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Project acronym:	**EURO-Laminopathies**
Project title:	**Nuclear Envelop-linked Rare Human Diseases: From Molecular Pathophysiology towards Clinical Applications**
Instrument:	**STREP**
Thematic priority:	**Life Science, Genomics and Biotechnology for Health**

MODIFIED FINAL ACTIVITY REPORT

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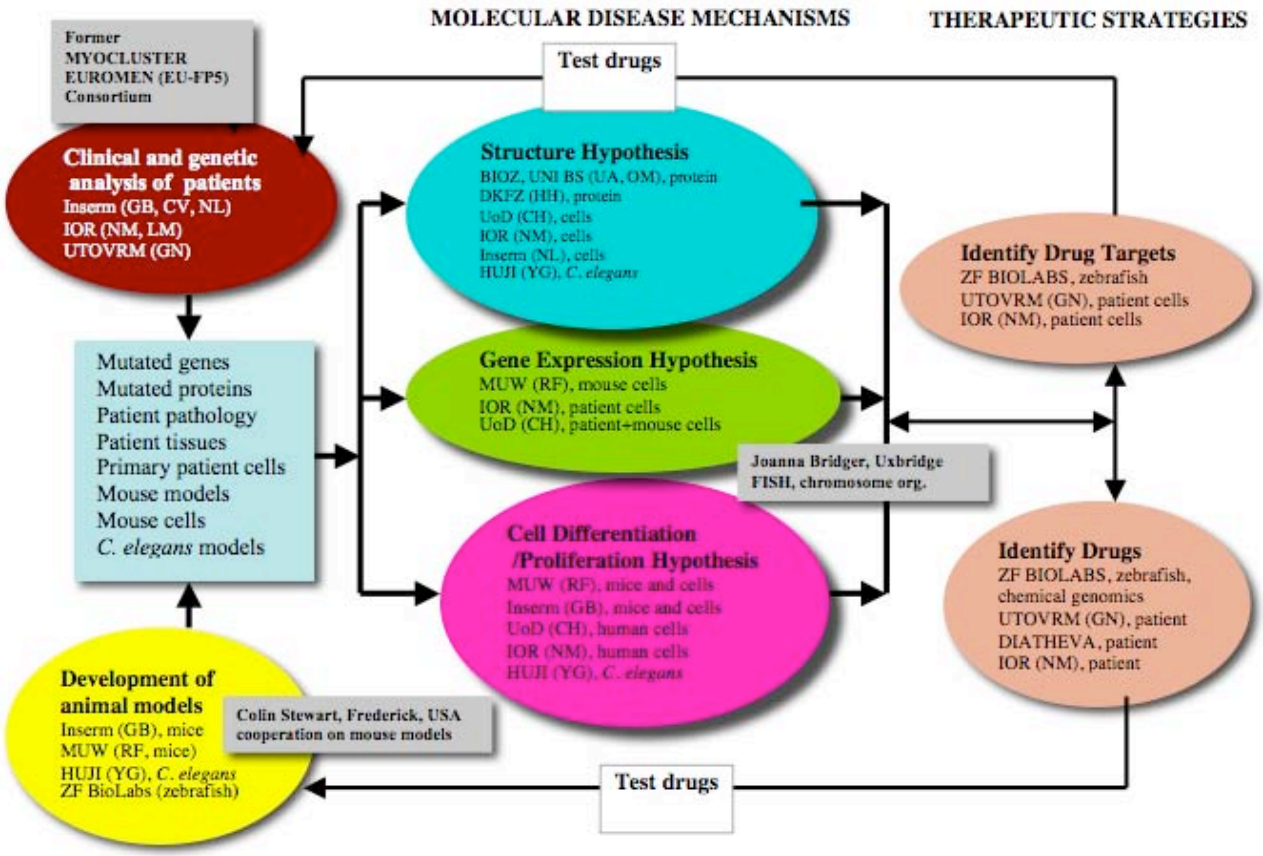
1 PROJECT EXECUTION

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*α); 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, new clinical subgroups 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 FPLD phenotype, we reported about a patient affected by a variant of Dunnigan syndrome characterized by partial lipodystrophy, insulin resistance and hypertrophic cardiomyopathy 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 dilated cardiomyopathy (DCM), 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 progeroid laminopathies, 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 nesprin-1 (SYNE1) and nesprin-2 (SYNE2). 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.

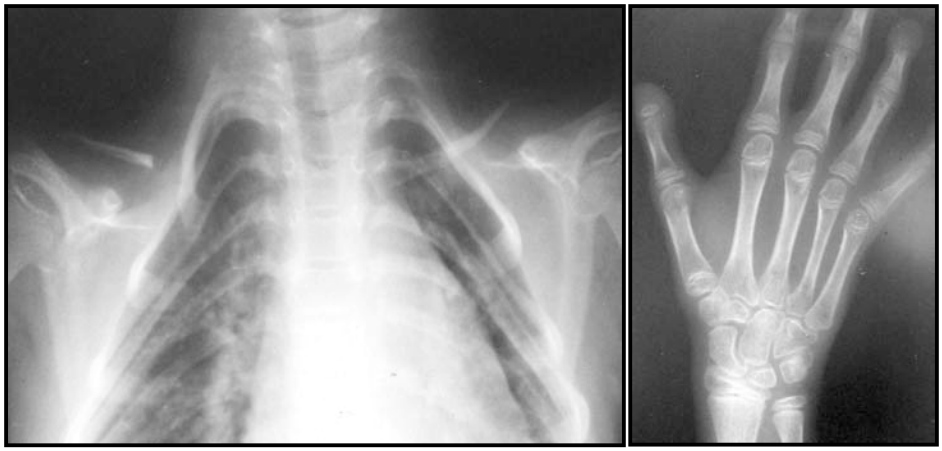


Figure 1: *Skeletal anomalies (clavicular hypoplasia, club-shaped terminal phalanges and acro-osteolysis in patients with progeroid syndromes, such as MADA*

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, LAP2α, ubiquitously or conditionally in muscle fibers and muscle stem cells. Furthermore, crosses of LAP2α-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::Ce-lamin 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.

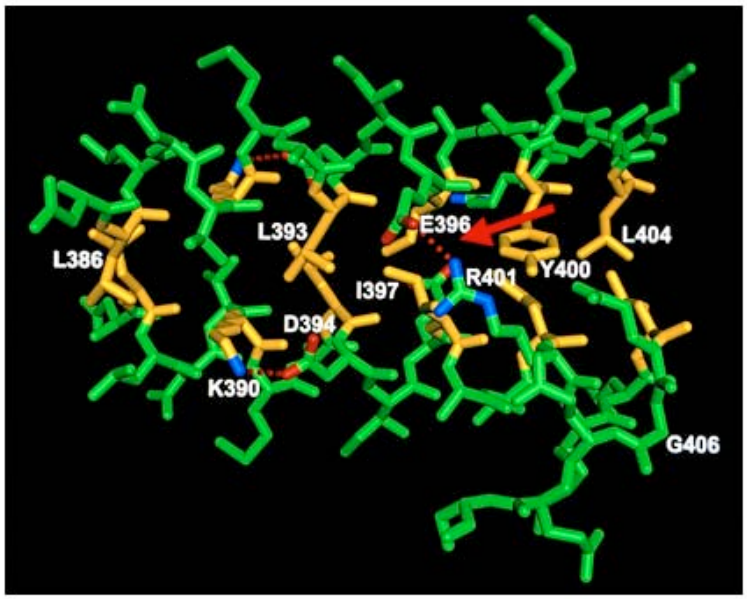
1.3.3 Molecular disease mechanisms

Using recombinantly expressed wild type and disease linked mutated lamin A/C and LAP2α 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.



***Inter*helical 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.

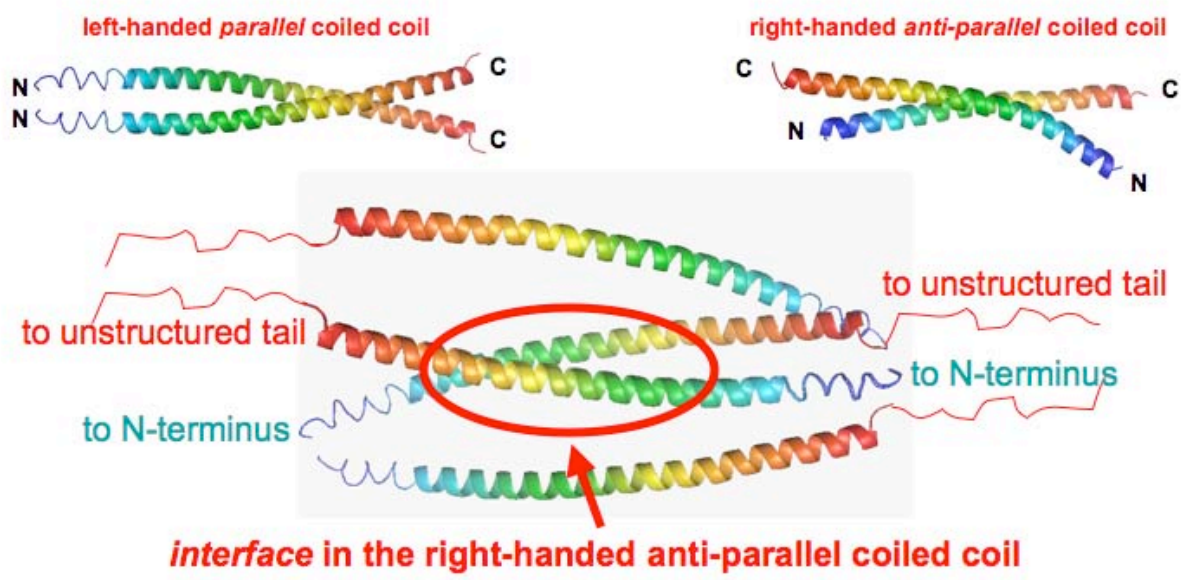


Figure 3: *Combining left-handed parallel and right-handed anti-parallel crystal structures of human lamin A rod fragments to model the interface between two anti-parallel two-stranded coiled coils such as they occur in tetrameric nuclear lamin and other IF protein complexes. Whereas human lamin A coil 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 lamin A assembly and specific interactions as well as the effects of disease causing mutations on these properties.

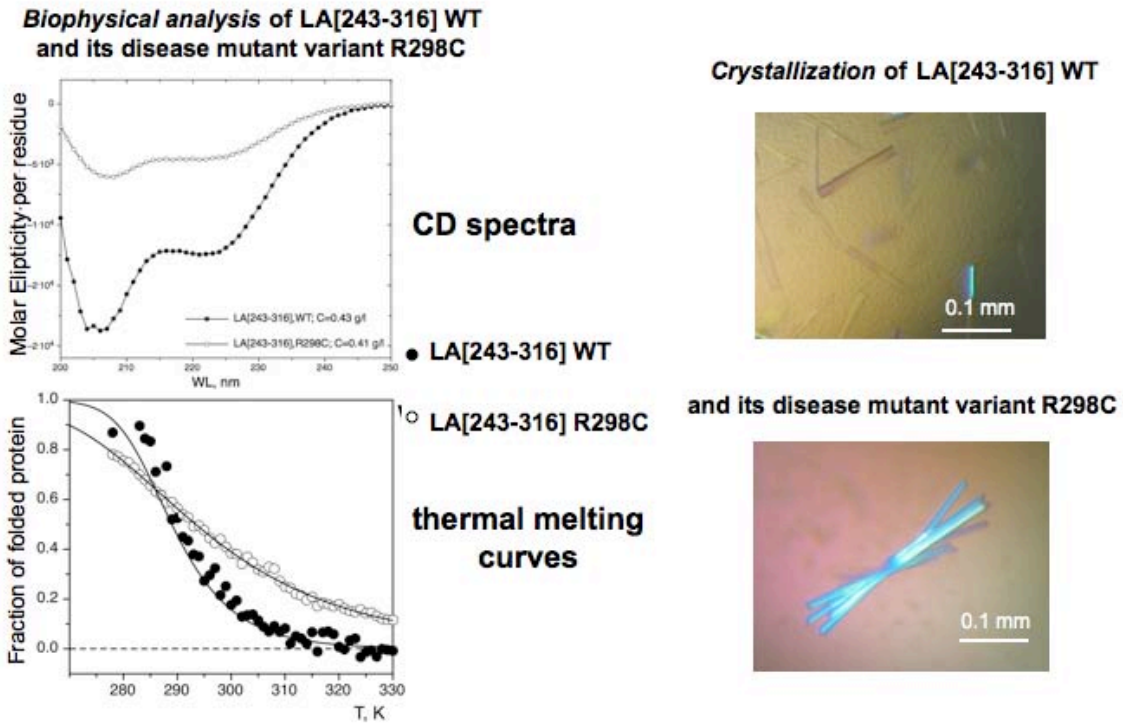


***Figure 4**: Biophysical and crystallographic analysis of human lamin A linker L2 construct (LA[243-316] together with the disease mutant variant LA[243-316/R298C]. CD spectra, thermal melting curves and crystals formed by the human lamin A fragments LA[243-316] WT and LA[243-316/R298C] mutant variant 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 supramolecular organization of the lamin filament. 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.

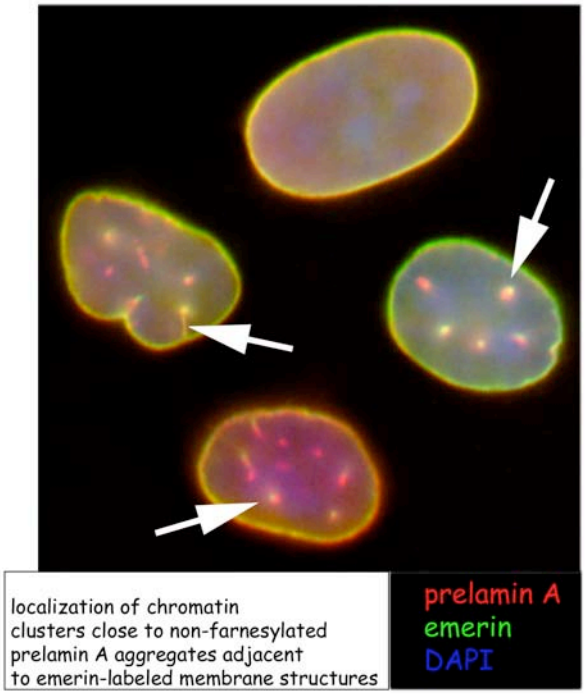


Figure 5 *Changes of heterochromatin in prelamin A over-expressing cells as shown by immunofluorescnce microscopy.*

We studied interactions of prelamin A with various nuclear components. We could demonstrate an interaction between prelamin A and LAP2α, which together formed complexes with the heterochromatin protein HP1alpha. High affinity for HP1α 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 HP1α, LAP2α and trimethyl-H3K9 in intranuclear aggregates. As shown previously, accumulation of farnesylated prelamin A caused loss of heterochromatin and uneven distribution of heterochromatin markers. Moreover, LAP2α 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 (AFCMe)), 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 Nesprin mutations (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-LAP2α 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 LAP2α 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 additon to the well known peripheral lamin network at the nuclear envelope, and that this nucleoplasmic pool of lamin A is stabilized by LAP2α. LAP2α-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. LAP2α-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/LAP2α 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.

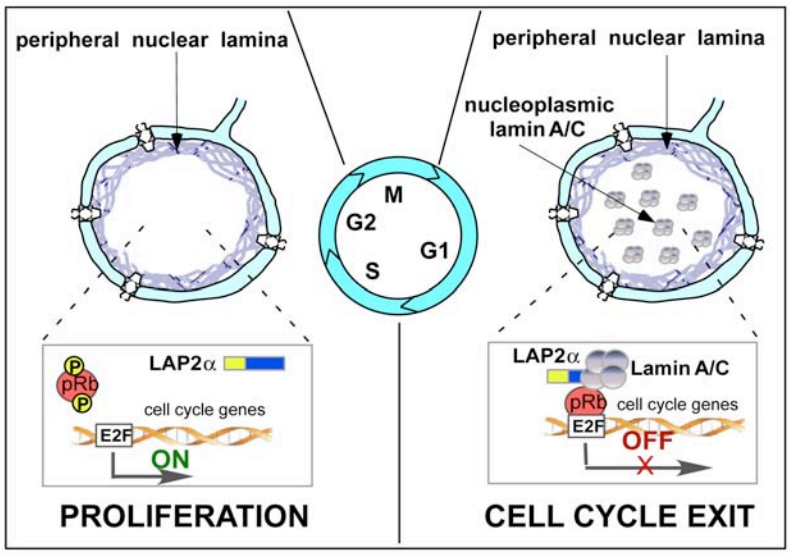


Figure 6: *Model depicting the molecular mechanism how lamins may affect cell cycle progression and differentiation of tissue progenitor cells. A nucleoplasmic pool of lamins A/C in the G1 phase of cycling cells, stabilized by LAP2α, regulates pRb-mediated cell cycle exit and initition of differentiation. Disease-causing lamin variants may affect the nucleoplasmic pool and thus, impair pRb regulation.*

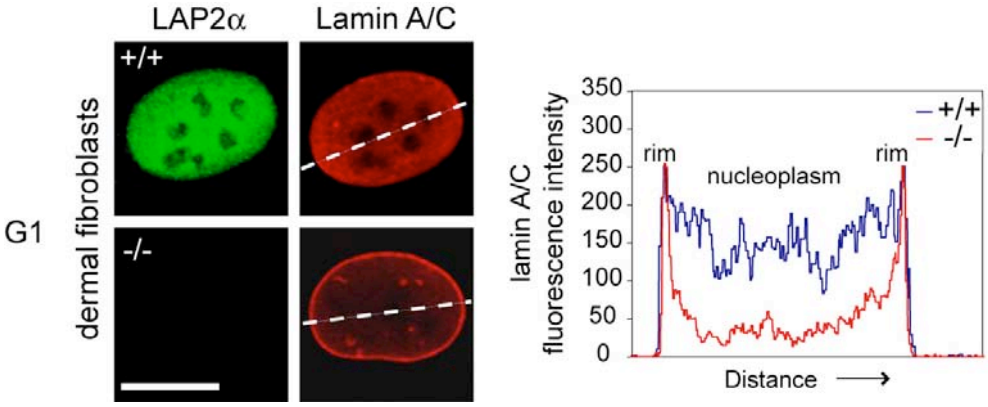


Figure 7: *Nucleoplasmic lamins A/C are lost in LAP2α-deficient fibroblasts. Left panel: immunofluorescent images of fibroblast nuclei from LAP2α$^{+/+}$ and LAP2α$^{-/-}$ mice stained for LAP2α (greeen) and lamins A/C (red). Bar is 10 μm. Right panel: Staining intensities of lamin A measuerd along the line shown in lef images. Note that nucleoplasmic versus rim staining is greatly reduced in LAP2α deficient cells (red line).*

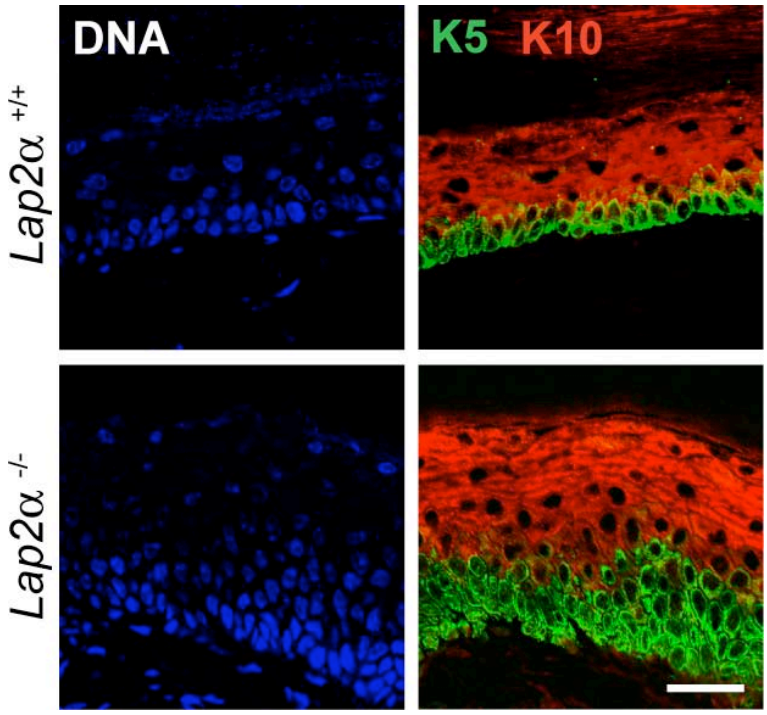


Figure 8: *Paw epidermis of LAP2α-deficient mice shows hyperplasia due to hyperproliferation of early tissue progenitor cells. Immunofluorescence images of paw epidermis sections from LAP2α$^{+/+}$ and LAP2α$^{-/-}$ are shown stained for DNA (blue), keratin 5 as marker for proliferating progenitor cells (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 LAP2α lead to premature entry into S-phase followed by a checkpoint arrest involving altered distribution and phosphorylation of pRb. Lamin A and LAP2α 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 N-acetyl-L-cysteine (NAC), 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 mitogen-activated protein kinases (MAPKs) 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 i*n 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*$^{-/-}$ mice, we could demonstrate the the joint administration of statins (pravastatin) and aminobiphosphonates (zoledronate) 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 preclinical proof 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, Ia 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 *C. elegans* cells show changes in nuclear architecture similar to HGPS fibroblasts and down regulating lamin expression in adult *C. elegans* 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 zebrafish laminopathy disease model 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 currently also testing drugs affecting TNFs *in vitro* and *in vivo* on a selected group of patients.

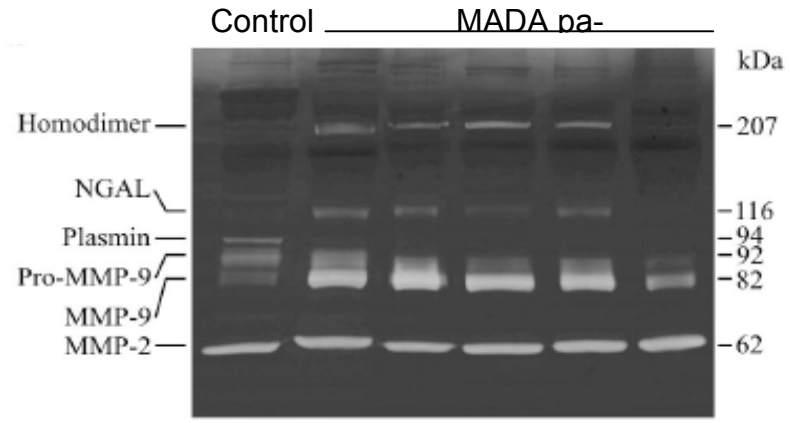


Figure 9: *Gelatinolytic (MMP) activity in serum from MADA pateints, detecting four forms (207 kDa, 116 kDa, 92 kDa and 82 kDa) that may be related to MMP-9, neutrophil gelatinase B-associated lipocalin (NGAL), pro-MMP-9 and active MMP-9, respectively. Note that in MADA serum the levels of active MMP-9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

2 Dissemination and Use

2.1 Exploitable Knowledge and Its Use

Exploitable knowledge	Exploitable result(s)	Sector(s) of application	Timetable for use	IPR protection	Owners
Standardized patient examination protocols	Methods	Medical	2008	A patent is possible in 2008 Publications	P3, P8
Mid-term status			2007 2008	Published	P3, P8
Final status			**2008** **2009**	**Published**	**P3, P8**
New animal models	Methods	Industrial Scientific community	2007 2008 2009	Publications	P1, P3, P10
Mid-term status	Disease models	Pharmaceutical industry for pre-clinical drug testing	2007 2008	Published	P1, P3
Final status			**2008** **2009** **2009 and beyond**	**Published** **to be published**	**P1, P3, P10**
Standardized clinical treatment protocols (SOP)	Methods	Medical	2009	Publications	P8, P11
Mid-term status			2007 2008	Published	P8, P11
Final status			**2008** **2008** **2009** **2009 and beyond**	**Patent** **Published** **to be published**	**P3, P8**
Novel targets and drugs for thera-	Method	Medical	2009	Publications	P3, P7, P8,

peutic intervention	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
Final situation			**2009** **2009 and beyond**	**Published** **to be published**	**P1, P3, P4, 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 LAP2α deficient mouse line. Our studies identified a phenotype of adult stem cells and early progenitor cells in regenerative tissues in LAP2α-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 LAP2α knockout

mice specifically ablating LAP2α 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 (LAP2α-deficient, dystrophin-deficient), and a double mutant (LAP2α-deficient and *Lmna*^{delK32} knock-in) line, were generated to study the functional relationship between LAP2α, 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-Pgk-neo-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 moleculalre 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).

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.

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 LAP2α knockout mouse revealed that defects in lamin A localization (caused by absence of LAP2α) 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

Type	Type of audience	Countries addressed	Partners responsible	Actual Date*
Project website	General public Journalists Researchers Higher education	Austria, France, Germany, Israel, Italy, England, Switzerland, Spain mainly	P9 / all	02/06

Type	Type of audience	Countries addressed	Partners responsible	Actual Date*
Project website update	General public Journalists Researchers Higher education	Austria, France, Germany, Israel, Italy, England, Switzerland, Spain mainly	P9 / all	Latest version 03/09
Press releases (press/radio/TV)		Austria	P1	03,05,10/08; 04/09
	General public	France	P3	2007-2008
	Journalists	England	P6	09/08
Websites of partners' institution	General public Journalists Researchers Higher education	Austria, France, Germany, Israel, Italy, England, Switzerland	P9 / all	02/06
Workshops and lab training	Young researchers collaborating with the project groups	Austria, France, Germany, Israel, Italy, England, Spain, Switzerland	P1, P2, P3, P4, P5, P6 P7, P8	not necessary due to the efficient communication platform
Communication platform was established	Young researchers collaborating with the project	Austria, France, Germany, Israel, Italy, 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, Italy, England, Switzerland, 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, Italy, England, Switzerland Spain	P1, P2, P4, P5	Published 2007-2009
Scientific publications	Researchers SME Industry	Austria, France, Germany, Israel, Italy, England, Switzerland, Spain	P1, P2, P3, P4, P5, P6 P7, P8, P10, P11	2006-2009
Posters	Researchers SME	Austria, France, Germany, Israel, Italy, England, Switzer-	P1, P2, P3, P4, P5, P6 P7, P8, P10,	2006-2009

Type	Type of audience	Countries addressed	Partners responsible	Actual Date*
	Industry	land, Spain, USA, others to be defined	P11	
Conferences	Researchers SME Industry	Austria, France, Germany, Israel, Italy, England, Switzerland, Spain USA, others to be defined	P1, P2, P3, P4, P5, P6 P7, P8, P10, P11	2006-2009
Lectures	Higher education	Austria, France, Germany, Israel, Italy, England, Switzerland, Spain	P1, P2, P3, P4, P5, P6 P7, P8	2006-2009 and beyond
Seminars	Researchers Higher education SME Industry	Austria, France, Germany, Israel, Italy, England, Switzerland, Spain	P1, P2, P3, P4, P5, P6 P7, P8, P10, P11	2006-2009 and beyond
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

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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:

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.

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.