The Center

The Center for Applied Medical Research (CIMA) is a biomedical research institution of the University of Navarra, based in Pamplona, Spain. CIMA’s research proudly aims to a deep translational patient orientation to solve unmet medical needs. CIMA synergizes with the contiguous University Hospital and benefits from the experience of the University Schools of Medicine, Sciences and Pharmacy. This organization of academic, research and clinical knowledge represents an ideal environment for the translation of biological science into its clinical application for the benefit of the patient.

Uniqueness

CIMA is a private research institution in Spain that combines solid basic biology science with early preclinical drug development capabilities within a context of clinical patient care. All is oriented to solve unmet medical problems for the benefit of the patient and the society.

CIMA has facilities and processes that allow target-based and phenotypic drug discovery and early development of new therapeutic agents, up to the lead optimization stage. Noteworthy are: the collection of biological samples; the animal facilities, which include from knock-out mice to larger species; the genomics, proteomics and bioinformatics unit; the imaging unit, providing services of microscopy, non-invasive image acquisition of laboratory animals and quantitative image analysis.

CIMA’s strategic linkage with the University Hospital enables and promotes the bidirectional flow of ‘bench-to-bed’ knowledge… and backwards.

Conscious of the complexity of this endeavor, CIMA operates in a context of Open Innovation, and willingly collaborates with other research institutions, biotechnology and pharmaceutical companies, public institutions and investors.

Vertical Programmes

Hepatology: this therapeutic area focuses on hepatocellular carcinoma, viral hepatitis and cirrhosis, as well as metabolic diseases of the liver.

Solid tumors and biomarkers: with focus on non-small cell lung cancer. Identification and validation of molecular biomarkers is also a priority, facilitated by the proximity to the hospital.

Oncohematology: with focus on leukemia, myeloma and lymphoma, exploring new targets and therapies.

Neurodegenerative diseases: with focus on Alzheimer’s, Parkinson’s, Huntingdon’s disease, finding new targets and exploring new therapies.

Cardiovascular diseases: working on two main fields: myocardial remodeling mechanisms and fibrinolysis, with new targets, new biologically active ‘leads’ and an special interest in non-invasive biomarkers to allow the early diagnosis of structural myocardial alterations involved in heart failure.

Transversal Programmes

Immunotherapy: immunotherapy of cancer and development of vaccines against viral infections and against different types of cancer.

Gene Therapy: development of viral vectors based on third-generation (‘gutless’) adenovirus and adeno-associated viral vectors, for defective monogenic disorders, cancer gene therapy and other diseases.

Cell Therapy and Regenerative Medicine: basic understanding of stem cell biology and use of stem cells and tissue engineering for cardiovascular diseases, diabetes and skin disorders. Among its resources, it has an accredited GMP facility.

Molecular Therapy: including the Small Molecule Discovery platform to de-risk the drug-discovery process, with expertise in Chemical Biology (identification of chemical probes –‘hits’- as pharmacological tools for target validation); Medicinal Chemistry-multifactorial optimization of proprietary ‘hit’ compounds to achieve lead molecules and perform in-vivo PoC, in terms of preliminary efficacy and safety. Aptamers and Peptides are also areas of expertise.

Funding

CIMA is funded like many other research sites through:

- individual and corporate philanthropy,
- public-private partnerships (PPP)
- and competitive research funds.

New products are spun off to newly created companies.

Pipeline

(*)CIMA or through its spin off companies.

- 4 molecules in clinical phases:  
  - Phase II: disitertide-P144- for scleroderma  
  - interferon alfa 5 for chronic hepatitis C  
  - cardiotrophin-1 in cold ischemia, organ transplantation and acute kidney injury.  
  - gene vector AAV-PBGD in acute intermittent porphyria (licensed to UniQure).

- Biotech platforms:
  - third generation adenoviral vectors (‘gutless’)
  - fusion proteins platform
  - cell therapy
  - aptamers

- Therapeutic leads: CM-352 as antifibrinolytic agent.
- Therapeutic hits for novel targets in the fields of neurodegeneration, prevention of stroke, myocardial fibrosis and Alzheimer’s disease.
- Identification and validation of novel targets.
- Diagnostic candidates: biomarkers in NSCLC & Myocardial Remodelling.
- Spin offs (Digna Biotech, Hepacly Therapeutics, Formune)

Collaborations &Partnerships

CIMA is open to various types of partnerships with academia and biopharmaceutical companies in order to facilitate the advancement of the research, with the ultimate goal of improving patient quality of life.

By joining capabilities and resources, this win-win cooperation facilitates the advancement in the different research stages, from target validation to lead optimization or early candidate development.
PROGRAMS

CIMA RESEARCH PROGRAMS

Neuroscience
Oncology
Cardiovascular Sciences
Gene Therapy

PRODUCTS / PROGRAMS LICENSED TO OTHER COMPANIES

Oncology
Cardiovascular Sciences
Rare Diseases
Gene Therapy

RESEARCH TECHNOLOGIES

Cell Therapy: centralized clinical trials unit
Cell lines & animal models
Biobank
Differential Technologies
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- Novel target in CNS: GluN3A in HD and drug abuse

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Alzheimer’s disease (AD) is particularly devastating since there is no cure, no way to prevent it and no proven way to slow its progression.

Management of AD represents a huge unmet need; thus, discovery and development of more effective therapies are critical for worldwide public health and health-care systems.


- Hitting two targets, epigenetic and non-epigenetic.
- *In vitro* Proof of Concept, using primary neuronal cultures: synergistic effect in related targets.
- *In vivo* Proof of Concept, using AD transgenic mice: behavior studies and AD pathological marks.

**Novel proprietary compounds** show *in-vivo* efficacy, and safety, using AD transgenic mice (Tg2576)

**Scope of the problem**

- Currently, approximately 18 million people worldwide are afflicted with this disease and it is projected to reach over 30 million by 2025.
- The current treatment options are only moderately effective. There is an unmet need for therapies that halt of substantially slow disease progression.
- Recent clinical trials of various disease-modifying therapies for AD failed to demonstrate benefit.

**Patient need addressed**

Substantially slow down the progression of Alzheimer’s disease and improve symptoms.

**Product Profile**

- Multifactorial optimization process guided to the discovery of the proprietary lead compound CM-414:
  - Binding affinities, for both targets, at nM range.
  - Efficacy, according to AD related markers (e.g. C99, pTau, …) in Tg2576 primary cultures, at low nM range
  - Crossing BBB and showing *in vivo* functional response - epigenetic mark - at brain level (hippocampus).
  - Safety window, efficacy vs toxicity, >2 log units.
- Effect of CM-414 in AD Tg2576 mice, after chronic treatment (3 weeks), showed a restoration of memory deficits in two different behavioral tasks: the Fear Conditioning and the Morris Water Maze test (A-C).
- The memory recovery induced by CM-414 was maintained after a washout period of 4 weeks in aged Tg2576 mice (Morris Water Maze test) (D-E)
- AD pathological marks analysis, from treated Tg2576 mice, showed significant decrease in amyloid (APP, C99 & Aβ42) and Tau pathology (F-G) as well as reversal in deficits in spine density (H).

![Graphs showing data](image)

(A) One-way ANOVA followed Sheffe test **(p<0.01). (B and D) Two-way repeated measures ANOVA followed Sheffe test. WT vehicle vs Tg2576 vehicle *** (p<0.001); Tg2576 CM-414 vs Tg2576 vehicle ### (p<0.001); (C and E-H) One-way ANOVA followed Sheffe test *(p<0.05)***(p<0.01) *** (p<0.001). Animals: 12-14 month-old. WT (n=12); Tg2576 vehicle (n= 9) & Tg2576 CM-414 40 mg/Kg (i.p.) (n=8).

**Intellectual Property:** Strong IP position. Patent filed (2013), four different chemical series.
Novel target in CNS: GluN3A in HD and drug abuse

- There is an unmet need for effective therapeutic strategies in Huntington’s disease (HD) and drug abuse.
- GluN3A is a novel target that regulates synapse plasticity and neuronal connectivity.
  - Its expression is reactivated in CNS pathologies including HD, cocaine and alcohol abuse.
  - Proof of Concept: Knock-Out mice in HD and shRNA in drug abuse.
  - Safety: lacking in healthy adult brain.

Scope of the problem
- HD is a rare disease: its average frequency ranges from estimated 4 to 10 individuals per 100,000 people, with symptoms usually occurring by late 40s. There is, at present, no cure and only one FDA-approved symptomatic treatment.
- Cocaine abuse is widespread and is becoming a major public health issue. The prevalence of cocaine use in the world is approximately 13 million people or 0.23% of the global population.

New target
- GluN3A subunits form part of NMDA receptors during early postnatal and juvenile stages, but are mostly absent in adult brain (human data).
- Adult reactivation of GluN3A is pathological: inhibits synaptic plasticity and triggers synapse loss (Neuron, 2009).

Clinical Impact
- Adult reactivation of GluN3A protein expression has been described:
  - in striatum of Huntington’s disease individuals (Nat Med, 2013)
  - after cocaine administration (J Neuroscience, 2013; Neuron, 2013)
  - after prenatal alcohol exposure (J Neuroscience, 2013).

Proof of Concept
- Suppressing aberrant reactivation blocks impaired plasticity (using shRNA in cocaine addiction), synapse loss and neurodegeneration (using GluN3A-KO mice in HD).

Safety
- Lacking in adult brain: lesser side-effects than previously failed approaches to target brain NMDA receptors.

Reference

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New epigenetic agents: therapeutic approach in cancer

- Epigenetic modifications are a major driver of biological complexity and can have a role in the development of a variety of disease treatments.
- Epigenetics is an emerging area covering a broad range of mode of actions. However, only four drugs are currently approved and eleven agents are in early-stage trials.
- Novel proprietary compounds binding two epigenetic targets have been developed:
  - more effective than reference epigenetic compounds vs different cancer cell lines.
  - HDAC-independent

Opportunity and Competitive Landscape

- The fact that the epigenome is dynamic is of particular relevance to drug development, as it implies that specific disease-associated epigenetic states may be reversible with treatment.
- To date, the most investigated therapeutic area in terms of epigenetics is cancer.
- Beyond cancer, epigenetic factors have been implicated in inflammatory, autoimmune, metabolic, neurological and cardiovascular disorders.
- Four drugs with epigenetic mechanisms of action are currently approved. All of them are anticancer drugs and they are only focused on 2 modes of action (HDACs and DNMT-irreversible).
- Mostly, research is focused on developing novel HDAC inhibitors.

Application Scope

- Cancer: a wide range of neoplastic diseases in which the epigenetics targets addressed are implied.

New agents

- Small molecules hitting two epigenetic HDAC-independent targets at low nM range MoA validated, through epigenetic marks, in cell lines and in-vivo. First-in-class.

Proof of Concept

- In vitro studies using our selected proprietary compounds (CM-272 and CM-579) @ 1μM.

- In vivo PoC and efficacy studies using CM-272 and ALL CEMO-1 line.

Intellectual Property

- Patent application is on-going
Novel therapeutic strategy in B-cell malignancies: targeting a key microenvironmental ion carrier machinery

- Despite recent outstanding advances in chemo-immunotherapy having shifted certain B-cell tumor subtypes from uniformly lethal to curable, most mature B-cell malignancies still remain incurable.
- The use of therapies combining direct B-cell targeting and immunotherapy implementing T-cell anti-tumor responses by repression of Tregs may improve cure rates of B-cell malignancies.
- A ion carrier numbered as 17 (IC17) has been found as a new target for tumor immunotherapy. Blocking IC17 function exerts a dual anti-tumor effect on a large number of B-cell malignancies by:
  - direct targeting of tumor B cells that are forced to undergo apoptosis.
  - triggering of T-cell anti-tumor responses through decreasing T\textsubscript{reg} numbers and potentiating effector T cells.
- **Indication**: B-cell malignancies (B-cell lymphoma, leukemia and multiple myeloma).

**Novel Approach**
- To establish a proof of principle for a new strategy to treat B-cell malignancies by blocking IC17 function.
- There are no similar dual therapies (potentiating T-cell mediated immunotherapy responses and directly induction of apoptosis) in cancer using a single molecule.

**Target Identification**
- Lymphocytes exhibit a network of ion channels and transporters in the plasma membrane that modulate intracellular concentration of ions which regulate intracellular pH (pHi). Higher pHi promotes proliferation whereas an acidic pHi favors apoptosis. Basal pHi values are higher in all B-cell lymphoma, leukemia and myeloma samples in comparison with non-tumoral B lymphocytes.
- Mice with targeted deletion of one of the master regulators of pHi, termed IC17, showed lymphocytes with abnormal pHi, which was associated with reduced regulated T cell (T\textsubscript{reg}) numbers.
- IC17 has been identified as a T\textsubscript{reg} function regulator and a potential target for tumor immunotherapy.

**Target Validation**
- **In vitro**, chemical probe: pIC17, a molecule binding an extracellular loop of IC17, showed different effects on T cell subsets (decreased T\textsubscript{reg} numbers and increased effector T cell numbers) and killed B-cell leukemia, lymphoma and myeloma cells.

**Figure 1.** pIC17 exerts opposite effects on T-cell subpopulations: it promotes proliferation of effector T cells and decreases survival of Tregs.

**Figure 2.** pIC17 induced apoptosis in human derived B-cell leukemia, lymphoma and myeloma cell lines.

**Figure 3.** Dose-dependent decrease in cell proliferation and increase in apoptosis after pIC17 exposure is observed in the sensitive lymphoma cells JEKO1 but not in resistant U266 cell

- pIC17 kills B-cell lymphoma cells by disrupting IC17 transport function.

**Figure 4.** Effects of pIC17 in sensitive lymphoma cells (JEKO1) and resistant myeloma cells (U266). a) Baseline, IC17 activity is higher in JEKO1 cells than in U266 cells. b) Basal JEKO1 pHi decreases upon pIC17 incubation, whereas U266 pHi remains unchanged. c) and d) Measurement of IC17 activity upon pIC17 incubation, which increases in JEKO1 cells but not in U266 cells.

- **In vivo** studies are on-going.

**Safety**
Studies to determine therapeutic window are on-going.

**Intellectual Property**
Patent application to be filled.

Contact: Jesús M. Hernández, MD, PhD | Chief Executive Officer | Email: jm hernández@unav. es; Tel: +34 948 194 700
Inhibitors of FOXP3 Transcription Factor for cancer therapy

- Immunoregulatory function of T regulatory cells (Treg) may hinder the induction of immune responses against cancer and infectious agents.
- FOXP3 transcription factor is essential for the specification and maintenance of Treg cells and it is considered its “master regulator”.
- Inhibition of FOXP3-NFAT interaction might lead to the impairment of specific functions of FOXP3 and Treg activity and thus, be beneficial in the development of vaccines and tumor therapies.
- F39, a peptide able to disrupt Foxp3/NFAT interaction has been found:
  - F39 interferes with the ability of FOXP3 to repress expression of IL2 and Treg immunosuppressive activity in vitro and in vivo.
  - F39 is able to improve T cell proliferation and cytokine production by effector T cells after TCR stimulation.
- A virtual screening is being conducted to select small molecules that may fit the Foxp3-NFAT hot spot and identify new pharmacological compounds.
- **Indication:** Chronic viral infections (HBV), Cancer (prostate, colorectal, breast...).

**Novel Approach**
- To establish a proof of principle for a new strategy to inhibit Treg cell activity by inhibiting Foxp3/NFAT interaction.
- There are no available compounds able to inhibit Treg activity. In vivo inhibition of Foxp3/NFAT interaction by F39 has shown antitumor activity in different tumor cells in mice.

**Target Identification**
- NFAT plays a pivotal role in the T cell activation-induced transcriptional response during Th cell differentiation. Its interaction with FOXP3 has been shown to be crucial to repress expression of IL2, upregulate expression of the Treg markers CTLA4 and CD25, and confer suppressor function to T lymphocytes.
- F39 inhibits Foxp3-NFAT interaction, impairs the activity of this cooperative complex (regulation of cytokine production by effector T cells, and immunosuppressive activity of Treg cells) and enhances T cell proliferation and cytokine production upon TCR stimulation.
- F39 exhibited antitumor efficacy in different mice tumor models.
- F39 has been identified as a T<sub>reg</sub> function regulator and a potential agent for tumor immunotherapy.

**Target Validation**
- In vitro, F39 binds to NFAT and inhibits Foxp3-NFAT interaction, improves cytokine production by effector T cells upon TCR stimulation and Impairs the Suppressor Function of FOXP3-Expressing.

**New Agents**
- Structure-activity relationship generated, together with structural information from plausible binding mode is being used to conduct a virtual screening to select small molecules that may fit the Foxp3-NFAT hot spot and identify new pharmacological compounds.

**Intell. Property**
- Patent application to be filled.

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Prevention of Stroke in AF patients: a new antithrombotic target

- **Atrial fibrillation** (AF) is a major cause of *stroke*, due to the formation of blood clotting in atrium, resulting from weaker heart beatings of AF patients.
- There is an urgent need to developed safer and more effective treatments to prevent stroke in AF patients.
- A novel target to prevent stroke has been identified (from biobank and patients data).
- **Target validation**
  - Chemical probes identified
  - *In vivo* proof of concept using three different thrombosis models
  - Safety: minimal bleeding risk and no effect on coagulation
- **Primary indication**: Prevention of cardioembolic stroke in atrial fibrillation patients.

### Scope of the problem

- 2.2 million individuals in US and 4.5 million in EU have atrial fibrillation.
- AF accounts for about 14% of all strokes. In US, strokes that result from AF are estimated in 200,000 per year.
- Current treatments to prevent stroke are based on anticoagulants (warfarin, dabigatran, rivaroxaban…), which are among the top-10 more toxic drugs, causing major bleeding in 5% of patients/year.
- Revenues worldwide for current treatment options in stroke prophylaxis secondary to AF, are >2,500M USD, and are estimated to reach 8,500M USD by 2018.

### Patient need addressed

Safer and more efficacious treatments to prevent stroke in AF patients.

### Target Identification

- A new stroke protective gene in AF has been identified in blood samples from 16 patients with AF and stroke vs AF without stroke.
- The gene has been validated in blood samples from 200 patients with AF and stroke vs AF without stroke.

### Target Validation

- Target knock-out mice show the desired biological response.
- Identified pharmacological tool compounds increase gene expression and show antithrombotic effect.
- *In vivo* Proof of Concept using three different thrombosis models.

### Safety

- No effect on coagulation
- No effect on bleeding. Minimal bleeding risk

### Intellectual Property

Patent application to be filed.

**Contact:** Jesús M. Hernández, MD, PhD | Chief Executive Officer | Email: jmhernandez@unav.es; Tel: +34 948 194 700
New treatment in heart failure: a novel antifibrotic target

- Heart Failure (HF) is a chronic, progressive condition in which the heart muscle is unable to pump sufficient quantity of blood through the arterial system to meet the body's needs for blood and oxygen.

- There is an unmet need to develop new antifibrotic therapies to treat HF since fibrosis is present even in the heart of those HF patients treated according to the current clinical practice guidelines, and it is associated with a bad prognosis.

- A novel target to treat myocardial fibrosis in HF has been identified (from biobank and patients data).
  - It is over-stimulated in the myocardium of HF patients
  - There is a related biomarker measurable in blood
  - A proof of concept has been developed: siRNA and chemical probe.

- Primary Indication: Heart Failure.

Scope of the problem
- In the US, over 5.7 million people are currently living with HF. An estimated 400,000 to 700,000 new cases of HF are diagnosed each year.
- About one in five people who have HF die within one year from diagnosis despite being treated in accordance with the standard guidelines.
- Global HF therapeutic market reached 4,068.5M USD in 2010 and it has been predicted to reach 5,104.1M in 2018.
- HF is the cause for 12-15 million medical visits per year and 6.5-7 million days of hospitalization per year.

Patient need addressed
To prevent the development of myocardial fibrosis that is associated with a detrimental impact on cardiac function and on clinical outcome in HF patients.

New target
- A new potential anti-fibrotic target over-stimulated in the myocardium of HF patients.
- Target activity correlates with a biomarker measurable in blood.

Proof of Concept

In vitro. Target inhibition prevents TGF-β induced collagen production in human fibroblasts.

Safety
- Homozygous constitutive knock-out mice are viable, with absence of gross abnormalities and no myocardial defects described.

Intellectual Property
- Patent application to be filed.

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- **Wilson’s Disease** is a rare autosomal recessive inborn error of copper metabolism caused by mutations in ATP7B, which encodes a copper-transporting adenosine triphosphatase.
- Current treatment options are only moderately effective and there is a need to develop long-term effective treatments.
- Adenoassociated viral (AAV) vectors are very promising tools for therapeutic gene delivery, since they are safe and they induce an efficient and long-term transduction.
- A new AAV vector, **AAV-ATP7B**, expressing the therapeutic gene ATP7B has been developed and tested in a Wilson’s disease animal models.

### Scope of the Problem
- Wilson’s disease (WD) is caused by mutations in the *ATP7B* gene located in chromosome 13 (patients are frequently compound heterozygous).
- Defective ATP7B protein prevents incorporation of copper into ceruloplasmin as well as biliary excretion of Cu excess. Cu accumulates progressively in the liver and later in the brain.
- WD affects approximately 1 in 30,000 people worldwide and is fatal unless detected and treated before serious illness from copper poisoning develops.
- Current treatment is based on penicillamine, other chelators and zinc.

### AAV vectors
- Safe, with no or only limited toxicity.
- Induce an efficient and long-term transduction in quiescent cells, a very important point to be effective in adult tissues.
- Genes carried by rAAV vectors have been efficiently transduced in skeletal muscle, heart, brain, joints, eyes and liver leading to stable expression at therapeutic levels.

### AAV-ATP7B

**AAV Vectors with different ATP7B variants and promoters have been generated.**

### Proof of Concept
- *In vivo.*

**A)**  

![Graph showing hepatic copper levels](image)

**B)**

![Histology images](image)

The administration of AAV-ATP7B in Wilson disease mice A) significantly reduces Cu accumulation in the liver and B) improves liver histology.

### Competitive Advantage

In collaboration with uniQure and Digna Biotech SL, a gene therapy product (AAV-PBGD) for the treatment of Acute Intermittent Porphyria has been developed in house and is currently being evaluated in phase I clinical trial.

### Intellectual Property
- Orphan drug designation application in process.
- Patent application in process.

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• **Apolipoprotein A-I (Apo-AI)** is the major protein constituent of the HDL particles.

• **Apo-AI fusion proteins** have an optimized pharmacokinetic/pharmacodynamic profile as they show the following properties:
  - Apo-AI increases the plasmatic half-life and the liver retention of the fused proteins.
  - **Blood-brain barrier active transport**, mediated by a saturable transporter that limits the amount of the fusion protein in the central nervous system.
  - **Modulation of activity**: Apo-AI activates MAPKs through ABCA1 and SR-BI and, therefore, modulates the therapeutic effect of fused proteins.

• **pSushi-IL-15-Apo** is a new proprietary triple fusion protein developed combining apolipoprotein A-I, IL-15 and IL-15Rα’s sushi, with:
  - Superior antitumor effect than IL-15 as a single agent.
  - Higher capacity than hIL15 to enhance antibody dependent cell cytotoxicity (ADCC) when combined with monoclonal antibodies.
  - Increased stability in circulation.
  - Facilitated trans-presentation.

• **Primary Indication**: adjuvant of tumor specific antigen antibodies in lymphoma, breast cancer and colorectal cancer treatment.

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<th>Medical need</th>
<th>Enhancement of the immune response against the tumors with emphasis in synergistic approaches with the current standard of care.</th>
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</thead>
</table>
| **Proof of Concept** | Immuno therapeutic effects against metastatic disease in *in vivo* tumor models, *(B16OVA lung metastasis of melanoma & MC38 colon cancer liver metastasis)*.  
• *In vitro* assays testing the ADCC capacity of Sushi-IL15-Apo in combination with cetuximab against colorectal cancer-derived cell lines. |
| **Safety** | • Toxicity is an on-target side effect; a therapeutic window has been found.  
• Repeated doses are feasible without desensitization or cumulative toxicity. |

**Product Profile**

• A plasmid encoding a triple fusion protein combining apolipoprotein A-I, IL-15 and the Sushi domain.

• Apo A-I acts as a natural vehicle that facilitates the IL-15/IL-15Ra sushi domain anchorage for trans-presentation of the cytokine.

• pSushi-IL-15-Apo is highly active and plasmid doses within the therapeutic range have been identified.

• These plasmid doses were tolerated and promoted the proliferation and accumulation of NK and memory CD8+ T cell in the spleen and in the liver (Fig. 1 and Fig. 2).

• In a classical cromium release assays it has been shown that human Sushi-IL15-Apo protein increases ADCC capacity of cetuximab against a tumoral cell line (Fig. 3).

![Fig. 1. pSushi-IL-15-Apo gene transfer to the liver ameliorates melanoma lung metastasis of B16OVA.](image1)

![Fig. 2. pSushi-IL-15-Apo hydrodynamic gene transfer is active against liver metastases of MC38 colon carcinoma.](image2)

![Fig. 3. Human Sushi-IL-15-Apo increases ADCC capacity of cetuximab in chromium release assays against HT-29 colon-carcinoma cells.](image3)

**Apo Platform:**

- **InterApo**: ApoA-I fused to IFNα.
- **Apo-Linker-144**: Apo-AI fused to P144, an inhibitor peptide of TGFβ.
- **Apo-CT1**: Apo-AI fused to cardiotrophin 1.
- Other fusion proteins are under development.

**Intellectual Property**

PCT/ES2009/070224. Conjugates for the administration of biologically active compounds (licensed to Digna Biotech).

New biomarker for lung cancer complement activation fragment C4d

- Lung cancer is the leading cause of cancer death worldwide.
- Lung cancer is often detected at advanced stages. No diagnostic marker has been proven useful in lung cancer clinical practice. The unmet need for lung cancer diagnostic tools is considered high.
- C4d, a complement activation fragment, has been identified as a new biomarker for the early diagnostic or prognostic evaluation of lung cancer patients.
- **Indication:** lung cancer: - new molecular marker in risk algorithms. - patient selection for invasive procedures.

Scope of the Problem
- In the United States, lung cancer incidence rate is the second highest among men and women and is the most common cause of cancer death in both sexes.
- Lung cancer comprises two subtypes: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter accounts for 80-85% of all cases and includes the two most frequent lung cancer types: adenocarcinomas and squamous cell carcinomas.
- Less than 20% of patients are diagnosed in early stages, when surgical intervention is possible. This explains the poor five-year survival rate (15-20% for all lung cancer tumors and less than 5% in metastatic cases).

Clinical need
- To significantly increase the percentage of early detected cases is an urgent need.
- Low-dose CT based lung cancer screening studies have reported high rates of detection of cancers in early stages and a reduction in mortality. In this context, the use of molecular markers may help in:
  - the implementation of population-based screening programs.
  - the confirmation of the presence of malignant cells.
  - the prediction of its evolution and its biological response to treatment.

Biomarker Identification
- The role of complement in the control of lung cancer cell growth has been evaluated: lung cancer cells efficiently activate the classical complement pathway.
- C4d, a complement activation fragment, is of value for detection and prognosis of lung cancer.

Biomarker Validation
- The value of C4d quantification for the early diagnostic or prognostic evaluation of lung cancer patients is sustained by the use of:
  - different biological samples (tumor tissue, bronchoalveolar lavage fluid and plasma)
  - a range of independent patient cohorts.
- C4d levels in bronchoalveolar lavage fluid and in plasma are increased in samples from lung cancer patients at both advanced (III and IV) and early (I and II) stages compared with control subjects.
- C4d levels in plasma are associated with increased lung cancer risk in asymptomatic individuals.

**Competitive Advantage**
- C4d is elevated irrespective of the lung cancer histology.
- C4d is not elevated in highly prevalent respiratory diseases such as COPD or emphysema.
- The analytical method is commercially viable and easy to implement in the clinical context.

**Intellectual Property**

**Reference**

Contact: Jesús M. Hernández, MD, PhD | Email: jm hernandez@unav.es; Tel: +34 948 194 700
Lung cancer is the leading cause of cancer death worldwide.

Prognostic markers may guide treatment decisions to improve survival of patients with lung cancer.

RNA metabolism score is a five-gene expression signature for the prognostic evaluation of patients with early stage lung cancer.

**Indication:** to determine the risk of recurrence in surgically-treated lung cancer patients.

**Scope of the Problem**

- Non-small cell lung cancer (NSCLC) accounts for 80-85% of all lung cancer cases and includes lung adenocarcinomas (ADC), the most frequent type of lung cancer.
- Surgery is the treatment of choice for early stage NSCLC. However, patients are at substantial risk for recurrence even after complete surgical resection (55%-70% of them will relapse within 5 years of diagnosis).
- To reduce the risk of recurrence, adjuvant chemotherapy is recommended for patients with resected stage II-IIIA lung cancer, although its benefit is low. The use of chemotherapy in stage I patients is still controversial.

**Clinical Need**

- The current staging system is not sufficient to determine the most appropriate treatment for each resectable lung cancer patient.
- A more accurate method for the identification of early-stage patients likely to have more aggressive tumors with occult metastases at the time of diagnosis is needed.
- Molecular prognostic markers may be used to identify those patients with surgically-treated NSCLC who are at high risk of developing regional or distant metastases and would benefit from adjuvant therapy.

**Prognostic Marker Identification**

- The RNA metabolism is involved in the pathogenesis of lung cancer.
- We have identified five RNA metabolism-related genes with prognostic value in patients with lung ADC.
- An RNA metabolism score based on the expression of these five genes has a strong prognostic capacity in these patients.

**Prognostic Marker Validation**

- The RNA metabolism score provides independent prognostic information for recurrence free survival and overall survival in patients with lung ADC.
- The score is able to differentiate high risk from low risk individuals in stages I and II, separately.
- The score is also able to differentiate risk populations of women with breast cancer.
- These results have been obtained using data from six independent patient’s cohorts (a total of 534 lung cancer patients and 589 breast cancer patients).

**Competitive Advantage**

- The prognostic information provided by the RNA metabolism score is independent of the tumor stage.
- The score retains its prognostic capacity in very early lung cancer stages (stage I).
- The score is based in only five genes, helping in its successful translation to the clinical practice.
- The RNA metabolism score can be applicable to other cancer types, such as breast cancer.

**Intellectual Property**

Methods and reagents for the prognosis of cancer. PCT/EP2013/062966. 21.06.2013 (licensed to Digna Biotech SL).

**Reference**


Contact: Jesús M. Hernández, MD, PhD | Email: jm hernandez@unav.es; Tel: + 34 948 194 700
CIMA RESEARCH PROGRAMS

Neuroscience
- Alzheimer’s Disease treatment: a novel approach
- Novel target in CNS:GluN3A in HD and drug abuse

Oncology
- New epigenetic agents: therapeutic approach in cancer
- Novel therapeutic strategy in B-cell malignancies: targeting a key microenvironmental ion carrier machinery
- Inhibitors of FOXP3 Transcription Factor for cancer therapy

Cardiovascular Sciences
- Prevention of Stroke in AF patients: a new antithrombotic target
- New treatment in heart failure: a novel antifibrotic target

Gene Therapy
- Gene therapy for Wilson’s Disease: AAV-ATP7B

PRODUCTS / PROGRAMS LICENSED TO OTHER COMPANIES

Oncology
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- New expression profile: prognosis of early stage lung cancer (licensed to Digna Biotech SL)

Cardiovascular Sciences
- New antihemorrhagic agents: a novel antifibrinolytic strategy (licensed to Digna Biotech SL)

Rare Diseases
- PBGD-conjugate: improving hemin efficacy in AIP treatment

Gene Therapy
- HC-Ad vectors: High-capacity adenoviral vectors (licensed to Digna Biotech SL)
- Gene therapy product for Acute Intermittent Porphyria: Phase I Clinical trial (licensed to Digna Biotech SL and UniQure)
**New antihemorrhagic agents: a novel antifibrinolytic strategy**

- **Hemorrhage** is a leading cause of death and cost associated with blood transfusion.
- There is a need for the improvement of current treatments of bleeding associated with surgery, trauma or other tissue damages.
- A novel target involved in fibrinolysis has been identified (from biobank and patients data).
- A proprietary novel compound to prevent major bleeding has been developed:
  - Small molecule entity (SME).
  - Efficacy: 30,000 times more effective than the currently available therapies.
  - Safety: No thrombus formation and no impact on coagulation.
- **Primary Indication:** **prophylaxis and acute treatment of bleeding in cardiac surgery.**
- **Life plan**
  - intravenous: cardiac surgery → other major surgeries → trauma and first-aid.
  - topical: trauma and first-aid → OTC → veterinary uses

### Scope of the problem
- Coronary artery bypass surgery: 470,000 procedures/year in the 7 Major Markets. Aprotinin withdrawal ($600M market niche) has generated demand and opportunity for safe and effective antifibrinolytics that significantly reduce the number of blood transfusions.
- Major surgeries: 100-120 million procedures every year in the 7 Major Markets, 2.5-3.5% with significant blood loss. Tranexamic acid (TXA) is used in 35-45% of surgeries.
- Hemorrhage is responsible for 50% deaths occurring within 24 h of traumatic injury.
- Annual expenditures on blood transfusion: $1.62M-$6.03M per hospital.

### Patient need addressed
- Prophylaxis and treatment of major bleeding in patients undergoing cardiac surgery

### Current Standard of Care & Competitive Landscape
- Antifibrinolytics are the Standard of Care for hemorrhage in surgery and trauma.
- TXA is the only commercially available agent, partially effective at high doses with significant side-effects. Current products in development are restricted to sealants (topical) and clotting factors (plasma derived compounds with higher risk of viral transmissions and thromboembolic complications).

### Product Profile
- A new mechanism of action that impacts on fibrinolytic function and not on coagulation.
- Multifactorial optimization process lead to compound **CM-352** that, along with **in vivo** PoC has optimal:
  - ADME/Tox profile.
  - Cardiovascular safety.
  - Off-target selectivity.
  - Pharmacokinetics and toxicokinetic properties.
- **CM-352** shows antifibrinolytic effect via a novel target, no impact on haemostasis and optimal profile for acute systemic administration (i.v.) with short half-life, ideal for short term control of bleeding.
- **CM-352** meets criteria to advance to IND-enabling studies.

### Intellectual Property
- Positive EESR report from EPO examiner, regarding novelty and inventive step.

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**Acute Intermittent Porphyria (AIP)** is a rare metabolic liver disorder resulting from mutations in the porphobilinogen deaminase (PBGD), which encodes for the third enzyme involved in the production of heme.

- **Current Standard of Care (SoC) in AIP** is **intravenous hemin administration**, which provides exogenous heme for the negative feedback inhibition of ALAS, decreasing further ALA and PBG production.
- **Hemin treatment effect** is slow and requires 3 or 4 daily infusions. Moreover, **side effects** as headache, thrombophlebitis, hepatosiderosis and decreasing sensitivity should be considered.
- A new molecule **rhPBGD-conjugate** has been developed with the following properties:
  - metabolizes serum PBG.
  - subcutaneous administration.
  - increased half-life in circulation than previous failed approaches rhPBGD (Zymenex A/S).

**Indication**: combined administration with hemin in AIP acute attacks.

**Scope of the problem**
- The SoC for acute attacks is the intravenous administration of hemin (**Normosang**), which restores hepatic heme deficiency and down-regulates stressed hepatic heme biosynthesis.
- A single dose of 250 mg of hemin contains 22.7 mg of iron, and iron overloads are therefore a potential problem in patients treated on numerous occasions. Thus, long-lasting treatment may be considered.
- Hemin administration induces the activity of the key enzyme of heme catabolism, the heme oxygenase, reducing the therapeutic efficacy of hemin administration.
- The effect of low doses of hemin (≤1 mg/kg/body weight) should be further explored as a dose that does not induce heme-oxygenase and reduce iron overload.

**Patient Need Addressed**
Low dose hemin treatment combined with our novel molecule rhPBGD-conjugate may maintain the same therapeutic efficacy reducing the time of onset of serum PBG and decreasing the risk of hepatosiderosis and other side effects as the induction of heme-oxygenase.

**Competitive Landscape**
New products under development may compete with **Normosang** and reduce its market share:
- Gene therapy, replaces liver transplantation in chronic acute porphyria.
- ALAS-siRNA, represses hepatic ALAS1 but did not restore regulatory heme pool and has no effect on heme precursors accumulated in the serum.

**Product Profile**
- The treatment with PBGD-conjugated replacement could be combined with hemin administration increasing the efficacy in the treatment of acute attacks and reducing the elevated toxic precursors accumulation in serum during the first 48 hours post-hemin treatment.
- PBGD-conjugated properties:
  - longer half-life in circulation (21.4 fold) than rhPBGD. (Fig. 1) and a single administration ensures protection throughout the acute attack.
  - subcutaneous administration ensures quick and long-lasting action (Fig. 2) and may be administered during the time of receipt and preparation of hemin.
  - crosses the blood brain barrier (Fig. 3).


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- Gene therapy product for Acute Intermittent Porphyria: Phase I Clinical trial (licensed to Digna Biotech SL and UniQure)
**High-Capacity adenoviral vectors (HC-Ad)**, also called helper-dependent or ‘gutless’ are a new class of gene therapy vectors that allow stable transfer of large DNA fragments *in vivo*.

A **new method** for production of high-quality HC-Ad with potential applications in humans has been developed, based on a **self-inactivating adenovirus** acting as a helper virus (HV).

**Indications**: monogenic diseases, cancer, liver cirrhosis and metabolic diseases.

### HC-Ad Platform
This new method for production of HC-Ad will facilitate the use of these vectors in basic and applied research and in the clinical setting.

### Competitive Advantage
- High transduction efficiency.
- Maintenance of gene expression for long periods of time after a single administration of the vector without the need of integration in the genome.
- Transfer of large DNA fragments (up to 36 Kb): Suitable for simultaneous expression of several therapeutic genes and incorporation of complex inducible systems.

### Therapeutic Approaches
- Gene supplementation in **monogenic diseases** when the DNA sequence required exceeds the cloning capacity of AAV vectors.
- Controlled expression of immunostimulatory cytokines.
- Long-term expression of polypeptides with anti-angiogenic and anti-proliferative functions.
- *In vitro* and *in vivo* gene correction through transference of specific recombinases together with genomic regions that facilitate the homologous recombination.
- Expression of monoclonal antibodies with therapeutic properties.

### Clinical Application
- Cancer: immunotherapy, anti-angiogenesis, inhibition of carcinogenic pathways.
- Liver cirrhosis: controlled, liver-specific expression of IGF-1.
- Metabolic diseases: porphyrias, hyperoxuluria, lysosomal storage diseases, hemophilia and alpha 1-anti-trypsin deficiency.

### An example of HC-Ad: The HC-Ad/RUm1L-12 vector
- HC-Ad/RUm1L-12 vector, designed to fight against primary or metastatic liver cancer, contains a liver-specific, mifepristone-inducible system for the expression of IL-12 that allows a tight control on the intensity and duration of cytokine expression.
- Following vector administration, the induction regime is adjusted based on the response to a low dose of mifepristone, to compensate for differences in viral transduction.
- This individualized protocol allows several cycles of IL-12 expression in the therapeutic range (figure a).
- A single cycle consisting on 10 daily inductions significantly extended the survival of animals and achieved eradication of hepatic tumors in 50% of them.
- A single dose of the chemotherapeutic agents oxaliplatin (OXP) administered 3 days before the initiation of IL-12 induction increased the rate of tumor eradication above 80%, whereas the same dose of drug had no significant antitumor effect by its own in this aggressive tumor model (figures b and c).

![Image of IL-12 expression](image)

**Intellectual Property**
- PCT/ES2009/070154. Self-inactivating helper adenoviruses for the production of high-capacity recombinant adenoviruses (licensed to Digna Biotech).

**References**

**Contact:** Jesús M. Hernández, MD, PhD | Email: jm hernandez@unav.es | Tel: +34 948 194 700
**Adenoassociated viral (AAV) vectors** are very promising tools for therapeutic gene delivery, since they are safe and they induce an efficient and long-term transduction.

In collaboration with uniQure a gene therapy product for the treatment of Acute Intermittent Porphyria has been developed and is currently being evaluated in **phase I clinical trial**.

A new **AAV expression system** has been developed and an **AAV production system** has been optimized.

- AAV tetracycline-inducible system
  - liver and brain specific expression.

### AAV vectors

- Safe, with no or only limited toxicity.
- Induce an efficient and long-term transduction in quiescent cells, a very important point to be effective in adult tissues.
- Genes carried by rAAV vectors have been efficiently transduced in skeletal muscle, heart, brain, joints, eyes and liver leading to stable expression at therapeutic levels.

**AAV-PBGD: gene therapy product for Acute Intermittent Porphyria (licensed to Digna Biotech and UniQure)**

- In collaboration with the Dutch company uniQure, a gene therapy product for the treatment of Acute Intermittent Porphyria has been developed.
- Orphan designation approved.
- Grant from the EU’s FP7 program (AIPGENE consortium) to bring this product forward to completion of a Phase I/II study in humans.
- The product is currently being evaluated in **phase I clinical trial** at the Clínica Universidad de Navarra and Hospital 12 de Octubre.
- Phase II clinical trial will be started in the near future.
- References:

**AAV Platform (CIMA)**

- A new AAV tetracycline-inducible system for liver and brain specific expression has been developed.
- Recombinant AAV viruses to express cytokines, shRNAs, antibodies and many other different genes have been constructed and produced for the treatment of infectious diseases, malignancies or hereditary metabolic disorders.
- Gene therapy products for rare diseases, hyperoxaluria and Wilson disease are under development.
- AAV vectors for different research groups at CIMA, Universidad de la Laguna, Universidad Complutense de Madrid, Centre Esther Koplovitz (CEK), San Raffaele Scientific Institute have been designed and produced.
- **Competitive Advantage**: Developed tools that allow designing therapeutic vectors according to the characteristics of the disease, such as tissue specificity or controlled expression.

**Intellectual Property**


**References**


PROGRAMS

CIMA RESEARCH PROGRAMS

Neuroscience
Oncology
Cardiovascular Sciences
Gene Therapy

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Rare Diseases
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RESEARCH TECHNOLOGIES

Cell Therapy: centralized clinical trials unit
Cell lines & animal models
Biobank
Differential Technologies
## RESEARCH TECHNOLOGIES

### Cell Therapy: centralized clinical trials unit

- **Cell lines & animal models**
- **Biobank**

### Differential Technologies

- Aptamers
- MicroPET
- Flow cytometry and cell sorting
- Imaging Unit
- Neurophysiology
Accredited GMP facility for production of more than 10 different products (6 different INDs) with trained personnel (cell therapy and gene therapy).

In house centralized Clinical Trial Unit with experience in Phase I trials.

Quality control/Quality assurance system developed in house that controls: infrastructure, personnel, registries, equipment, process control, control of primary supplies, traceability, record of events, quality monitoring. System accredited and approved by the Spanish Agency of Medical Products.

Standard operating procedures (SOPs) for all processes performed under GMP conditions.

Large animal facility for both murine and large animal models allowing preclinical studies to test cell therapies.

Approaches:
- Clinical Trials with Mesenchymal Stem Cells (MSC) and other cell therapy products.
- Clinical Trials with dendritic cells (DC) vaccines.

**Clinical Trials Ongoing**

<table>
<thead>
<tr>
<th>Code</th>
<th>Abbreviated Title</th>
<th>Phase</th>
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<td>Percutaneous implant of autologous skeletal myoblast in chronic myocardial infarction.</td>
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<td>2006-000679-14</td>
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<td>Lymphoma Idiotipic Vaccine</td>
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<td>2009-009879-35</td>
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<td>DEND/GM</td>
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<td>CSM/CROH</td>
<td>Treatment of fistula in Chron's Disease: implant of autologous MSC from adipose tissue.</td>
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<td>2009-014278-16</td>
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<td>EPC/CIRR</td>
<td>Autologous endothelial progenitor cells from BM mononuclear cells for liver cirrhosis.</td>
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<td>2009-017757-36</td>
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<td>LEA/VIT</td>
<td>Autologous melanocytes monolayer in amniotic membranes in stable vitiligo.</td>
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<td>2009-017829-19</td>
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<td>LFNK</td>
<td>Combined immunotherapy with autologous effector lymphocytes in non-Hodgkin lymphoma in rituximab maintenance therapy after response to first-line chemotherapy.</td>
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<td>CDCC/2010</td>
<td>Vaccination with autologous DC in locally advanced high risk colon adenocarcinoma</td>
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<td>2010-024043-32</td>
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<td>CD-AdNS3</td>
<td>Therapeutic vaccination in chronic hepatitis C with autologous DC transduced with recombinant adenovirus expressing hepatitis derived NS3 protein.</td>
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<td>10-148/10-149</td>
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<td>CMM/PRG/ART</td>
<td>Treatment of arthritis with intra-articular injection of bone marrow MSC and PRGF.</td>
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<td>Immunotherapy with DC and hiltonol in patients with solid tumors.</td>
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**Treatments not included in Clinical Trials: comporative use or established therapies.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Patients Included</th>
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<td>Bone marrow mononuclear cells for peripheral ischemia</td>
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<td>Mesenchymal bone marrow stem cells for GVHD</td>
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<td>Mesenchymal bone marrow stem cells for knee arthrosis</td>
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<td>Mesenchymal bone marrow stem cells for necrosis of the hip</td>
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<td>Mesenchymal bone marrow stem cells for Diabetes Mellitus type I</td>
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<td>Mesenchymal stem cells derived from adipose tissue for decubitus ulcer</td>
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</tr>
<tr>
<td>Epidermal layers enriched in melanocytes for treatment of vitiligo</td>
<td>59</td>
</tr>
<tr>
<td>Limbo-corneal cells for limbic deficiencies</td>
<td>12</td>
</tr>
<tr>
<td>Pulsed DC with tumor lysate for glioblastoma</td>
<td>15</td>
</tr>
<tr>
<td>Pulsed DC with tumor lysate for colon cancer</td>
<td>5</td>
</tr>
<tr>
<td>Pulsed DC with tumor lysate for breast cancer</td>
<td>15</td>
</tr>
<tr>
<td>Pulsed DC with tumor lysate for pediatric tumors</td>
<td>1</td>
</tr>
<tr>
<td>LF-NK for colon cancer</td>
<td>10</td>
</tr>
</tbody>
</table>

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RESEARCH TECHNOLOGIES

Cell Therapy: centralized clinical trials unit

Cell lines & animal models

Biobank

Differential Technologies

- Aptamers
- MicroPET
- Flow cytometry and cell sorting
- Imaging Unit
- Neurophysiology
Cell lines

- Isolation and cell culture of various types of liver cells: hepatocytes, stellate cells, macrophages, endothelial cells.
- B-cell chronic lymphocytic leukemia (B-CLL): human cell lines recapitulate the phenotypical, histopathological and molecular features of the transformation of B-CLL to chemotherapy resistant RS.

Animal models

Murine models

- Model of liver transplantation (rat and mice)
- Generation of chimeric mice with humanized liver
- A murine model of mucosa-associated lymphoid tissue (MALT) lymphoma

Non-Human Primate Models

- Models of bilateral Parkinsonism after MPTP chronic administration.
- Histopathological analysis of brain tissue sections (conventional immunohistochemistry and confocal immunofluorescence).
RESEARCH TECHNOLOGIES

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- Neurophysiology
Biobank-integrated samples and data are available for any research project approved by an ethics committee (Integrated Collections).

Additionally, there are a number of historic human samples collections that could be used in the context of a scientific collaboration or under specific transfer condition (Associate Collections).

### Integrated Collections

<table>
<thead>
<tr>
<th>Collection</th>
<th>No. cases</th>
<th>Samples</th>
<th>Data*</th>
<th>Registry</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal cancer</td>
<td>197</td>
<td>Tumoral / Non-tumoral tissue, plasma, blood</td>
<td>CD-AP</td>
<td>Integrated</td>
<td>Open</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>68</td>
<td>Tumoral / Non-tumoral tissue</td>
<td>CD-AP</td>
<td>Integrated</td>
<td>Open</td>
</tr>
<tr>
<td>Bone cancer and soft tissue</td>
<td>61</td>
<td>Tumoral / Non-tumoral tissue</td>
<td>CD-AP</td>
<td>Integrated</td>
<td>Open</td>
</tr>
<tr>
<td>Neurological cancer</td>
<td>23</td>
<td>Tumoral tissue, CSF</td>
<td>CD-AP</td>
<td>Integrated</td>
<td>Open</td>
</tr>
<tr>
<td>Advanced lung and urologic cancer</td>
<td>224</td>
<td>Serum, plasma, blood</td>
<td>CD-AP and M</td>
<td>Integrated</td>
<td>Open</td>
</tr>
<tr>
<td>Radiotherapy-treated cancer</td>
<td>258</td>
<td>Serum, plasma, blood</td>
<td>CD-AP and M</td>
<td>Integrated</td>
<td>Open</td>
</tr>
<tr>
<td>Nasal Polyps</td>
<td>95</td>
<td>Polyps, plasma, blood</td>
<td>CD</td>
<td>Integrated</td>
<td>Open</td>
</tr>
<tr>
<td>Chronic renal failure grade IV</td>
<td>91</td>
<td>Serum, plasma</td>
<td>CD and biochemistry of kidney</td>
<td>Integrated</td>
<td>Closed</td>
</tr>
<tr>
<td>Cardiovascular Risk in Asymptomatic Adults</td>
<td>1960</td>
<td>Serum, plasma, DNA</td>
<td>CD and biochemistry of CV risk</td>
<td>Associate</td>
<td>Closed</td>
</tr>
<tr>
<td>Different types of liver disorders: hepatitis, cirrhosis, liver tumours, liver transplant, steatosis</td>
<td>874**</td>
<td>Tumoral / Non-tumoral tissue, serum, PBMC, blood, urine</td>
<td>CD-AP and M</td>
<td>Integrated</td>
<td>Open</td>
</tr>
<tr>
<td>Surplus diagnostic tissue from hematological malignancies</td>
<td>251</td>
<td>DNA, Carnoy’s fixed cell pellets, RNA</td>
<td>CD, cytogenetic and molecular</td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>Attention Deficit Hyperactivity Disorder (ADHD)</td>
<td>7</td>
<td>Plasma, DNA</td>
<td>Integrated</td>
<td>Open</td>
<td></td>
</tr>
</tbody>
</table>

*Data: CD-AP: clinical diagnosis and possibility of anatomical pathology; M: monitoring.

** 874 registered donors since 2009. Previously, around 13.323.
<table>
<thead>
<tr>
<th>Collection</th>
<th>Samples</th>
<th>Data*</th>
<th>Registry</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunotherapy of cancer. Samples from cancer patients treated with immunotherapy.</td>
<td>Serum, PBMC, tumoral tissue</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>Cancer biomarkers, University Hospital Laboratory of Biochemistry. Surplus diagnostic samples from cancer patients.</td>
<td>Serum, plasma, PBMC, urine</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>Pediatric tumors</td>
<td>Plasma, PBMC, tumoral tissue, gDNA, RNA</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>University Hospital Laboratory of Allergology. Surplus diagnostic samples from patients with well-characterized allergic process.</td>
<td>Serum</td>
<td></td>
<td>A+I</td>
<td>Open</td>
</tr>
<tr>
<td>Surplus diagnostic samples from pemphigus patients.</td>
<td>Serum</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>Thrombosis. Atrial fibrillation and eventual cardioembolic stroke patients.</td>
<td>Auricules, plasma</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>Atherothrombosis. Patients at cardiovascular risk and/or vulnerable plaque.</td>
<td>Serum, plasma, carotid wall or plaque</td>
<td></td>
<td>Associate</td>
<td>Closed</td>
</tr>
<tr>
<td>University Hospital Laboratory of Hematology. Surplus diagnostic samples from hematology patients.</td>
<td>Serum, plasma, gDNA</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>Pediatrics: deafness, Fragile X syndrome, anorexia, rheumatoid arthritis, obesity.</td>
<td>Serum, plasma, gDNA</td>
<td></td>
<td>Associate</td>
<td>Closed</td>
</tr>
<tr>
<td>ABONUS. Surplus samples from the Department of Nutritional Sciences.</td>
<td>Serum, plasma, urine, adipose tissue</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>Metabolic research: health individuals, overweight, obesity and metabolic syndrome patients.</td>
<td>Serum, plasma, bile and non-tumoral tissues.</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>University Hospital Laboratory of Immunology. Surplus diagnostic samples from immunology patients.</td>
<td>Plasma, PBMC, DNA and non-tumoral tissue</td>
<td></td>
<td>CONSULT</td>
<td>Open</td>
</tr>
<tr>
<td>Neurogene library. Patients with neurological disorders.</td>
<td>Serum, plasma, DNAg, RNA</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
<tr>
<td>Brain biobank (collaboration with the Regional Biobank of Neurological Tissues in Navarre).</td>
<td>Brain tissue processed according to protocol for neuroanatomy studies.</td>
<td></td>
<td>Associate</td>
<td>Closed</td>
</tr>
<tr>
<td>University Hospital Laboratory of Ophthalmology. Hypermetropic patients.</td>
<td>gDNA</td>
<td></td>
<td>Associate</td>
<td>Open</td>
</tr>
</tbody>
</table>

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# RESEARCH TECHNOLOGIES

## Cell Therapy: centralized clinical trials unit

## Cell lines & animal models

## Biobank

## Differential Technologies

- Aptamers
- MicroPET
- Flow cytometry and cell sorting
- Imaging Unit
- Neurophysiology
Aptamers

Aptamers are single-stranded nucleic acid oligomers which acquire a complex three-dimensional structure that is determined by the nucleotide sequence. Aptamers are selected via SELEX (Systematic Evolution of Ligands by Exponential Enrichment) and Cell-SELEX, a complex technique consisting of multiple phases of binding, partition and elution.

Competitive advantages:
- Exhibit specificity and avidity comparable or exceeding that of antibodies
- Can be generated against most targets, proteins and small molecules
- Synthesized chemically; they are not cell-based products.
  - Development & manufacturing - cost effective
  - Vastly simpler regulatory approval process

Applications:
- Aptamers with therapeutic application, special interest in cancer immunotherapy.
- Agonistic aptamers binding to receptors that boost immune response.
- Antagonistic aptamers binding to receptors that inhibit immune response.
- Bivalent and bi-specific aptamers.
- Aptamers-siRNA chimeras, to inhibit specifically transcripts targeted cells.
MicroPET

Infrastructure
- PET-GMP lab:
  - 18MeV cyclotron: $^{18}$F, $^{11}$C and $^{13}$N
  - $^{68}$Ga generator
  - Fully equipped QC lab: radio-HPLC, radio-TLC, gamma spectrometry, GC...
  - Automatic modules for chemical synthesis
  - Shielded class B hot cells
  - Shielded class A dispensing hot cells
- MicroPET where we routinely study mice, rats and monkeys
- Gamma counter
- Whole body cryostat and digital quantitative autoradiography

Research Capabilities
- Radiosynthesis Platform; custom synthesis of radiolabelled probes
- In vivo biodistribution studies (PET): evolution, treatment response, follow-up
- Ex vivo biodistribution studies --> high spatial resolution
- In vivo pharmacokinetics and competition studies

PET radiodrugs availability
- $^{18}$F-FDG, $^{11}$C-choline, $^{18}$F-DOPA, $^{18}$F-FLT, $^{68}$Ga-NOTA-tagged peptides, $^{11}$C-DTBZ, ...
- $^{99m}$Tc-RP and $^{125}$I, $^{131}$I- labelled proteins
Flow cytometry and cell sorting

Discovery
- Ex vivo screening of drug efficacy and toxicity in pluricellular primary samples of patients with hematological malignancies. Patients will be fully characterized (mandatory for differential diagnosis) and in addition the tumor clone will be sorted and stored (if further molecular characterization is required to correlate chemosensitivity or chemoresistance with unknown genetic abnormalities)
- Sorting of non-apoptotic (chemoresistant) subclones in cell lines exposed to drugs for subsequent molecular characterization: understanding chemoresistance

Clinical translational research
- Comprehensive flow cytometry core for immunophenotypic diagnostics in clinical trials
- Measurement of treatment efficacy through minimal residual disease (MRD) monitoring in leukemias, lymphomas and multiple myeloma
- Patient-specific sequential immunophenotypic screening and cell sorting of paired baseline (diagnosis) and MRD tumor cells to unravel the molecular mechanism of chemoresistance
- Monitoring of immune modulation in patients or primary samples treated with immunotherapeutic drugs

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Imaging Unit

Hardware
- Spinning disk confocal microscope, for high-throughput time-lapse microscopy
- High-resolution X-Ray computed tomography (MicroCT)
- High-resolution ultrasonography
- 2D and 3D systems for bioluminescence and fluorescence detection in whole animals

Software
- Software for emphysema, COPD quantification (clinical and preclinical) in X-ray computed tomography
- Detection and tracking of Lung nodules (preclinical) in microCT images of mice
- In vivo high-throughput quantification of cell migration
- Semi-automated tracking of individual neurons for longitudinal survival analysis: testing for longitudinal effects in neurons. 
  \[ \text{Proc Natl Acad Sci U S A. 2005 Mar 8;102(10):3840-5}. \]
- Atlas based segmentation of brain structures in MRI
- Automated quantification of nuclearly, cytoplasmic and membrane based immunopositive cells
- Fiber/collagen detection based in Sirius read and Masson’s trichrome staining
- Dynamic quantification of wound healing in time-lapse phase contrast microscopy
- Quantification of glomerulal mesangial expansion in histological sections
- Colocalization of fluorescent markers in fixed and living cells
- Tracking and morphological characterization of migrating cells in 2D and 3D
- Traction force microscopy for the measurement of cell traction forces
- Quantification of adipose tissue content on histological sections
Neurophysiology

The Animal Neurophysiology laboratory

Capabilities
- The lab can perform a wide range of neurophysiological recordings *in vivo* (with video-monitoring of motor activity):
  - in an acute setting in anesthetized animals
  - in chronical settings in awake animals
- Simultaneous recording of single unit action potentials and local field potentials or EEG in different structures (simultaneous acquisition of up to 32 channels with optimal sampling rates).
- Deliver auditory, somatosensory or visual stimuli synchronized to the recording equipment, allowing the recording of different modalities of evoked/induced responses both at central (i.e. cortical and subcortical potentials) and peripheral (i.e. nerve conduction) levels.
- We can perform a wide range of signal analysis: advanced spike sorting procedures, single unit activity bursting and oscillation indexes, auto-correlation and cross-correlation, FFT, imaginary and phase coherence, time-frequency transforms, cross-frequency coupling, independent component analysis and detrended fluctuation analysis. All the analysis tools described in this section can also be applied to human data.

Areas and species covered
- Experience in the recording and analysis of neural activity in cortical and subcortical areas, including the hippocampus, motor and auditory cortex, basal ganglia, inferior colliculus and thalamus.
- Different animal species: mice (wildtype and transgenic animals), rats and non-human primates (*macaca fascicularis*).

Clinical neurophysiology laboratory (located in the University Hospital)
- Human neurophysiology laboratory routinely performs: EEG, High density EEG, Polisomnographic recordings, evoked potentials, event related potentials, electromyography and electroneurography, transcranial magnetic stimulation, reflexology and other motor control studies.