologue, Villejuif, France), allowing tetracyclin-dependent expression of the disease lamin A variant. This model will allow us to regulate the expression of the transgene and thus to study the effects of variations of truncated prelamin A production and assess the effficacy of novel drugs under controlled conditions. 2) A knock-in progeria model in collaboration with Bernard Malissen (CIML, Marseille, France) in which a the singly point mutation identified in human *LMNA* was introduced into the mouse *Lmna* together with an upstream Lox-Pgkneo-stop-Lox cassette. Expression of the mutated lamin A (progerin) can be induced in specific tissues, by crossing this mice with mice expressing Cre recombinase in these tissues. In this way progerin could be exoressed in the endothelium (*tie-1* Cre) or smooth muscle cells (smooth muscle actin Cre), which are affected in progeria patients.

Standardized clinical treatment protocols (SOP) / Methods:

P3/INSERM/Gisèle Bonne: In patients with muscle and cardiac involvement, the treatment has to aim at preventing the cardiologic risks, i.e. the cardiac sudden death, by implantation of ICD when patients are in need of a device to treat arhythmias and conduction defects. The treatment of dilated cardiomyopathy and heart failure are according to the cardiologic standards.

P3/INSERM/Corinne Vigouroux: Concerning patients with lipodystrophy and/or insulin resistance, the treatment has to aim at reducing the metabolic consequences (diabetes, dyslipidemia) and the cardio-vascular risks. Therapeutic molecules are not specific for laminopathies, except thiazolidinediones, which were recently reported to have a particular interest in treating insulin resistance in lipodystrophic patients.

P3/INSERM/Nicolas Lévy: As the Medical Genetics and Cell Biology Department of Marseille's Timone Hospital investigate patients exhibiting progeria and progeroid syndromes, a detailed protocol of investigation was set up including clinical examination, imaging, biological parameters recording, cell and molecular biology investigations performed both on PBMC, immortalized lymphoblastoïd lines as well as on cultures on skin fibroblast.

P8/UTOVRM/Giuseppe Novelli: In collaboration with Nicolas Lévy (Hôpitaux de Marseille, France), we developed a therapeutic protocol based on the capacity to reduce, prevent or delay the more severe symptoms of MADA and HGPS diseases. The clinical trial is taken place in the

Fondazione Policlinico di Tor Vergata, Department of "Medicina Interna" and Department of "Medicine of Laboratory", U.O.C. of Medical Genetics. We evaluate the efficacy and the tolerance of pravastatin (statin) and zoledronic acid (bisphosphonate) in association in a non-randomized and open label study. Given the small number of patients in EU (15 HGPS children and 7 Italian MADA patients), a double-blind placebo controlled protocol cannot be envisaged and we decided for a protocol with a Single Group Assignment, that compare the obtained results with historical control.

We established the HGPS and MADA inclusion and exclusion criteria, the posology and the modality of administration of both drugs, a flowchart containing programmed visits, clinical examinations and laboratory data. Moreover, we envisaged different primary and secondary outcomes in relation to main disease signs. The results obtained in this pilot phase will be analyzed through only descriptive analysis (in terms of position, variability, form, etc.) because of the poor number of available patients. In case this analysis will have no significant result, it will be used as a basis for the eventual establishment of a new and more appropriate sample.

P11/DIATHEVA: The generated *LMNA* mutation detection kit and the pre-lamin A antibodies are commercially exploited by DIATHEVA, which sells these products. Knowledge having a potential for direct and immediate industrial or commercial applications in research or for developing, creating or marketing a product or process or for creating or providing a service have not been generated.

Novel targets for therapeutic intervention / Small molecules:

P3/INSERM/Gisèle Bonne: The N-acetyl cysteine, a precursor of gluthatione, was successfully tested in mouse models to slow down the progression of contractile dysfunction and fibrosis. Further analysis in mice are still necessary to complety evaluate this moleculaire on long term basis, before to envisage any test in patients.

P3/INSERM/Corinne Vigouroux: The treatment with leptin needs to be evaluated in lipodystrophic patients with *LMNA* mutations and leptin deficiency, given its efficiency against metabolic disorders in other lipodystrophic syndromes. In France, we have obtained the authorization of the health agency (Afssaps) to treat patients with leptin through a named-patient program; we are still waiting for the drug delivery by the Amylin laboratory (USA).

P3/INSERM/Nicolas Lévy: A 3-year therapeutic trial began running in Marseille in September 2008 with the authorization of AFFSAPS and a financial support of French Minstry of Health (Clinic Reseach Hospital Program) and AFM. 15 progeria childrens coming from all Europa will be given the drug combination (ZoPra: zoledronate + pravastatin) designed to partially block the prenylation pathway and to reduce the amount of progerin (see Varela et al., 2008). The same protocol will be applied to mandibulo-acral dysplasia patients in Roma (Pr. Guiseppe Novelli).

<u>Patent:</u> WO 2008/003864 A1 (10th january 2008) "Combination of an HMG-CoA reductase inhibitor and a farnesyl-pyrophosphate synthase inhibitor for the treatment of diseases related to the persistance and/or accumulation of prenylated proteins". Owners: Aix-Marseille 2 University, Oviedo Université (Spain), AFM, Marseille Hospital Administration (AP-HM). Inventors: Nicolas Lévy, Pierre Cau, and Carlos Lopez-Otin.

P7/IOR/Nadir Maraldi: The treatment of laminopathies bearing progeroid features and lipodystrophy with different drugs may be monitored by checking the staining pattern of trimethylated-histone 3 on lysine 9 which we have established as a biomarker of heterochromatin disorganization in laminopathies. Partial or complete rescue of the cellular phenotype corresponds to recovery of proper trimethylation-of histone 3 on lysine 9.

P8/UTOVRM/Giuseppe Novelli: Farnesyltransferase inhibitors (FTIs) block farnesylation of prelamin A that accumulates in MADA nuclei. Since FTI ABT-100 treatment of MADA cells in vitro did not show any significant improvement, we developed, together wiith P3, a combined treatment with statins and aminobisphosphonates that inhibit both farnesyltransferase and geranylgerabyltransferase activity. Both drugs are authorized chemical substances widely used in therapeuthic protocols. The statins are cholesterol-lowering agents and nitrogen-bisphosphonates (NBPs) are used in osteoporosis and tumor treatment. We used a combination of pravastatin and zoledronate in cell culture tests, producing much better effects (rescue of misshapen nuclei and heterchromatin effects) than single treatment. Thus, this combinatorial treatment can be further tested in preclinical trials.

Novel pathways and functions of Lamin complexes / Methods:

P1/MUW/Roland Foisner: The characterisation of the LAP2 α knockout mouse revealed that defects in lamin A localization (caused by absence of LAP2 α) affect adult stem cells in regenerative tissues, supporting the emerging concept that an impairment of tissue progenitor cells contributes to the disease pathology.

P3/INSERM/Gisèle Bonne: The characterisation of the KI-*Lmna*^{DelK32} mouse model demonstrate that the *Lmna* delK32 mutation alter the lamin filament formation, which lead to reduce level of lamin in the muscle and promote muscle maturation defect both in skeletal and heart muscle. In addition, this mutation alters the whole structure of the nuclei, which in turn modify the gene expression important in the postnatal period.

P7/IOR/Nadir Maraldi: Involvement of lamin A/C and prelamin A in chromatin remodeling has been demonstrated. A direct correlation between prelamin A processing rate and cellular aging has been shown, which has implications in basic mechanisms regulating not only pathological, but also normal aging processes. The use of specifc antibodies directed to prelamin A allows the identification of pathological impairment of prelamin A processing as well as aging-related prelamin A accumulation. Based on our studies, we can divide the wide group of lamin-linked disorders (laminopathies) into two subgroups: a) laminopahies with progeroid features or lipodystrophy tracts characterized by prelamin A accumulation, b) laminopathies with selective involvement of skeletal muscle or myocardium characterized by mutations that do not affect prelamin A maturation.

P8/UTOVRM/Giuseppe Novelli: An altered bone extracellular matrix (ECM) remodelling could also play a pivotal role in MADA and contribute to the observed bone. We found that the serum level of active metalloproteinase (MMP-9), which is involved in bone development, remodelling and homeostasis, is significantly increased in MADA sera compared with healthy controls.

2.2 Dissemination of Knowledge

| Туре | Type of audi- ence | Countries addressed | Partners responsible | Actual Date* |
|-----------------|-----------------------|---|----------------------|-----------------|
| | General public | | | |
| o o an mande | Austria, France, | | | |
| | Researchers | Germany, Israel, Italy, England, Switzer- | | |
| Project website | Higher education | land, Spain mainly | P9 / all | 02/06 |

| Туре | Type of audience | Countries addressed | Partners responsible | Actual Date* |
|---|---|--|--|--|
| Project website update | General public Journalists Researchers Higher education | Austria, France, Germany, Israel, It- aly, England, Switzer- land, Spain mainly | P9 / all | Latest version 03/09 |
| | | Austria | P1 | 03,05,10/0 8; 04/09 |
| Press releases (press/radio/TV) | General public Journalists | France England | P3 P6 | 2007-2008 09/08 |
| Websites of partners' institution | General public Journalists Researchers Higher education | Austria, France, Germany, Israel, It- aly, England, Switzer- land | P9 / all | 02/06 |
| Workshops and lab training | Young researchers collaborating with the project groups | Austria, France, Germany, Israel, It- aly, England, Spain, Switzerland | P1, P2, P3, P4, P5, P6 P7, P8 | not neces- sary due to the efficient communi- cation plat- form |
| Communication plat- form was established | Young researchers collaborating with the project | Austria, France, Germany, Israel, It- aly, England, Spain, Switzerland | P1, P2, P3, P4, P5, P6 P7, P8, P10 | To be continued |
| Clinical and genetic data of patients on UMD-LMNA database | Researchers | Austria, France, Germany, Israel, It- aly, England, Switzer- land, Spain | P3, P7, P8 | Latest version 03/09 |
| Atomic structure, Lamin assembly, Nuclear architecture, Chromatin organisation, Tissue on project website | Researchers | Austria, France, Germany, Israel, It- aly, England, Switzer- land Spain | P1, P2, P4, P5 | Published 2007-2009 |
| Scientific publications | Researchers SME Industry | Austria, France, Germany, Israel, It- aly, England, Switzer- land, Spain | P1, P2, P3, P4, P5, P6 P7, P8, P10, P11 | 2006-2009 |
| Posters | Researchers SME | Austria, France, Germany, Israel, It- aly, England, Switzer- | P1, P2, P3, P4, P5, P6 P7, P8, P10, | 2006-2009 |

| Туре | Type of audi- ence | Countries addressed | Partners responsible | Actual Date* |
|--|---|--|--|------------------------------|
| | Industry | land, Spain, USA, others to be defined | P11 | |
| Conferences | Researchers SME Industry | Austria, France, Germany, Israel, It- aly, England, Switzer- land, Spain USA, oth- ers to be defined | | 2006-2009 |
| Lectures | Higher education | Austria, France, Germany, Israel, It- aly, England, Switzer- land, Spain | P1, P2, P3, P4, P5, P6 P7, P8 | 2006-2009 and be- yond |
| Seminars | Researchers Higher education SME Industry | Austria, France, Germany, Israel, It- aly, England, Switzer- land, Spain | P1, P2, P3, P4, P5, P6 P7, P8, P10, P11 | 2006-2009 and be- yond |
| International project specific symposium | Researchers SME Industry | worldwide | P1, P2, P3, P4, P5, P6 P7, P8, P10, P11 | 01/09 |
| Public lecture | General public | Austria | P1 | None on this topic |

Table 2

2.3 Brief Description of Major Activities (modified)

The consortium vividly transferred their newly generated knowledge on "Nuclear Envelop-linked Rare Human Diseases: From Molecular Pahtophysiology towards Clinical Applications" to the scientific community, industry, students, patient organisations, and to science journalists in numerous talks and posters at international conferences. Consortium members were invited for talks, seminars and lectures at national and international institutions, meetings and conferences. The young group members were especially encouraged to and did present their projects and results at the annual consortium meetings and at external conferences.

Partner 3 Nicolas Lévy and Partner 6, Chris Hutchision published information about their research on TV (France 2 and 3 and BBC). The coordinator, Roland Foisner, will publish information about the rpoejct EURO-Laminopathies on HEALTH TV, funded by the EU. Several partners also published press releases that appeared in newspapers and magazines.

Most of the partners were involved in the organisation and co-organisation of internationally recognized meetings, workshops and conferences.

One patent was filed by partner 3:

Patent: WO 2008/003864 A1 (10th january 2008) "Combination of an HMG-CoA reductase inhibitor and a farnesyl-pyrophosphate synthase inhibitor for the treatment of diseases related to

the persistance and/or accumulation of prenylated proteins". Owners: Aix-Marseille 2 University, Oviedo Université (Spain), AFM, Marseille Hospital Administration (AP-HM). Inventors: Nicolas Lévy, Pierre Cau, and Carlos Lopez-Otin.

In addition many protocols, methods and new insights into the molecular pathways of lamins in health and disease were published in 57 papers in scientific journals with a high impact factor in the last 18 months.

The consortium acquired third party funding in order to organise the international symposium (=EMBO workshop) on the topic "The Multiple Faces of Lamins in Aging & Diseases", which took place from 6th to 9th January 2009 in Vienna (Austria). P1, 3, 5 and 8 were respnsible for the scientific program of this symposium and invited internationally highly acknowledged and young researches in the field of nuclear organisation and disease. P9 was responsible for all organisational issues during the submission, the preparatory, the executive and conclusion phase of the symposium, with a financial support of about Euro 41.000 by European and Austrian organisations, including EMBO, Landes Bioscience, Pharmaceutical companies, Austrian Academy of Sciences and Vienna Convention Office.

With the EMBO workshop on "The Multiple Faces of Lamins in Aging & Diseases" the consortium managed to organise a very successful event at the end of the EURO-Lamiopathies network project, which at the same time established a sustainable discussion platform for future projects.

Throughout the EURO-Laminopathies project a communication platform has evolved that allowed particularly the young group members in the partners' laboratories to efficiently learn state of the art techniques and apply them successfully in their work. This also formed the basis for intense collaborations. All partners thus, maintained a vivid exchange of knowledge, tools and methods by email, phone and personal contacts at meetings and conferences. All partners agreed to continue to cultivate this platform.

2.4 Publication list

Joint publications within EURO-Laminopathies

- Ben-Harush K, Wiesel N, Frenkiel-Krispin D, Moeller D, Soreq E, <u>Aebi</u> U, <u>Herrmann</u> H, <u>Gruenbaum</u> Y, Medalia O. (2009) The supramolecular organization of the *C. elegans* nuclear lamin filament. **J Mol Biol.** 13;386(5):1392-402.
- Kapinos LE, Schumacher J, Mücke N, Machaidze G, <u>Aebi</u> U, Strelkov SV, <u>Herrmann</u> H. (2009) Biophysical characterization of head-to-tail complexes formed by human lamin A, B1 and B2 "mini-dimer" constructs. **J Mol Biol**, submitted.
- Mrosek M, Müller SA, Skegro D, Rigden DJ, <u>Foisner R</u>, Lustig A, <u>Mayans O.</u> (2009) Biophysical characterization of the intrinsically disordered nuclear protein LAP2a. In preparation.
- Dominici S, Fiori V, Magnani M, Schena E, Capanni C, Camozzi D, D'Apice MR, Le Dour C, Auclair M, Caron M, Novelli G, Vigouroux C, Maraldi NM, Lattanzi G. (2009) Different prelamin A forms accumulate in human fibroblasts: a study in experimental models and progeria. Submitted. **Eur J Histochemistry**, 2009, 53: 43-52.
- Kreplak L, <u>Herrmann</u> H, <u>Aebi</u> U. (2008) Tensile properties of single desmin intermediate filaments. **Biophys**. J. 94, 2790-2799.
- Wiesel N, Mattout A, Melcer S, Melamed-Book N, <u>Herrmann</u> H, Medalia O, <u>Aebi</u> U, <u>Gruenbaum</u> Y. (2008) Laminopathic mutations interfere with the assembly, localization and dynamics of

- nuclear lamins. Proc. Natl. Acad. Sci. USA, 105, 180-185.
- Mattioli E, Columbaro M, Capanni C, Santi S, <u>Maraldi</u> N, D'Apice MR, <u>Novelli G</u>, Riccio M, Squarzoni S, <u>Foisner R</u>, <u>Lattanzi G</u>. (2008) Drugs affecting prelamin A processing: Effects on heterochromatin organization. **Exp. Cell Res**. 314, 453-462.
- Rowat A, Lammerding J, <u>Herrmann H, Aebi U.</u> (2008) Towards an integrated understanding of the structure and mechanics of the cell nucleus. **Bioessays** 30, 226-236.
- Capanni C, Del Coco R, Squarzoni S, Columbaro M, Mattioli E, Camozzi D, Rocchi A, Scotlandi K, <u>Maraldi N, Foisner R, Lattanzi G. Prelamin A is involved in early steps of muscle differentiation. Exp Cell Res.</u> 2008 Dec 10;314(20):3628-37.
- Mattioli E, Columbaro M, Capanni C, Santi S, <u>Maraldi</u> N, D'Apice MR, <u>Novelli</u> G, Riccio M, Squarzoni S, <u>Foisner</u> R, <u>Lattanzi</u> G. (2008) Drugs affecting prelamin A processing:Effects on heterochromatin organization. **Exp. Cell Res.** 314, 453-462.
- Capanni C, Del Coco R, Squarzoni S, Columbaro M, Mattioli E, Camozzi D, Rocchi A, Scotlandi K, <u>Maraldi N, Foisner R, Lattanzi G</u>. (2008) Prelamin A is involved in early steps of muscle differentiation. **Exp Cell Res.** 314:3628-3637.
- Herrmann H, Strelkov SV, Aebi U. (2008) The intermediate filament protein family: from structure to function. **J Clin Invest**, in press.
- Lattanzi G, Columbaro M, Mattioli E, Cenni V, Camozzi D, Wehnert M, Santi S, Riccio M, Del Coco R, Maraldi NM, Squarzoni S, Foisner R, Capanni C. (2007) Pre-Lamin A processing is linked to heterochromatin organization. J. Cell. Biochem. 102, 1149-1159.
- Lombardi F, Gullotta F, Columbaro M, Filareto A, D'Adamo M, Vielle Ae, Guglielmi V, Maria Nardone A, Azzolini V, Grosso E, <u>Lattanzi</u> G, D'Apice MR, Masala S, <u>Maraldi</u> NM, Sbraccia P, <u>Novelli</u> G. (2007) Compound heterozygosity for mutations in LMNA in a patient with a myopathic and lipodystrophic Mandibuloacral Dysplasia type A phenotype. **J Clin Endocrinol Metab**. 92(11), 4467-71.
- Meaburn KJ, Cabuy E, <u>Bonne</u> G, <u>Levy</u> N, Morris GE, <u>Novelli</u> G, Kill IR, <u>Bridger</u> JM. (2007) Primary laminopathy fibroblasts display altered genome organization and apoptosis. **Aging Cell.** 6 (2):139-53.
- Margalit A, Brachner A, Gotzmann J, <u>Foisner F, Gruenbaum Y.</u> (2007) Barrier-to-autointegration factor a BAFfling little protein. **Trends Cell Biol**. 17, 202-208.
- Snyers L, Vlcek S, Dechat T, Skegro D, Korbei B, Gajewski A, <u>Mayans</u> O, Schöfer C, <u>Foisner</u> R. (2007) Lamina-associated Polypeptide 2 alpha forms Homo-trimers via its C-terminus and oligomerization is unaffected by a disease causing mutation. **J. Biol. Chem.** 282, 6308-6315.
- Pekovic V, Harborth J, Broers JLV, Ramaekers FCS, van Engelen B, Lammens M, von Zglinicki T, Foisner R, Hutchison C, Markiewicz E. (2007) Nucleoplasmic LAP2α Ipha-lamin A complexes are required to maintain a proliferative state in human fibroblasts, **J. Cell Biol**. 176/2, 163-172.
- Goldie KN, Wedig T, Mitra A, <u>Aebi</u> U, <u>Herrmann</u> H, Hoenger A. (2007). Dissecting the 3-D structure of intermediate filaments by cryo-electron tomography. **J Struct Biol** 158:378-85.
- Herrmann H, Baer H, Kreplak L, Strelkov SV, <u>Aebi</u> U. (2007) Intermediate filaments: from cell architecture to nanomechanics. **Nature Rev Mol Cell Biol** 8:562-73.
- <u>Foisner</u> R, <u>Aebi</u> U, <u>Bonne</u> G, <u>Gruenbaum</u> Y, <u>Novelli</u> G. (2007) Nuclear envelope-linked rare human diseases: from molecular pathophysiology towards clinical applications. **Neuromuscular Disorders** 17:655-60.

- Bär H, Mücke N, Katus HA, <u>Aebi</u> U, <u>Hermann</u> H. (2007) Assembly defects of desmin disease mutants carrying deletions in the alpha-helical rod domain are rescued by wild type protein. **J Struct Biol.** 158, 107-115.
- Kirmse R, Portet S, Mücke N, <u>Aebi</u> U, <u>Herrmann</u> H, Langowski J. (2007) A quantitative kinetic model for the in vitro assembly of intermidiate filaments from tetrameric vimentin. **J Biol Chem.** 282, 18563-18572.
- Parry DA, Strelkov SV, Burkhard P, <u>Aebi</u> U, <u>Herrmann</u> H. (2007) Towards a molecular description of intermediate filament structure and assembly. **Exp Cell Res.** 313, 2204-2216.
- Foeger N, Wiesel N, Lotsch D, Mücke N, Kreplak L, <u>Aebi</u> U, <u>Gruenbaum</u> Y, <u>Herrmann</u> H. (2007). Solubility properties and specific assembly pathways of the B-type lamin from Caenorhabditis elegans. **J Struct Biol**. 155, 340-350.
- Dorner D, Vlcek S, Foeger N, Gajewski A, Makolm C, Gotzmann J, <u>Hutchison CJ, Foisner R.</u> (2006) Lamina-associated polypeptide 2alpha regulates cell cycle progression and differentiation via the retinoblastoma-E2F pathway. **J. Cell Biol.** 173, 83-93.
- Sokolova AV, Kreplak L, Wedig T, Mucke N, Svergun DI, <u>Herrmann</u> H, <u>Aebi</u> U, Strelkov SV. (2006) Monitoring intermediate filament assembly by small-angle x-ray scattering reveals the molecular architecture of assembly intermediates. **Proc Natl Acad Sci USA** 103:16206-11.
- Broers JL, Ramaekers FC, <u>Bonne</u> G, Ben Yaou R, <u>Hutchison</u> CJ. (2006) Nuclear lamins: laminopathies and their role in premature ageing. **Physiol Rev** 86(3), 967-1008.
- Mittelbronn M, Hanisch F, Gleichmann M, Stötter M, Korinthenberg R, Wehnert M, Bonne G, Rudnik-Schöneborn S, Bornemann A. (2006) Myofiber degeneration in autosomal dominant Emery-Dreifuss muscular dystrophoby (AD-EDMD) (LGMD1B). Brain Pathol. 16, 266-272.

Additional publications from each partner

- Renard D, Milhaud D, Bessis D, Esteves-Vieira V, Boyer A, Roll P, Bourgeois P, <u>Levy</u> N, De Sandre-Giovannoli A. (2009) Novel LMNA mutation in a familial case of atypical Werner syndrome presenting with severe atherosclerosis and acute ischemic disease. **Stroke** 40(2):e11-4.
- Caron M, <u>Vigouroux</u> C, Bastard JP, Capeau J. (2009) Antiretroviral-related dysfunction and lipodystrophy in HIV-infected patients: alteration of the PPARgamma-dependent pathways, **PPAR Research** 2009:507141.
- Mejat A., Decostre V, Li J, Renou L, Kesari A, Hantai D, Stewart CL, Xiao X, Hoffman E, <u>Bonne</u> G, Misteli T. (2009), Lamin A/C-mediated neuromuscular junction defects in Emery-Dreifuss muscular dystrophy. **J Cell Biol** 184 31-44.
- Rauner M, Sipos W, Goettsch C, Wutzl A, <u>Foisner R</u>, Pietschmann P, Hofbauer LC. (2009) Inhibition of Lamin A/C Attenuates Osteoblast Differentiation and Enhances RANKL-Dependent Osteoclastogenesis. **J. Bone Miner. Res.** 24:78-86.
- Tilgner K, Wojciechowicz K, Jahoda C, <u>Hutchison</u> C, Markiewicz E. (2009) Dynamic complexes of A-type lamins and emerin influence adipogenic capacity of the cell via nucleocytoplasmic distribution of {beta}-catenin. **J Cell Sci.** Feb 1;122(Pt 3):401-13.
- Capanni C, Del Coco, Camozzi D, Columbaro M, Schena E, Merlini L, Squarzoni S, <u>Maraldi NM</u>, <u>Lattanzi</u> G. (2009) Emerin-prelamin A interplay in human fibroblasts. **Biol Cell**, submitted.

- Araújo-Vilar D, <u>Lattanzi</u> G, González-Méndez B, Costa-Freitas AT, Prieto D, Columbaro M, Mattioli E, Victoria B, Martínez-Sánchez N, Ramazanova A, Fraga M, Beiras A, Forteza J, Domínguez-Gerpe L, Calvo C, Lado-Abeal J. (2009) Site-dependent differences in both prelamin A and adipogenic genes in subcutaneous adipose tissue of patients with type 2 familial partial lipodystrophy. **J Med Genet**. 46:40-48.
- Perrot A, Hussein S, Ruppert V, Schmidt HH, <u>Wehnert MS</u>, Duong NT, Posch MG, Panek A, Dietz R, Kindermann I, Böhm M, Michalewska-Wludarczyk A, Richter A, Maisch B, Pankuweit S, Ozcelik C. (2009) Identification of mutational hot spots in LMNA encoding lamin A/C in patients with familial dilated cardiomyopathy. **Basic Res Cardiol.** 104(1):90-9.

- Mulky A, Cohen TV, Kozlov SV, Korbei B, <u>Foisner</u> R, Stewart CL, Kewal Ramani VN. (2008) The mouse LEM domain proteins aemerin and LAP2α are dispensible for HIV-1 and MLV infection. **J. Virol.** 82, 5860-5868.
- Naetar N, Korbei B, Kozlov S, Kerenyi MA, Dorner D, Kral R, Gotic I, Fuchs P, Cohen T, Bittner R, Stewart CL, <u>Foisner</u> R. (2008) Loss of LAP2α-lamin A complexes causes erythroid and epidermal progmitor hyperproliferation. **Nat. Cell Biol.**, published online 10.1038/ncb1793.
- Naetar N, Korbei B, Kozlov S, Kerenyi MA, Dorner D, Kral R, Gotic I, Fuchs P, Cohen TV, Bittner R, Stewart CL, <u>Foisner</u> R. (2008) Loss of nucleoplasmic LAP2α lpha-lamin A complexes causes erythroid and epidermal progenitor hyperproliferation. **Nat Cell Biol.** 10:1341-1348.
- Lu W, Gotzmann J, Sironi L, Jaeger VM, Schneider M, Luke Y, Uhlen M, Szigyarto CA, Brachner A, Ellenberg J, <u>Foisner</u> R, Noegel AA, Karakesisoglou I. (2008) Sun1 forms immobile macromolecular assemblies at the nuclear envelope. **Biochim Biophys Acta.** 1783:2415-2426.
- Shumaker DK, Solimando L, Sengupta K, Adam SA, Grunwald A, Strelkov S, <u>Aebi</u> U, Cardoso MC, Goldman RD. (2008) The highly conserved nuclear lamin Ig-fold binds to PCNA: its role in DNA replication. **J Cell Biol** 181:269-20.
- Renou L, Stora S, Ben Yaou R, Volk M, Sinkovec M, Demay L, Richard P, Peterlin B, <u>Bonne</u> G, (2008) Heart-hand syndrome of Slovenian type: a new kind of laminopathy. **J Med Genet** 45, 666-671.
- Quijano-Roy S, Mbieleu B, Bönnemann CG, Jeannet PY, Colomer J, Clarke NF, Cuisset JM, Roper H, De Meirleir L, D'Amico A, Ben Yaou R, Nascimento A, Barois A, Demay L, Bertini E, Ferreiro A, Sewry CA, Romero NB, Ryan M, Muntoni F, Guicheney P, Richard P, Bonne G, Estournet B. (2008) De novo LMNA mutations cause a new form of Congenital Muscular Dystrophy (L-CMD). **Annals of Neurology**. 64:177-186.
- Bonne G, Lombes A, Decostre V. (2008) Les maladies du muscle. Myopathies: nouvelles approches thérapeutiques. La Science au Présent 2008. **Encyclopedia Universalis** France.
- Béréziat V, Cervera P, Verpont MC, Le Dour C, Antuna-Puente B, Dumont S, Somja-Azzi LM, Vantyghem MC, Capeau J, <u>Vigouroux C</u>. (2008) Remodeling of subcutaneous adipose tissue from lipodystrophic patients with LMNA mutations: fibrosis and mitochondrial alterations but no inflammation, submitted.
- Vantyghem MC, Vincent-Desplanques D, Defrance-Faivre F, Capeau J, Fermon C, Valat AS, Lascols O, Pigny P, Hecart AC, Delemer B, Vigouroux C, Wémeau JL. (2008) Fertility and

- obstetrical complications in women with LMNA-related familial partial lipodystrophies. **J Clin Endocrinol Metab**. 93, 2223-2229.
- Pereira S, Massacrier A, Roll P, Vérine A, Etienne-Grimaldi MC, Poitelon Y, Robaglia-Schlupp A, Jamali S, Roeckel-Trevisiol N, Royer B, Pontarotti P, Lévêque C, Seagar M, Lévy N, Cau P, Szepetowski P. (2008) Nuclear localization of a novel human syntaxin 1B isoform. **Gene**. 1;423(2):160-71.
- Varela I, Pereira S, Ugalde AP, Navarro CL, Suárez MF, Cau P, Cadiñanos J, Osorio FG, Foray N, Cobo J, de Carlos F, <u>Lévy</u> N, Freije JM, López-Otín C. (2008) Combined treatment with statins and aminobisphosphonates extends longevity in a mouse model of human premature aging. **Nat Med.** 14(7):767-72.
- <u>Pereira S, Bourgeois P, Navarro C, Esteves-Vieira C, Cau P, De Sandre-Giovannoli A, Lévy N.</u> (2008) HGPS and related premature aging disorders: from genomic identification to the first therapeutic approaches. **Mechanisms of Aging and Development**, 129:449-459.
- Hamadouche T, Poitelon Y, Genin E, Chaouch M, Tazir M, Kassouri N, Nouioua S, Chaouch A, Boccaccio I, Benhassine T, De Sandre-Giovannoli A, Grid D, <u>Levy</u> N, Delague V. (2008) Founder effect and estimation of the age of the c.892C>T (p.Arg298Cys) mutation in LMNA associated to Charcot-Marie-Tooth subtype CMT2B1 in families from North Western Africa. **Annals of Human Genetics** 72:590-7.
- Navarro CL, Poitelon Y, <u>Lévy</u> N. (2008) A-type lamins and progeroïd syndromes : persistent farnesylation with dramatic effects. **Med Sci.** 24 (10), 833-40.
- Meshorer E, <u>Gruenbaum</u> Y. (2008) Gone with the Wnt/Notch: stem cells in laminopathies, progeria and aging. **J. C ell Biol**. 181,9-13.
- Schmitz ML, <u>Herrmann</u> H. (2008) Functional Architecture of the Cell Nucleus. Biochimica et Biophysica Acta (BBA) **Molecular Cell Research.** Volume1783, Issue 11, Pages 2041-2222.
- Olins AL, Zwerger M, <u>Herrmann</u> H, Zentgraf H, Simon AJ, Monestier M, and Olins DE. (2008) The human granulocyte nucleus: Unusual nuclear envelope and heterochromatin composition. Eur J Cell Biol. 87(5), 279-290.
- Zwerger M, <u>Herrmann</u> H, Gaines P, Olins AL, Olins DE. (2008) Granulocytic nuclear differentiation of lamin B receptor-deficient mouse EPRO cells. **Exp Hematol**. [Epub ahead of print] PMID: 18495328.
- Willis ND, Wilson RG, <u>Hutchison</u> CJ. (2008) Lamin A: a putative colonic epithelial stem cell biomarker which identifies colorectal tumours with a more aggressive phenotype. **Biochem Soc Trans**. (Pt 6):1350-3. Review.
- Pekovic V, <u>Hutchison</u> CJ. (2008) Adult stem cell maintenance and tissue regeneration in the ageing context: the role for A-type lamins as intrinsic modulators of ageing in adult stem cells and their niches. **J Anat.** Jul;213(1):5-25. Review
- Cenni V, Bertacchini J, Beretti F, <u>Lattanzi</u> G, Bavelloni A, Riccio M, Ruzzene M, Marin O, Arrigoni G, Parnaik V, <u>Wehnert M, Maraldi NM</u>, de Pol A, Cocco L, Marmiroli S. (2008) Lamin a Ser404 is a nuclear target of Akt phosphorylation in C2C12 cells. **J Proteome Res.** 7(11):4727-4735.
- Camozzi D, Pignatelli S, Valvo C, <u>Lattanzi</u> G, Capanni C, Dal Monte P, Landini MP. (2008) Remodelling of the nuclear lamina during human cytomegalovirus infection: role of the viral proteins pUL50 and pUL53. **J Gen Virol**. 89, 731-40.

- Zini N, Avnet S, Ghisu S, <u>Maraldi</u> NM, Squarzoni S, Baldini N, <u>Lattanzi</u> G. (2008) Effects of prelamin A processing inhibitors on the differentiation and activity of human osteoclasts. **J Cell Biochem**. 105:34-40.
- Maraldi NM, Capanni C, Lattanzi G, Camozzi D, Facchini A, Manzoli FA. (2008) SREBP1 interaction with prelamin A forms: a pathogenic mechanism for lipodystrophic laminopathies. **Adv Enzyme Regul**.;48:209-23.
- Morais P, Magina S, Ribeiro MD, Rodrigues M, Lopes JM, Thanh HL, <u>Wehnert M</u>, Guimarães H. (2008) Restrictive dermopathy-a lethal congenital laminopathy. Case report and review of the literature. **Eur J Pediatr.**
- Rankin J, Auer-Grumbach M, Bagg W, Colclough K, Nguyen TD, Fenton-May J, Hattersley A, Hudson J, Jardine P, Josifova D, Longman C, McWilliam R, Owen K, Walker M, Wehnert M, Ellard S. (2008) Extreme phenotypic diversity and nonpenetrance in families with the LMNA gene mutation R644C. **Am J Med Genet A.** 46A(12):1530-42
- Di Masi A, D'Apice MR, Ricordy R, Tanzarella C, <u>Novelli</u> G (2008). The R527H muta-tion in LMNA gene causes an increased sensitivity to ionising radiation. **Cell Cycle**. In press.
- Lombardi F, Fasciglione GF, D'Apice MR, Filareto A, Vielle A, D'Adamo M, Sbraccia P, Marini S, Novelli G. (2008) Increased release and activity of MMO-9 in patients with Man-dibuloacral Displasia type A, a rare premature ageing sindrome. **Clin Genet**. In press.
- Thill M, Nguyen TD, Wehnert M, Fischer D, Hausser I, Braun S, Jackisch C. (2008) Restrictive dermopathy: a rare laminopathy. **Arch Gynecol Obstet.** 278(3):201-8.
- Dominici S, <u>Fiori</u> V, Magnani M, Schena E, Capanni C, Camozzi D, D'Apice MR, Le Dour C, Auclair M, Caron M, Novelli G, Vigouroux C, Lattanzi G. (2008) Different prelamin A forms are detected by specific antibodies in human fibroblasts: a study in ex-perimental models and progeria. European **Journal of Histochemistry**. In press.

- Vlcek S, <u>Foisner</u> R. (2007) A-type lamin networks in light of laminopathic diseases. Biochim. **Biophys. Acta** 1773, 661-674.
- Dorner D, Gotzmann J, <u>Foisner</u> R. (2007). Nucleoplasmic lamins and their interaction partners, LAP2α lpha, Rb, and BAF, in transcriptional regulation. **FEBS Journal** 274, 1362-1373.
- Vlcek S, <u>Foisner</u> R. (2007) Lamins and Lamin-associated proteins in ageing and disease. **Curr. Opin. Cell Biol.** 19, 298-304.
- Schirmer E. <u>Foisner</u> R. (2007) Proteins that associate with lamins: Many Faces, Many Functions. **Exp. Cell Res.**, 313, 2167-2179.
- Bonne G, Leturcq F, Recan-Budiartha D, Ben Yaou R. (2007) Emery-Dreifuss Muscular Dystrophy. Gene Reviews at GeneTests: **Medical Genetics Information Resource [database online]**. Vol. Available at www.genetests.org. Seattle, 1997-2007: University of Washington.
- Muchir A, Pavlidis P, <u>Bonne</u> G, Hayashi YK, Worman HJ. (2007) Activation of MAPK in hearts of EMD null mice: similarities between mouse models of X-linked and autosomal dominant Emery Dreifuss muscular dystrophy. **Hum Mol Genet**. 16:1884-1895.
- Worman HJ, <u>Bonne</u> G. (2007) "Laminopathies": a wide spectrum of human diseases. **Exp Cell Res** 313, 2121-33.

- Capeau J, Magré J, Lascols O, Caron M, Béréziat V, <u>Vigouroux</u> C. (2007) Primary lipodystrophies. **Ann. Endocrinol**. (Paris) 68, 10-20.
- Moreau F, Boullu-Sanchis S, <u>Vigouroux</u> C, Lucescu, C, Lascols O, Sapin R, Ruimy D, Guerci B, Pinget M, Jeandidier N. (2007) Efficacy of pioglitazone in familial partial lipodystrophy of the Dunnigan type: a case report. **Diabetes Metab**. 33, 385-389..
- Vantyghem MC, Faivre-Defrance F, Marcelli-Tourvieille S, Fermon C, Evrard A, Bourdelle-Hego MF, <u>Vigouroux</u> C, Defebvre L, Delemer B, Wemeau JL. (2007) Familial partial lipodystrophy due to the LMNA R482W mutation with multinodular goiter, extrapyramidal syndrome and primary hyperaldosteronism. **Clin. Endocrinol**. (Oxf.) 67, 247-9.
- Decaudain A, Vantyghem MC, Guerci B, Hécart AC, Auclair M, Reznik Y, Narbonne H, Ducluzeau PH, Donadille B, Lebbé C, Béréziat V, Capeau J, Lascols O, <u>Vigouroux</u> C. (2007) New Metabolic Phenotypes in Laminopathies: LMNA Mutations in Patients With Severe Metabolic Syndrome. J **Clin Endocrinol Metab**. 92, 4835-4844.
- Caron M, Auclair M, Donadille B, Béréziat V, Guerci B, Laville M, Narbonne H, Bodemer C, Lascols O, Capeau J, <u>Vigouroux</u> C. (2007) Human lipodystrophies linked to mutations in A-type lamins and to HIV protease inhibitor therapy are both associated with prelamin A accumulation, oxidative stress and premature cellular senescence. **Cell Death Differ**. 14, 1759-1767.
- Caron M, <u>Vigouroux</u> C, Bastard JP, Capeau J. (2007). Adipocyte dysfunction in response to antiretroviral therapy: clinical, tissue and in-vitro studies. **Curr Opin HIV AIDS**, 2: 268-273
- Shalev SA, De Sandre-Giovannoli A, Shani AA, <u>Lévy</u> N. (2007) An association of Hutchinson-Gilford progeria and malignancy. **Am J Med Genet A**. 143 (16), 1821-6.
- Margalit A, Neufeld E, Feinstein N, Wilson KL, Podbilewicz B, <u>Gruenbaum Y.</u> (2007) Barrier-to-autointegration factor (BAF) is required for cell fusion timing, vulva formation, germ cell devel-opment and survival, DTC migration and adult muscle integrity in C. elegans. **J. Cell Biol**. 178, 661-73.
- Geiger S, Bär H, Ehlermann P, Wälde S, Rutschow D, Zeller R, Ivandic B, Zentgraf H, Katus H, <u>Herrmann</u> H, Weichenhan D. (2007) Incomplete nonsense-mediated decay of mutant lamin A/C mRNA provokes dilated cardiomyopathy and ventricular tachycardia. **J Mol Med.** 86, 281-289.
- Salpingidou G, Smertenko A, Hausmanowa-Petrucewicz I, Hussey PJ, <u>Hutchison</u> CJ. (2007) A novel role for the nuclear membrane protein emerin in association of the centrosome to the outer nuclear membrane. **J Cell Biol**. 178(6), 897-904.
- Araújo-Vilar D, Lado-Abeal J, Palos-Paz F, <u>Lattanzi</u> G, Bandín MA, Bellido D, Domínguez-Gerpe L, Calvo C, Pérez O, Ramazanova A, Martínez-Sánchez N, Victoria B, Costa-Freitas AT. (2007) A novel phenotypic expression associated with a new mutation in LMNA gene, characterized by partial lipodystrophy, insulin-resistance, aortic stenosis and hypertrophic cardiomyopathy. **Clin Endocrinol** (Oxf) 69: 61-68.
- Maraldi NM, Mazzotti G, Rana R, Antonucci A, Di Primio R, Guidotti L. (2007) The nuclear envelope, human genetic diseases and ageing. **Eur. J. Histochem**. 51, 117-24.
- Maraldi NM, Lattanzi G. (2007) Involvement of prelamin A in laminopathies. Crit. Rev. Eukaryot. Gene Expr. 17, 317-34.
- Maraldi NM, Mattioli E, <u>Lattanzi</u> G, Columbaro M, Capanni C, Camozzi D, Squarzoni S, Manzoli, F.A. (2007) Prelamin A processing and heterochromatin dynamics in laminopathies. **Adv. Enzyme Regul.** 47, 154-67.

- Kandert S, Lüke Y, Kleinhenz T, Neumann S, Lu W, Jaeger VM, Munck M, <u>Wehnert M, Müller CR, Zhou Z, Noegel AA, Dabauvalle MC, Karakesisoglou I.</u> (2007) Nesprin-2 giant safeguards nuclear envelope architecture in LMNA S143F progeria cells. **Hum Mol Genet.** 1;16(23):2944-59.
- Zhang Q, Bethmann C, Worth NF, Davies JD, Wasner C, Feuer A, Ragnauth CD, Yi Q, Mellad JA, Warren DT, Wheeler MA, Ellis JA, Skepper JN, Vorgerd M, Schlotter-Weigel B, Weissberg PL, Roberts RG, Wehnert M, Shanahan CM. (2007) Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. *Hum Mol Genet.* 1;16(23):2816-33
- Muchir A, Pavlidis P, Decostre V, Herron AJ, Arimura T, <u>Bonne</u> G, Worman HJ. (2007) Acitivation of MAPK pathways links LMNA mutations to cardiomyopathy in Emery-Dreifuss muscular dystophy. **J Clin Invest.** 117, 1282-1293.
- Ben Yaou R, Toutain A, Arimura T, Demay L, Massart C, Peccate C, Muchir A, Llense S, Deburgrave N, Leturcq F, Litim KE, Rahmoun-Chiali N, Richard P, Babuty D, Récan-Budiartha D, Bonne G. (2007) Multitissular involvement in a family with LMNA and EMD mutations: Role of digenic mechanism? **Neurology**. 29, 1883-1894.
- Mehta IS, Figgitt M, Clements CS, Kill IR, <u>Bridger</u> JM. (2007) Alterations to Nuclear Architecture and Genome Behaviour in Senescent Cells. In press **Annals of NY Academy of Sciences**.
- Naetar N, Hutter S, Dorner D, Dechat T, Korbei B, Gotzmann J, Beug H, <u>Foisner R.</u> (2007) LAP2alpha-binding protein LINT-25 is a novel chromatin-associated protein involved in cell cycle exit. **J. Cell Sci**. 120, 737-747.
- Melcer S, Gruenbaum Y, Krohne G. (2007) Invertebrate lamins. Exp Cell Res. 313, 2157-2166.
- Cohen M, Santarella R, Wiesel N, Mattaj I, <u>Gruenbaum</u> Y. (2007) Electron microscopy of lamin and the nuclear lamina in Caenorhabditis elegans. **Methods Cell Biol**. in press.
- Mattout A, Goldberg M, Tzur Y, Margalit A, <u>Gruenbaum</u> Y. (2007) Specific and conserved sequences in D. melanogaster and C. elegans lamins and histone H2A mediate the attachment of lamins to chromosomes. **J Cell Sci.** 120, 77-85.
- Bär H, Goudeau B, Wälde S, Casteras-Simon M, Mücke N, Shatunov A, Goldberg YP, Clarke C, Holton JL, Eymard B, Katus HA, Fardeau M, Goldfarb L, Vicart P, <u>Herrmann</u> H. (2007) Conspicuous involvement of desmin tail mutations in diverse cardiac and skeletal myopathies. **Hum Mutat.** 28, 374-386.
- Maraldi NM, Capanni C, Mattioli E, Columbaro M, Squarzoni S, Parnaik WK, Wehnert M, Lattanzi G. (2007) A pathogenic mechanism leading to partial lipodistrophy and prospects for pharmacological treatment of insulin resistance syndrome. **Acta Biomed**. 78, 207-215.

- Smith GC, Kinali M, Prasad SK, <u>Bonne</u> G, Muntoni F, Pennell DJ, Nihoyannopoulos P. (2006) Primary myocardial dysfunction in autosomal dominant EDMD. A tissue doppler and cardiovascular magnetic resonance study. **J Cardiovasc Magn Reson.** 8, 723-730.
- Muntoni F, <u>Bonne</u> G, Goldfarb LG, Mercuri E, Piercy RJ, Burke M, Ben Yaou R, Richard P, Récan D, Shatunov A, Sewry CA, Brown S. (2006) Disease severity in dominant Emery Dreifuss is increased by mutations in both emerin and desmin proteins, **Brain** in press.

- Muchir A, Massart C, van Engelen BG, Lammens M, <u>Bonne G</u>, Worman HJ. (2006) Proteasome-mediated degradation of integral inner nuclear membrane protein emerin in fibroblasts lacking A-type lamins. Biochem Biophys Res Commun. 351, 1011-1017.
- Meune C, van Berlo JH, Anselme F, <u>Bonne</u> G, Pinto YM, Duboc D. (2006) Primary Prevention of Sudden death in patients with Lamins A/C gene mutations. **New England Journal of Medecine**, 354 (2), 209-210.
- Foster HA, Stokes P, Forsey K, Leese HJ, <u>Bridger JM</u>. (2006) Intranuclear lamin A foci in nuclei of early embryos. **Chromosome Research** In press.
- Foster HA, Stokes P, Forsey K, Leese HJ, <u>Bridger JM</u>. (2006) Lamins A and C are present in the nuclei of early porcine embryos, with lamin A being distributed in large intranuclear foci. **Chromosome Research**. In press.
- Gotzmann J, <u>Foisner</u> R. (2006) A-type lamin complexes and regenerative potential: a step towards understanding laminopathic diseases? **Histochem. Cell Biol.** 125, 33-41.
- Mattout A, Dechat T, Adam SA, Goldman RD, <u>Gruenbaum</u> Y. (2006) Nuclear lamins, diseases and aging. **Curr. Opi. Cell Biol** 18, 335-341.
- Melcer S, <u>Gruenbaum</u> Y. (2006) Nuclear morphology: When round kernels do the Charleston. **Current Biology.** 6, R195-197.
- Prokocimer M, Margalit A, <u>Gruenbaum</u> Y. (2006) The nuclear lamina and its proposed roles in tumorigenesis: Projection on the hematologic malignancies and future targeted therapy. **J. Struc. Biol.** 155, 351-360 (2006).
- Tzur YB, Wilson KL, <u>Gruenbaum</u> Y. (2006) SUN-domain proteins: the 'velcro' that links nucleus and cytoskeleton. Nat. Rev. **Cell Mol. Biol.** 7, 782-788.
- Tzur YB, Margalit A, Melamed-Book N, <u>Gruenbaum</u> Y. (2006) Matefin/SUN-1 is a nuclear envelope receptor for CED-4 during Caenorhabditis elegans apoptosis. **Proc. Nat. Acad. Sci. USA** 103, 13397-13402.
- Mattout A, Dechat T, Adam SA, Goldman RD, <u>Gruenbaum</u> Y. (2006) Nuclear lamins, diseases and aging. **Curr. Opi. Cell Biol.** 18, 335-341.
- Schumacher J, Reichenzeller M, Kempf T, Schnolzer M, <u>Herrmann</u> H. (2006) Identification of a novel, highly variable amino-terminal amino acid sequence element in the nuclear intermediate filament protein lamin B(2) from higher vertebrates. **FEBS Lett.** 580:6211-6.
- Navarro CL, Cau P, <u>Lévy</u> N. (2006) Molecular bases of progeroid syndromes. **Hum Mol Genet.** 15, 151-161.
- De Sandre-Giovannoli A, <u>Lévy</u> N. (2006) Altered splicing in prelamin A-associated premature aging phenotypes. **Prog Mol Subcell Biol** 44,199-232.
- Maraldi NM, Lattanzi G, Capanni C, Columbaro M, Mattioli E, Sabatelli P, Squarzoni S, Manzoli FA. (2006) Laminopathies: a chromatin affair. **Adv Enzyme Regul.** 46, 33-49.
- Maraldi NM, Lattanzi G, Capanni C, Columbaro M, Merlin L, Mattioli E, Sabatelli P, Squarzoni S, Manzoli FA. (2006) Nuclear envelope proteins and chromatin arrangement: a pathogenic mechanism for laminopathies. **Eur J Histochem**. 50, 1-8.
- Capeau J, Magré J, Caron M, Lagathu C, Bastard JP, <u>Vigouroux</u> C. (2006) Genetic and acquired lipodystrophies: from fat redistribution to insulin resistance and aging. **Future Lipidol.** 1, 593-604.

- Capeau J, <u>Vigouroux</u> C, Magré J, Lascols O, Caron M, Bastard JP. (2006) Lipodystrophic syndromes: congenital or acquired diseases of adipose tissue. **C. R. Biol**. 329, 639-52.
- Capeau J, Caron M, <u>Vigouroux</u> C, Cervera P, Kim M, Maachi M, Lagathu C, Bastard JP. (2006) Lipodystrophies related to antiretroviral treatment of HIV infection. **Med. Sci. (Paris)** 22, 531-36.

3 Publishable Exploitable Results (modified)

P3/INSERM/Nicolas Lévy and P8/UTOVRM/Giuseppe Novelli started a 3-year therapeutic trial in Marseille and Rome, based on a newly developed treatment of progeria and MADA patients with a drug combination (ZoPra: zoledronate + pravastatin). A patent was filed for the treatment:

<u>Patent:</u> WO 2008/003864 A1 (10th january 2008) "Combination of an HMG-CoA reductase inhibitor and a farnesyl-pyrophosphate synthase inhibitor for the treatment of diseases related to the persistance and/or accumulation of prenylated proteins". Owners: Aix-Marseille 2 University, Oviedo Université (Spain), AFM, Marseille Hospital Administration (AP-HM). Inventors: Nicolas Lévy, Pierre Cau, and Carlos Lopez-Otin.

We also considered requesting the orphan medicinal product designation for this drug combination (pravastatin/zoledronate mixture) but at the moment it seems unclear whether designation can be given to a treatment rather than to a product.

P11/DIATHEVA: The generated *LMNA* mutation detection kit and the pre-lamin A antibodies are commercially exploited by DIATHEVA, which sells these products. Knowledge having a potential for direct and immediate industrial or commercial applications in research or for developing, creating or marketing a product or process or for creating or providing a service have not been generated.

Other generated knowledge does not have the potential for a direct and immediate industrial or commercial application in research or for developing, creating or marketing a product or process or for creating or providing a service. Results, however, were and will be published in high impact scientific journals.